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COMPARATIVE EVALUATION OF MACERATION AND SOXHLET EXTRACTION TECHNIQUES USING ACETONE AND AQUEOUS ACETONE: A COMPREHENSIVE STUDY ON THE EXTRACTIVE YIELD OF AZADIRACHTA INDICA AND TERMINALIA CHEBULA

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ABSTRACT

The extraction process is critical for isolating bioactive compounds from medicinal plants. This study compares the effects of extraction method (maceration vs. Soxhlet) and solvent polarity (100% acetone vs. 70% aqueous acetone) on the yield and phytochemical profile of Azadirachta indica (Neem) leaf and Terminalia chebula (Haritaki) fruit. Dried plant materials underwent eight extraction protocols. Percentage yield was calculated gravimetrically, and extracts were screened for major secondary metabolites. Soxhlet extraction with 70% aqueous acetone yielded the highest quantity of crude extract, with Haritaki achieving a remarkable 15.9% yield. Phytochemical analysis showed solvent-specific partitioning: pure acetone was superior for extracting Neem's terpenoids and steroids, while aqueous acetone enhanced the recovery of Haritaki's tannins and phenolics. The choice of extraction method and solvent polarity significantly impacts both the yield and chemical composition of plant extracts. These findings provide a critical framework for optimizing protocols to target specific bioactive compounds.

KEYWORDS: Plant Extraction, Solvent Polarity, Maceration, Soxhlet Extraction, Azadirachta indica, Terminalia chebula.

1. INTRODUCTION

1.1 The Paramount Role of Extraction in Natural Product Research

The journey from a medicinal plant to a therapeutic agent begins with extraction. This initial step is arguably the most critical in natural product research, as it dictates the success of all subsequent isolation, characterization, and bioactivity assays. An efficient extraction protocol must achieve a high yield of target bioactive compounds while minimizing the co-extraction of inert or interfering materials, preserving the chemical integrity of labile constituents, and being economically and environmentally viable. The reproducibility and scalability of the extraction process are fundamental to the quality control and standardization of herbal drugs and nutraceuticals.[1]

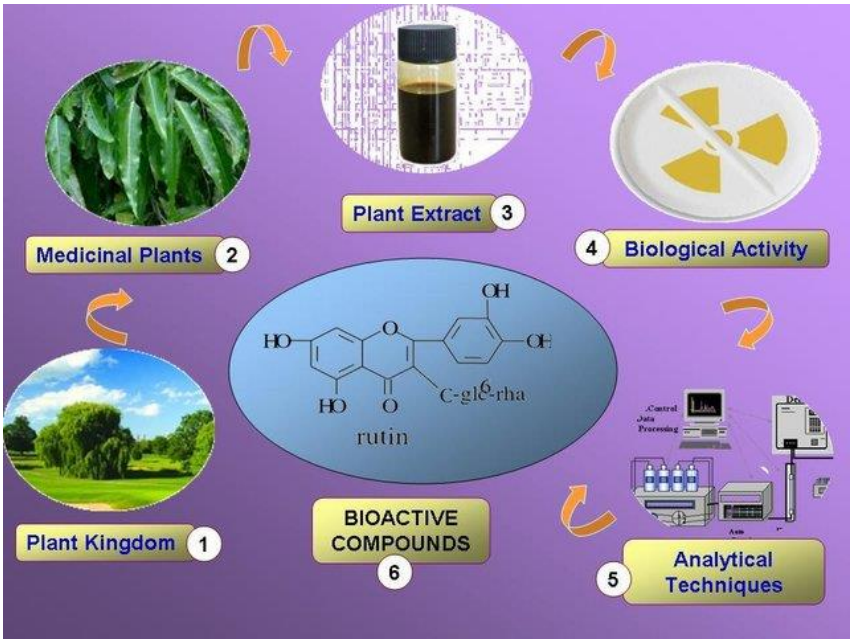

Fig. 01: Role of Extraction.

1.2 Historical Trajectory and Modern Extraction Techniques

The history of plant extraction is as old as medicine itself, with traditional methods like decoction (boiling in water), infusion (steeping, as in tea), and tincture preparation (using hydroalcoholic solutions) forming the bedrock of ancient pharmacopeias. The advancement of modern pharmacognosy has introduced a suite of sophisticated techniques, each with distinct mechanisms and advantages. These include percolation (continuous flow of solvent), maceration (passive soaking), Soxhlet extraction (continuous reflux and percolation), and

modern green techniques like Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), and Supercritical Fluid Extraction (SFE). Despite the advent of these novel methods, maceration and Soxhlet extraction endure as gold standards in both academic and industrial laboratories due to their operational simplicity, minimal equipment requirements, and excellent reproducibility, providing a reliable baseline for comparative studies.[2-4]

Fig 02: Traditional method of extraction.

Fig 03: Modern method of extraction.

1.3 The Scientific Rationale of Solvent Selection

The principle of "**like dissolves like**" is the cornerstone of solvent selection. The efficiency of a solvent in extracting specific compounds is primarily governed by its polarity, which can be quantified by its dielectric constant or its empirically derived polarity index (P′). Non-polar solvents (e.g., hexane, petroleum ether; P′ < 2) are ideal for non-polar compounds like fixed oils, waxes, and some terpenes. Medium-polarity solvents (e.g., chloroform, ethyl acetate, acetone; P′ ~4-5.5) are effective for alkaloids, aglycones, and less polar terpenoids. Polar solvents (e.g., ethanol, methanol; P′ ~5-6) and aqueous mixtures are required for extracting polar compounds such as flavonoids, tannins, phenolic acids, and glycosides. Water (P′ = 9), the most polar solvent, efficiently extracts highly polar compounds like sugars, amino acids, and certain polysaccharides. The use of binary solvent systems, such as aqueous-organic mixtures, is a powerful strategy to modulate overall polarity and hydrogen-bonding capacity, often leading to synergistic extraction effects for a wider spectrum of metabolites.[5-8]

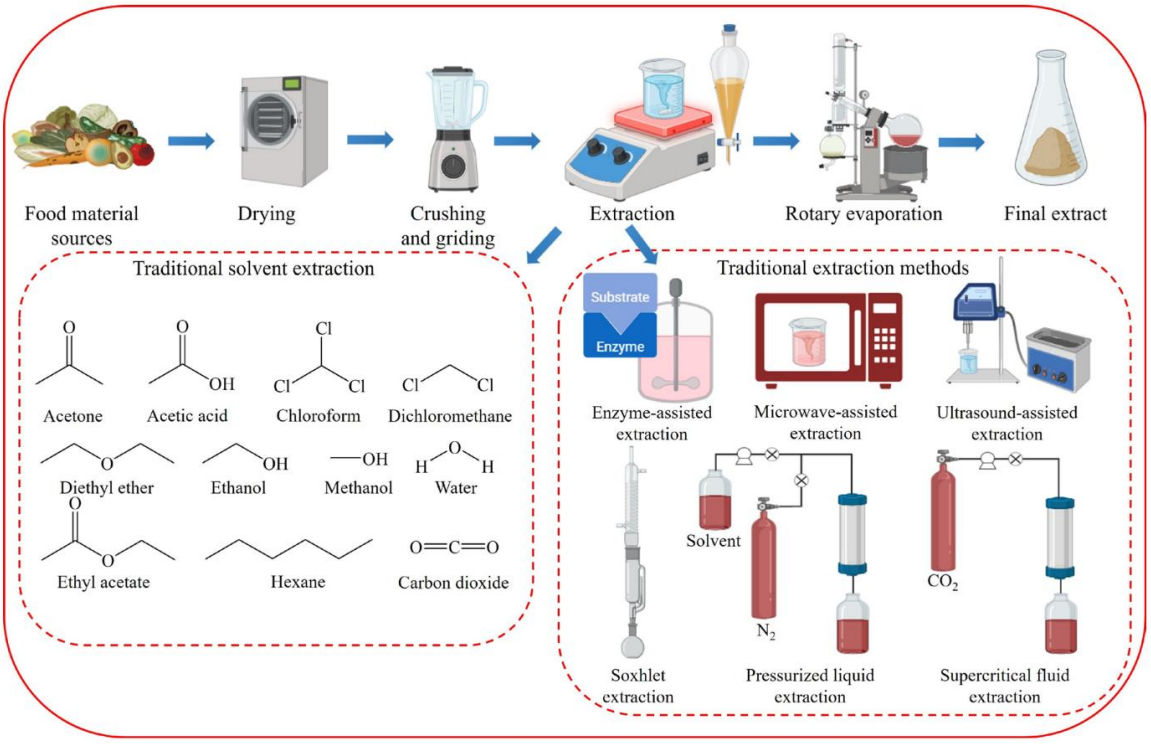

Fig. 04: Choice of solvents for extraction.

Table 1: Polarity Index and Applications of Common Extraction Solvents.

Solvent	Polarity Index (P′)	Dielectric Constant (ε)	Typical Classes of Extracted Compounds
n-Hexane	0.1	1.9	Lipids, Waxes, Fatty Acids, Pigments (chlorophyll)
Toluene	2.4	2.4	Alkaloids, Resins
Diethyl Ether	2.8	4.3	Alkaloids, Terpenoids, Coumarins
Chloroform	4.1	4.8	Alkaloids, Steroids, Terpenoids
Ethyl Acetate	4.4	6.0	Flavonoids, Terpenoids, Phenolic compounds
Acetone	**5.1**	**20.7**	**Terpenoids, Phenolics, Polar pigments**
Ethanol	5.2	24.3	Polyphenols, Flavonoids, Alkaloids, Glycosides
Methanol	5.1	32.7	Similar to ethanol, higher extraction efficiency
Water	**9.0**	**80.1**	**Tannins, Saponins, Carbohydrates, Amino Acids**

1.4 Maceration vs. Soxhlet Extraction: A Mechanistic Comparison

Maceration: This is a cold, passive extraction process where the powdered plant material is soaked in a solvent for an extended period (typically 72 hours) with occasional agitation. Its primary advantages are its simplicity, low energy consumption, and suitability for thermolabile compounds that may degrade upon heating. The major drawback is its inherent inefficiency; the process relies on diffusion and concentration gradients, which plateau as the solvent becomes saturated with solutes, leading to incomplete extraction.[9-12]

Fig 05: Maceration.

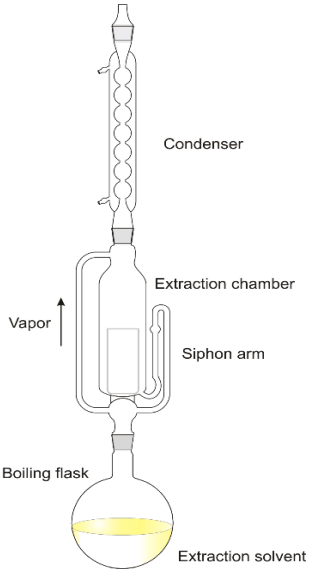

Fig 06: Soxhlet extraction.

Soxhlet Extraction: This is a continuous, hot extraction technique. The apparatus repeatedly cycles fresh, distilled solvent through the plant matrix via siphoning action. This ensures the plant material is always in contact with pure solvent, maintaining a high concentration gradient and driving the extraction to near-exhaustion. Its main advantages are high efficiency, automation, and lower solvent consumption per gram of extract. The significant disadvantage is the application of sustained heat, which can lead to the thermal degradation of heat-sensitive compounds like certain vitamins, enzymes, and unstable polyphenols.

1.5 Acetone and Aqueous Acetone as Extraction Solvents

Acetone (P′ = 5.1) is a versatile, moderately polar, water-miscible solvent with a strong ability to dissolve a wide range of medium-polarity secondary metabolites. It is particularly effective for terpenoids, limonoids (e.g., azadirachtin in Neem), and many phenolic compounds. Its low boiling point (56°C) facilitates easy removal by evaporation. The preparation of 70% aqueous acetone introduces a powerful hydrogen-bonding component (water) to the solvent system. This mixture is exceptionally effective at solubilizing highly polar and high-molecular-weight polyphenols, such as the complex hydrolyzable tannins (e.g., chebulinic and chebulagic acid) prevalent in Terminalia chebula. The water molecules help to swell the plant cells, improving solvent penetration and mass transfer.

1.6 Pharmacological Significance of the Model Plants

Azadirachta indica (Neem): Often called "the village pharmacy," Neem is a reservoir of biologically active limonoids and triterpenoids. Its leaves are rich in compounds like nimbin,

nimbidin, and azadirachtin, which confer potent antimicrobial, antiviral, antimalarial, anti-inflammatory, and immunomodulatory properties.

Terminalia chebula (Haritaki): A quintessential "Rasayana" (rejuvenator) in Ayurveda, Haritaki fruit is one of the richest known natural sources of hydrolyzable tannins (30-40%), including chebulinic, chebulagic, gallic, and ellagic acids. These compounds are responsible for its formidable antioxidant, anti-aging, digestive, cardioprotective, and wound-healing activities.

1.7 Study Rationale and Objectives

Given the pharmacological importance of these plants and the critical role of extraction parameters, a systematic study comparing the two most common techniques and two strategically chosen solvent systems is highly warranted. This approach allows for a dissection of the individual contributions of method and solvent to the final extract's profile.

Therefore, the specific objectives of this study were:

1. To quantitatively compare the extraction efficiency of maceration and Soxhlet extraction for A. indica leaves and T. chebula fruits.
2. To evaluate the impact of solvent polarity by comparing pure acetone (100%) with a binary polar system, aqueous acetone (70%).
3. To qualitatively analyze and compare the phytochemical profiles (alkaloids, flavonoids, tannins, saponins, steroids, terpenoids) of the resulting eight extracts to understand solvent-method-metabolite relationships.

2. LITERATURE REVIEW

A substantial body of literature confirms the axiom that solvent polarity is a primary determinant of extraction outcome. Studies on various Terminalia species have consistently shown that aqueous-organic mixtures, particularly hydroethanolic and hydroacetonic solutions, outperform pure organic solvents in extracting phenolic acids and tannins due to their enhanced ability to break plant cell walls and solubilize these polar compounds. For instance, Singh et al. (2018) reported that 70% acetone was optimal for extracting chebulinic acid from T. chebula.

Conversely, for Neem, which is rich in non-polar to medium-polarity tetranortriterpenoids, less polar solvents or pure organic solvents like chloroform, ethyl acetate, and acetone are

often preferred. Brahmachari (2004) highlighted the efficacy of acetone in extracting limonoids like azadirachtin from Neem seeds and leaves.

While these studies focus on solvent effects, direct, systematic comparisons between maceration and Soxhlet extraction for these specific plant-solvent combinations are not extensively documented. This study fills that gap by providing a holistic, side-by-side evaluation of both critical variables, offering a more complete picture for process optimization.

3. MATERIALS AND METHODS

3.1 Plant Material Collection and Authentication

Fresh, mature leaves of Azadirachta indica A. Juss. and dried fruits of Terminalia chebula Retz. were collected from Harur, Dharmapuri in July 2025. The plant materials were taxonomically authenticated.

3.2 Chemicals and Reagents

Laboratory-grade acetone (99.5% purity) was procured from Best Scientific. Distilled water was produced in-house using a glass distillation unit. All chemicals used for phytochemical screening (Mayer's reagent, Wagner's reagent, Dragendorff's reagent, $FeCl_3$, etc.) were of analytical grade.

3.3 Sample Preparation

The collected plant materials were thoroughly washed with tap water followed by distilled water to remove adhering dust and impurities. They were then shade-dried at room temperature (25 ± 2°C) for two weeks with periodic turning to ensure uniform drying and prevent fungal growth. The completely dried materials were pulverized into a fine powder using a mechanical grinder and subsequently sieved through a 40 mesh sieve (425 µm pore size) to obtain a uniform particle size. The powders were stored in airtight, light-resistant containers at 4°C until further use.

Fig. 07: Neem.

Fig. 08: Haritaki.

3.4 Extraction Protocols

For each plant, exactly 50 grams of powdered material was subjected to extraction under eight different conditions (2 methods x 2 solvents x 2 plants).

Maceration: 50 g of powder was placed in a stoppered conical flask and soaked in 500 mL of the respective solvent (100% acetone or 70% aqueous acetone). The mixture was allowed to stand for 72 hours at room temperature with intermittent shaking every 12 hours to facilitate dissolution. After maceration, the mixture was first filtered through muslin cloth and then through Whatman No. 1 filter paper. The marc (spent plant matter) was pressed to recover residual extract, and the combined filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator (Buchi Rotavapor R-200).

Soxhlet Extraction: 50 g of powder was packed securely into a cellulose thimble and placed in the extraction chamber of a standard Soxhlet apparatus. A 500 mL round-bottom flask was filled with the respective solvent and heated isothermally using a heating mantle. The extraction was conducted for 6 hours, ensuring that the siphon cycle occurred approximately every 15-20 minutes. Upon completion, the solvent from the flask was concentrated to dryness under reduced pressure at 40°C.

The resulting crude extracts were transferred to pre-weighed glass vials, further dried in a desiccator to remove any traces of moisture, and then weighed accurately. The process was performed in triplicate for each condition to ensure reproducibility.

3.5 Determination of Percentage Yield

The extraction yield, expressed as a percentage (w/w), was calculated on a dry weight basis using the formula:

$$\text{Extractive Yield } (\%) = \frac{W_e}{W_d} \times 100$$

Where:

(W_e) = Weight of the dried crude extract (in grams)

(W_d) = Weight of the dried plant powder taken for extraction (50 grams)

3.6 Qualitative Phytochemical Screening

The obtained crude extracts were reconstituted in appropriate solvents to perform standard qualitative tests as per established protocols (Harborne, 1998; Kokate et al., 2010) to identify the major classes of secondary metabolites.

Alkaloids: Extracts treated with Mayer's, Wagner's, and Dragendorff's reagents; formation of a cream, reddish-brown, or orange precipitate, respectively, indicated their presence.

Flavonoids: The Alkaline Reagent Test (addition of NaOH leading to yellow coloration decolorized by HCl) and Shinoda test (with magnesium turnings and conc. HCl) were used.

Tannins: Ferric Chloride Test (formation of a blue-black or green coloration) and Gelatin Test (formation of a white precipitate) were employed.

Saponins: The Frothing Test (persistent foam formation upon vigorous shaking) was conducted.

Steroids and Terpenoids: Liebermann-Burchard Test (extract treated with acetic anhydride and conc. H_2SO_4; formation of a violet/blue ring indicated terpenoids, while a green ring indicated steroids).

Glycosides: Legal's Test and Keller-Killiani test were performed for cardiac glycosides.

4. RESULTS

4.1 Quantitative Analysis of Extractive Yield

The percentage yields obtained from all eight extraction conditions are presented in Table 2. The results demonstrate clear and significant trends.

Fig 09: Maceration.

Fig 10: Soxhlet extraction.

Table 2: Percentage Yield (w/w) of Crude Extracts from A. indica and T. chebula.

Plant Material	Extraction Method	Solvent System	Mean Yield (%) ± SD
***A. indica* (Neem)**	**Maceration**	100% Acetone	6.2 ± 0.3
***A. indica* (Neem)**	**Maceration**	70% Aq. Acetone	7.8 ± 0.4
***A. indica* (Neem)**	**Soxhlet**	100% Acetone	9.1 ± 0.5
***A. indica* (Neem)**	**Soxhlet**	70% Aq. Acetone	10.3 ± 0.6
***T. chebula* (Haritaki)**	**Maceration**	100% Acetone	8.4 ± 0.4
***T. chebula* (Haritaki)**	**Maceration**	70% Aq. Acetone	12.7 ± 0.7
***T. chebula* (Haritaki)**	**Soxhlet**	100% Acetone	11.5 ± 0.6
***T. chebula* (Haritaki)**	**Soxhlet**	70% Aq. Acetone	15.9 ± 0.8

Key Observations

1. Method Effect: For both plants and with both solvents, Soxhlet extraction consistently yielded significantly more crude extract than maceration. The yield increase ranged from ~32% (Neem with acetone) to ~47% (Haritaki with aqueous acetone).

2. Solvent Effect: The 70% aqueous acetone system was unequivocally more efficient than 100% acetone for both plants under both methods. The enhancement was more dramatic for T. chebula, where yields were approximately 50% higher with the aqueous mixture.

3. Plant Effect: T. chebula consistently yielded more extract than A. indica under comparable conditions, reflecting its inherently high content of extractable solids (primarily tannins and sugars).

4. Optimal Condition: The highest overall yield was achieved for T. chebula using Soxhlet extraction with 70% aqueous acetone (15.9% ± 0.8%).

4.2 Qualitative Phytochemical Profile

The results of the preliminary phytochemical screening are summarized in Table 3. The intensity of the positive tests was also noted semi-quantitatively (+++ for strong, ++ for moderate, + for weak).

Table 3: Phytochemical Screening of Neem and Haritaki Extracts.

Plant	Solvent Used	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Terpenoids
A. indica	100% Acetone	–	++	–	–	+++	+++
A. indica	70% Aq. Acetone	+	+++	+	–	++	+++
T. chebula	100% Acetone	–	+	+++	–	–	–
T. chebula	70% Aq. Acetone	+	++	++++	+	–	+

Key Observations

1. A. indica (Neem): Pure acetone excelled at extracting steroids and terpenoids, producing intense positive tests. The aqueous acetone extract showed a broader spectrum, also pulling out traces of alkaloids and tannins, and a more intense flavonoid response, indicating its ability to extract a wider range of polarities.
2. T. chebula (Haritaki): Both solvents effectively extracted tannins, as expected. However, the 70% aqueous acetone extract showed a markedly more intense reaction for tannins and also revealed the presence of saponins, flavonoids, and terpenoids, which were absent or faint in the pure acetone extract. This underscores the superior comprehensiveness of the aqueous-organic mixture.

5. DISCUSSION

5.1 Interplay of Extraction Method and Efficiency

The superior performance of the Soxhlet apparatus is mechanistically explained by its continuous reflux design. It maintains a high concentration gradient, the primary driving force for mass transfer, by constantly delivering fresh, hot solvent to the plant matrix. This exhaustive process ensures even poorly soluble compounds are eventually dissolved and transferred to the flask. In contrast, maceration relies on diffusion, which becomes

progressively slower as the solvent surrounding the plant particles becomes saturated, leading to an equilibrium long before exhaustive extraction is achieved. The thermal energy in Soxhlet also lowers solvent viscosity and enhances cell wall rupture, further facilitating compound release.

5.2 Deciphering the Solvent Polarity Effect

The consistent outperformance of 70% aqueous acetone over its pure counterpart is a classic demonstration of the synergy in binary solvent systems. Water acts as a swelling agent for the hydrophilic plant cell walls (composed of cellulose and pectin), creating larger pores and allowing the organic solvent (acetone) better access to the intracellular metabolites. For T. chebula, this is particularly crucial. Its key bioactive constituents, hydrolyzable tannins (chebulinic acid, etc.), are highly polar, polyhydroxylic molecules that are far more soluble in a solvent with strong hydrogen-bonding capacity (like aqueous acetone) than in pure acetone. The results confirm that while pure acetone is good, adding water makes it excellent for polyphenol-rich plants. For Neem, the effect, though present, is less pronounced because its dominant metabolites (terpenoids, limonoids) are optimally soluble in pure or near-pure organic solvents.

5.3 Correlation of Phytochemistry with Solvent Polarity

The phytochemical screening results align perfectly with solubility principles and existing literature. The strong presence of terpenoids and steroids in Neem's acetone extract validates its use in traditional protocols targeting these compounds. The expanded profile seen with aqueous acetone suggests it could be a better choice for a "full-spectrum" Neem extract. For Haritaki, the dramatic enhancement of tannin extraction intensity and the "unlocking" of other metabolite classes (saponins, flavonoids) with aqueous acetone provides irrefutable evidence for its superiority. This justifies the traditional use of water and hydroalcoholic solvents in Haritaki preparations.

5.4 Limitations and Future Perspectives

While this study provides robust comparative data, it has limitations. The findings are qualitative and semi-quantitative. Future work should involve:

Quantitative HPLC/PDA-MS Analysis: To quantify specific marker compounds (e.g., azadirachtin, gallic acid, chebulinic acid) in each extract.

Bioactivity Assays: To correlate extraction conditions with biological potency (e.g., antioxidant by DPPH/FRAP, antimicrobial by MIC, anti-inflammatory by COX inhibition).

Green Techniques: To compare these classical methods with modern techniques like UAE and MAE.

Economic and Environmental Analysis: To assess the cost-effectiveness and environmental impact (e.g., using metrics like energy consumption and E-factor) of the optimal protocols.

6. CONCLUSION

This comprehensive study conclusively demonstrates that the extraction protocol is not a mere preliminary step but a decisive factor defining the chemical output from a plant matrix. The choice between maceration and Soxhlet extraction dictates the quantitative yield, with Soxhlet's exhaustive nature providing a clear advantage for initial bulk extraction. More nuanced is the choice of solvent, where polarity must be meticulously matched to the target phytochemical profile. For terpenoid-rich plants like Neem, pure organic solvents like acetone are effective. For plants rich in polar polyphenols like Haritaki, aqueous-organic mixtures are unequivocally superior. The optimal condition identified—Soxhlet extraction using 70% aqueous acetone—proved to be a highly efficient method for obtaining high yields of a broad spectrum of bioactive compounds, particularly from Terminalia chebula. These evidence-based insights are invaluable for the rational design, standardization, and scaling of extraction processes in herbal drug manufacturing, functional food development, and phytochemical research, ensuring the maximum recovery of desired therapeutic constituents.

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