

# Comprehensive Quality Evaluation: Flow Characteristics, Microscopy and Phytochemical Screening of *Terminalia chebula*

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## ABSTRACT

**Introduction:** *Terminalia chebula* are widely recognized for their medicinal value in traditional and modern systems of medicine. The present study was undertaken to evaluate the phytochemical profile and microscopic characteristics of *Terminalia* plant material in order to establish its identity, quality, and therapeutic relevance. Phytochemical are abundantly present at varying concentrations in numerous medicinal plants. Microscopic examination of the powdered drug showed characteristic diagnostic features such as distinct epidermal cells, lignified fibers, stone cells, xylem vessels with spiral and pitted thickenings, calcium oxalate crystals, and starch grains, which serve as reliable markers for authentication. The powder exhibited fair flow behavior, as indicated by a Carr's compressibility index of 18.00% and a Hausner's ratio of 1.22. Proximate analysis revealed the presence of essential nutritional components, including moisture content, ash values, crude protein, crude fat, crude fiber, and carbohydrate content, providing insight into the physicochemical stability and compositional quality of the plant material. Fluorescence analysis was conducted under visible and UV light using different physicochemicals and solvents. **Methods:** Powdered *Terminalia chebula* was evaluated using physicochemical tests: powder characterization, extractive value, alcohol, and water-soluble matter, Ash value and LOD. Flow properties were determined using standard pharmacopeial parameters, including bulk density, tapped density, angle of repose, Carr's compressibility index, and Hausner's ratio. **Result:** Organoleptic characters of Haritaki revealed dark brown color, characteristic odor, astringent taste and fine texture. Physicochemical parameters resulted in water-soluble extractive (67.32±0.83), alcohol-soluble extractive (42.6±1.20), total ash (3.18±0.12), acid insoluble ash (2.78±0.66) and LOD (6.37±0.53) respectively. The phytochemical analysis shows the presence of alkaloids, glycosides, tannins, flavonoids, terpenoids, carbohydrates and phenols.

**Keywords:** Microscopic, fluorescence, phytochemicals, alkaloids, flow property, Carr's index

## INTRODUCTION

Medicinal plants continue to constitute a primary healthcare resource for a substantial proportion of the global population, particularly in developing regions where the historical reliance on herbal remedies remains uninterrupted. Increasing scientific and economic interest in botanical therapeutics is evident across both industrialized and developing nations, reflecting their expanding clinical and commercial relevance. For millennia, plant-based substances have provided the foundational framework for traditional medical systems, and they remain a critical source for the discovery of novel pharmacological agents. Although certain purported therapeutic attributes have been reassessed or disproven, contemporary use of medicinal plants is still anchored in cumulative empirical knowledge acquired over centuries of practical application. Traditional medicine represents the cumulative therapeutic wisdom derived from successive generations of practitioners within indigenous healing systems. Such formulations typically incorporate botanical materials, mineral substances, and other natural components. Within this broader framework, **herbal medicines** specifically refer to those

traditional interventions in which plant-based preparations constitute the principal therapeutic agents<sup>1</sup>. Traditional or folk medical systems encompass culturally embedded therapeutic practices, conceptual frameworks, and healing beliefs that are not founded on empirical biomedical validation, yet are employed to diagnose, manage, and prevent disease within a specific sociocultural milieu. These modalities are inherently shaped by the epistemological foundations and value orientations of their originating culture, rendering both their risk perception and health rationale context-dependent. When such historically rooted healing paradigms are appropriated by contemporary societies beyond their native cultural boundaries, they are reclassified within modern discourse as **complementary, non-conventional, or alternative medical systems**<sup>2</sup>. The global utilization of herbal therapeutics and phyto-derived nutraceuticals has accelerated markedly, with an increasing proportion of the population turning to these agents for the management of diverse health conditions across a range of national healthcare systems<sup>3</sup>.

The demand for medicinal plant resources continues to rise, accompanied by a growing global acceptance of their therapeutic value. Beyond their curative

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potential, plants constitute fundamental ecological assets, sustaining life systems and enabling the persistence of human and non-human species alike. Medicinal flora, in particular, function as key biological indicators of environmental integrity. Human populations remain heavily reliant on raw botanical sources to support healthcare needs, maintain physiological well-being, and manage disease. Their medicinal application is attributed to complex biochemical properties, including synergistic interactions among phytoconstituents. Such interactions may enhance therapeutic efficacy, neutralize adverse effects, or modify the activity of individual components. Bioactive compounds isolated from plants have demonstrated exceptional potential in addressing otherwise refractory diseases, including malignancies, and many phytochemicals exhibit pronounced prophylactic capabilities by impeding the onset or progression of specific disorders. According to the World Health Organization, traditional medicinal plants are classified as naturally occurring botanical materials utilized with minimal or no industrial modification for therapeutic purposes within specific local or regional contexts. The employment of herbal remedies has persisted for millennia across both developing and industrialized nations, largely owing to their natural origin and their relatively lower incidence of adverse effects when compared with conventional pharmaceutical interventions<sup>4</sup>. Herbal therapeutics is increasingly being acknowledged within contemporary pharmacotherapy, not merely because of their longstanding traditional use but also due to the expanding scientific evidence substantiating their clinical effectiveness. Numerous phytochemicals have demonstrated significant potential in the management of persistent and complex disorders, including diabetes, malignancies, cardiovascular pathologies, and neurodegenerative diseases. Ongoing investigations into their pharmacodynamics, pharmacokinetic behavior, and safety parameters have further clarified their capacity to complement and in some cases augment conventional medical treatments. Nevertheless, despite their pharmacological advantages, the broader incorporation of herbal formulations into modern healthcare systems remains impeded by persistent challenges. Core limitations include inadequate standardization, limited large-scale clinical validation, possible herb drug interactions, and regulatory ambiguities. Emerging innovations in advanced formulation science, nanotechnology-based delivery systems, and integrative medical frameworks, however, are progressively addressing these constraints and facilitating more systematic and safe utilization of herbal medicines in clinical practice<sup>5</sup>.

Plants contribute substantially to a wide spectrum of industrial sectors, including pharmaceuticals, fine chemicals, cosmetics, and raw material production. Their role is particularly critical in innovative drug discovery, where medicinal flora serve as a foundational reservoir of novel bioactive molecules. Historically, plant-derived compounds have been indispensable in addressing life-threatening illnesses such as cancer and viral diseases, including hepatitis and HIV/AIDS. A considerable proportion of breakthrough therapeutic agents introduced over the past five decades those that have significantly transformed modern clinical practice originate from plant metabolites or their semi-synthetic derivatives. These bioactive constituents embody the inherent therapeutic potential of natural products. Recognizing their efficacy, safety profile, and affordability, the World Health Organization advocates for the integration of herbal medicines into national healthcare systems, emphasizing their accessibility to the general population and their long-established clinical reliability when compared with synthetic pharmaceuticals. Consequently, systematic exploration of biologically active compounds from natural sources, particularly medicinal plants, continues to yield pharmacologically valuable agents that remain central to treating a wide range of human disorders<sup>6</sup>.

*Terminalia chebula* Retz. (syn. Willd.), commonly referred to as yellow myrobalan, chebulic myrobalan, or haritaki, originates from the South Asian region, extending from India and Nepal through southwestern

China (Yunnan) and further south to Sri Lanka, Malaysia, and Vietnam. The genus *Terminalia* comprises over 100 species, generally represented by large trees distributed across various tropical ecosystems. Members of this genus are recognized as valuable sources of secondary metabolites, including cyclic triterpenoids and their derivatives, flavonoids, tannins, and diverse aromatic compounds. The species has long been a principal therapeutic agent in the Ayurvedic, Unani, Amchi, and Homeopathic systems of medicine and is widely utilized not only in the Indian subcontinent but also throughout several Asian and African nations. Its extensive ethnomedicinal use is attributed to the broad pharmacological profile conferred by its bioactive constituents. The significant therapeutic effects associated with *T. chebula* are largely due to its rich phytochemical composition, encompassing polyphenols, terpenoids, flavonoids, anthocyanins, alkaloids, glycosides, and other complex plant metabolites. *Terminalia chebula* is widely distributed across South and Southeast Asia, including India, Sri Lanka, Nepal, Bhutan, Bangladesh, Pakistan, Myanmar, Cambodia, Laos, Vietnam, Indonesia, Malaysia, the Philippines, Thailand, Turkey, and Egypt. In China, the species occurs naturally in western Yunnan and is also cultivated in regions such as Fujian, Guangdong, Guangxi (Nanning), and Taiwan (Nantou). Within India, it is commonly encountered along the sub-Himalayan belt, extending from the Ravi River through West Bengal and Assam, and can be found at elevations reaching approximately 1,500 meters. Propagation generally occurs via seeds. After maturation, the fallen fruits are collected, dried thoroughly, and subsequently processed by removing the hardened pericarp<sup>7</sup>.

*T. chebula* is a medium-sized deciduous tree that can reach up to 30 meters in height. It has widely spreading branches and a broad, rounded crown. The species is found at elevations between 1500 and 2000 meters, typically thriving in clay-rich and shaded soils. Its leaves are elliptic-rhombic with a pointed tip and a heart-shaped base, entire margins, smooth upper surfaces, and a yellowish pubescence on the underside. The tree bears monoecious flowers that are dull white to yellow and possess a strong, unpleasant smell, arranged in terminal spikes or short panicles. The fruits are smooth, ellipsoid to ovoid drupes, yellow to orange-brown in colour, each containing a single angled stone<sup>8,9</sup>.

The fruits of *Terminalia chebula* are known to contain a high concentration of tannic acid. This tannic fraction is predominantly composed of chebulic acid, chebulagic acid, corilagin, and gallic acid, and is classified as a pyrogallol-type (hydrolyzable) tannin. Extensive phytochemical analyses have identified fourteen distinct hydrolyzable tannin components in the fruit, including gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucose, 1,6-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose, and terchebulin. Certain reports estimate the tannic acid content of *T. chebula* fruits to be as high as 32%. Notably, the levels of tannin vary considerably depending on geographical and environmental factors. In addition to its tannin profile, the fruit contains fructose, amino acids, succinic acid,  $\beta$ -sitosterol, resin, as well as anthraquinone- and sennoside-type purgative principles. Furthermore, the plant has yielded a range of other secondary metabolites, including flavonol glycosides, triterpenoids, coumarin-gallic acid conjugates such as chebulin, and several other phenolic compounds<sup>10</sup>.

Phytochemical investigations of *Terminalia chebula* have revealed a diverse spectrum of bioactive constituents, underscoring its pharmacological significance. The plant is particularly rich in anthraquinone derivatives, including ethadiaoic acid and sennosides, along with complex glycosidic compounds such as 4.2.4-chebylyl-D-glycopyranose. Various terpenes and terpinols further contribute to its chemical profile. Notably, the concentration and composition of tannins exhibit significant variation depending on the geological and environmental conditions in which the plant grows. In addition

to major tannins, several secondary polyphenolic metabolites such as corilagin, galloyl glucose, punicalagin, terflavin A, and maslinic acid have been identified, along with simple biomolecules including fructose, amino acids, succinic acid,  $\beta$ -sitosterol, and resinous materials. The presence of anthraquinone-based purgative principles aligns with the plant's traditional therapeutic applications. Furthermore, comprehensive lipid profiling has led to the isolation of twelve distinct fatty acids, among which palmitic, linoleic, and oleic acids constitute the predominant components. *T. chebula* also contains an array of triterpenoid glycosides, such as chebulosides I and II, arjunenin glucoside, 2 $\alpha$ -hydroxymicromeric acid, and other 2 $\alpha$ -hydroxy triterpenoid derivatives. Its leaves, in particular, have been shown to possess high levels of polyphenolic compounds, including punicalin, punicalagin, and terflavins B, C, and D, further affirming the plant's status as a potent reservoir of pharmacologically relevant metabolites<sup>11</sup>.

*Terminalia chebula* is regarded as one of the most important medicinal plants in the Ayurvedic Materia Medica and is traditionally prescribed for conditions such as asthma, bleeding piles, sore throat, and gout. In Thai traditional medicine, the plant is valued for its carminative, astringent, and expectorant properties. Classical Ayurvedic texts, particularly those of Vagbhata, describe *T. chebula* as a principal remedy for disorders associated with aggravated **vata**. The well-known polyherbal formulation *Triphala* comprising *T. chebula*, *T. Bellerica* and *Embllica officinalis* is widely used as a laxative in chronic constipation, a colon detoxifier, a digestive regulator for poor assimilation, and a general rejuvenating agent. Contemporary studies indicate that *Triphala* enhances appetite and exhibits anticancer and detoxifying activities, earning it recognition as one of the most versatile Ayurvedic formulations. It is also prescribed as a cardiogenic and for the management of candidal infections.

The fruits of Haritaki (*T. chebula*) are extensively employed both externally and internally. When applied topically as a paste, the fruit promotes wound healing, reduces inflammation, and cleanses ulcers. In cases of erysipelas and other dermatological disorders, it prevents the accumulation of pus, while Haritaki oil is particularly effective in the treatment of burns. Its decoction is traditionally instilled in cases of conjunctivitis due to its anti-inflammatory effects. Gargling with the decoction provides relief in stomatitis and throat ailments. *Triphala* also has several external uses, including as a hair wash, a tooth-cleaning agent for pyorrhea or bleeding gums, and a wound-cleansing rinse for chronic non-healing ulcers. A fine powder of the drug is used to strengthen the gums, and aqueous extracts serve as an effective anticaries mouth rinse<sup>11,12</sup>.

The topical application of *Terminalia chebula* fruit paste mixed with water has been shown to exhibit significant anti-inflammatory and analgesic effects, along with notable wound-cleansing and healing properties. Its powdered form is traditionally employed as an astringent for managing loose or bleeding gums and for treating oral ulcers. Internally, the plant is used to enhance appetite, stimulate liver function, and act as a stomachic and gastrointestinal prokinetic agent. It is also administered in cases of chronic diarrhea and serves as a mild laxative. In dermatological conditions characterized by secretions—such as allergic reactions, urticaria, and other erythematous disorders the herb offers therapeutic benefits. Additionally, *T. chebula* is prescribed as an adjuvant in the management of chronic fevers. It is also reputed to mitigate the adverse effects associated with the consumption of fatty, creamy, and oily foods<sup>13</sup>.

## MATERIAL AND METHODS

### Collection of Sample

**Dried fruit pericarp of *Terminalia chebula* Retz.** was obtained from a local market in Moradabad and their identity was confirmed through

**available literature and authenticated by** emeritus scientist of CSIR-NIScPR, New Delhi, India. A **specimen voucher no.** (NIScPR/RHMD/Consult/2019/3525-26-3) was deposited in the Department of Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NIScPR, New Delhi, India, for future reference. The rhizomes were then **mechanically crushed, sieved into a coarse powder, and stored in an airtight container** for further analysis.

### Organoleptic Characters

The **organoleptic properties** of the crude drug were **examined using sensory evaluation**, assessing its **color, odor, and taste**, as well as its **size, shape, surface characteristics, and type of fracture**<sup>14</sup>.

### Powdered Microscopical Studies

For **powder microscopy**, different **slides were prepared from powdered crude herb** Haritaki using **water and glycerine** as mounting media. The sample was then **cleared by gentle heating in a chloral hydrate solution** before observation. The slides were then stained with safranin, mounted with glycerine on glass slides, and examined under 10X and 40X objectives using a Brightfield microscope equipped with an analyser. Photomicrographs of the observed **British Pharmacopoeia** microscopic features were captured and preserved for future reference. The diagnostic characteristics identified were found to be in accordance with the standards specified in the respective volumes of the Ayurvedic Pharmacopoeia of India<sup>15,16</sup>.

### Physiochemical Evaluation

The **ash content, alcohol and water soluble extractive values, and volatile oil content** of the test sample were determined according to the procedures outlined in the **Ayurvedic Pharmacopoeia of India (API)** and the. Additionally, **fluorescence analysis of the rhizome powder** was carried out by treating it with various **chemical reagents** and examining the results under ultraviolet and **daylight** conditions<sup>17,18,19</sup>.

**Determination of foreign organic matter:** 10 g sample of crude drug powder was evenly spread in a thin layer, and any foreign matter was identified and removed either by visual inspection or with the help of a magnifying lens (6x and 10x). Following the removal of extraneous materials, the remaining sample was passed through a sieve no. 250 to eliminate any dust particles. The collected foreign matter was then weighed, and its quantity was calculated in grams per 100 g of the air-dried sample.

$$\text{Foreign organic matter (\%)} = \frac{\text{Weight of foreign organic matter}}{\text{Weight of air dried drug}} \times 100$$

**Determination of ash values:** Ash value is a key parameter used in quality assessment, as it reflects the presence of inorganic impurities within a sample. It helps determine the sample's quality, purity, and authenticity. Various types of ash values are evaluated, including total ash, acid-insoluble ash, water-soluble ash, and sulphated ash.

### Determination of total ash

An accurately weighed 2 g portion of air-dried crude drug was placed into a pre-weighed silica crucible. The sample was then incinerated in a muffle furnace at a temperature not exceeding 450°C until all carbon content was eliminated. Once incineration was complete, the crucible was allowed to cool in a desiccator and then weighed. The percentage of total ash was calculated in relation to the air-dried sample. This procedure was performed in triplicate, and the average value was determined<sup>20</sup>.

$$\text{Ash value (\%)} = \left[ \frac{W3 - W1}{W2 - W1} \right] \times 100$$

Where, W1 = Weight of empty crucible (g)

W2= Weight of crucible + Sample before ashing (g)

W3 = Weight of crucible + Ash (g)

### Determination of acid insoluble ash

The ash obtained from the previous method was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. After boiling, the mixture was allowed to cool and then filtered using Whatman filter paper no. 41 (ashless). The residue was thoroughly washed with hot water to ensure complete removal of the acid. The filter paper containing the acid-insoluble residue was carefully transferred to a pre-weighed crucible and incinerated until a constant weight was achieved. Following incineration, the crucible was cooled in a desiccator, weighed, and the percentage of acid-insoluble ash was calculated. This procedure was repeated three times to ensure accuracy.

$$\text{Acid insoluble ash (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where, W1 = Weight of crucible containing ash (g)

W2 = Weight of empty crucible (g)

W3 = Weight of powder taken for obtaining total ash (g)

### Determination of water soluble ash

The total ash obtained from the earlier procedure was boiled in a waterbath with 25 ml of water for 5 minutes. After cooling, the mixture was filtered using ashless Whatman filter paper no. 41 and washed with hot water. The filter paper containing the water-insoluble residue was transferred to a pre-weighed crucible and incinerated in a muffle furnace at a temperature not exceeding 450 °C until a constant weight was reached. Once incineration was complete, the crucible was cooled in a desiccator and weighed. The weight of the insoluble residue was subtracted from the total ash weight, and the resulting difference represented the water-soluble ash. The percentage of water-soluble ash was then calculated accordingly. The procedure was repeated thrice<sup>21, 22</sup>.

$$\text{Water insoluble ash (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where, W1 = Weight of crucible containing ash (g)

W2 = Weight of empty crucible (g)

W3 = Weight of powder taken for obtaining total ash (g)

Water soluble ash (%) = [Total ash (%) – Water insoluble ash (%)]

**Determination of LOD (Loss on Drying):** Approximately 5 grams of the prepared air-dried sample was precisely weighed using a pre-dried and tared flat weighing bottle. The powdered materials were evenly spread inside the bottle and placed in a drying oven. The drying process was conducted at a temperature of 100 ± 2°C for duration of 5 hours. Once the drying was complete, the bottle was immediately sealed, allowed to cool in a desiccator to room temperature, and then reweighed. The moisture content was calculated relative to the air-dried sample. This procedure was repeated until a constant weight was achieved. The percentage loss on drying was then calculated, and the average value from three separate readings was taken<sup>23, 24</sup>.

$$\text{Loss on drying (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where W1 = Weight of bottle containing sample (g)

W2 = Weight of bottle along with residue of sample after drying (g)

W3 = Weight of sample taken (g)

**Determination of extractive values:** Extractive value is a measure of the content of the drug extracted by solvents. Extractive value can be water soluble, ethanol soluble and ether soluble extractives.

### Determination of ethanol soluble extractive

Approximately 5 g of the coarsely powdered, air-dried drug was subjected to cold maceration with 100 mL of ethanol in an airtight flask to ensure minimal solvent loss. The mixture was agitated for 6 hours and subsequently allowed to stand undisturbed for an additional 18 hours to facilitate exhaustive diffusion of ethanol-soluble constituents. Following extraction, the macerate was filtered through Whatman No. 1 filter paper to obtain a clear filtrate. An aliquot of 25.0 mL of this filtrate was carefully measured and evaporated to dryness in a previously tarred beaker. The resulting residue was further dried in a hot-air oven maintained at 110 °C to achieve a constant weight. The ethanol-soluble extractive value was then calculated and expressed as a percentage of the air-dried drug, providing an estimate of the concentration of ethanol-extractable phytoconstituents<sup>25</sup>.

$$\text{Ethanol soluble extractive value (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where, W1 = Weight of beaker with extract (g)

W2 = Weight of empty beaker (g)

W3 = Weight of sample taken (g)

### Determination of ether soluble extractive value

An accurately weighed 5 g portion of the coarsely powdered, air-dried drug was extracted by cold maceration with 100 mL of petroleum ether in a sealed container for 24 hours, during which the mixture was shaken intermittently for the first 6 hours and subsequently allowed to stand without disturbance for the remaining 18 hours. The extract was then passed through Whatman No. 1 filter paper to obtain a clear filtrate. From this, a 25.0 mL aliquot was carefully measured and evaporated to dryness in a pre-tarred beaker, after which the residue was dried in an oven maintained at 110 °C to constant weight. The ether-soluble extractive percentage was then determined based on the dried residue<sup>26</sup>.

$$\text{Ether soluble extractive value (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where, W1 = Weight of beaker with extract (g)

W2 = Weight of empty beaker (g)

W3 = Weight of sample taken (g)

### Determination of water soluble extractive value

A carefully weighed 5 g sample of the air-dried powdered drug was transferred into a glass-stoppered flask and subjected to maceration with 100 mL of chloroform water (1:99). The mixture was intermittently shaken for 6 hours and subsequently allowed to stand undisturbed for an additional 18 hours. Filtration was carried out immediately thereafter, ensuring no loss of solvent during the process. A 25 mL aliquot of the resulting filtrate was quantitatively evaporated to dryness in a tared flat-bottomed petridish, followed by drying at 105 °C, cooling in a desiccator, and final weighing. The water-soluble extractive value was computed as a percentage of the air-dried drug, and the reported value represents the mean of three independent determinations<sup>26</sup>.

$$\text{Water soluble extractive value (\%)} = \left[ \frac{W1 - W2}{W3} \right] \times 100$$

Where, W1 = Weight of beaker with extract (g)

W2 = Weight of empty beaker (g)

W3 = Weight of sample taken (g)

Water soluble ash (%) = [Total ash (%) – Water insoluble ash (%)]

### Fluorescence analysis

Fluorescence analysis of the dried powdered Haritaki was conducted following a standard procedure. For this evaluation, a small quantity of the finely powdered test drug was placed on a piece of black chart paper. The sample was then treated with various chemical reagents, including 1N NaOH, 1N HCl, 50% KOH, 50% H<sub>2</sub>SO<sub>4</sub>, concentrated H<sub>2</sub>SO<sub>4</sub>, 50% HNO<sub>3</sub>, concentrated HNO<sub>3</sub>, iodine solution, and acetic acid. A few drops of each reagent were added to the powder and mixed gently with a glass rod. The treated samples were examined under a UV chamber at short-wave UV (254 nm), long-wave UV (364 nm), and visible light (fluorescent tube). The resulting color changes and fluorescence characteristics were observed and documented accordingly<sup>27</sup>.

**Phytochemical screening of the active ingredients:** The qualitative and quantitative phytochemicals present in Haritaki fruit pericarp were analysed as follows.

**Qualitative Analysis of Phytochemicals:** The plant extract was subjected to preliminary phytochemical screening to identify the presence of various bioactive constituents, including alkaloids, tannins, flavonoids, carbohydrates, steroids, saponins, and proteins.

**Preparation of plant extract by cold maceration:** Initially, the Haritaki was thoroughly washed multiple times with tap water to eliminate adhering impurities and given a final rinse with distilled water. The cleaned material was then cut into small pieces and sun-dried for 2–3 days. The dried Ginger was pulverized using a mortar and pestle to obtain a fine powder suitable for extraction. For the extraction procedure, 20 g of the powdered Ginger was subjected to aqueous extraction for 8 hours. After maceration, the residue was removed by filtration, and the filtrate was concentrated through solvent evaporation. The resulting extract was then placed in a hot air oven maintained at 50–60 °C until a solid extract was obtained<sup>28</sup>.

**Tests for flavonoids:** About 5 ml of aqueous plant extract was taken in test tube and few drops of sulphuric acid were added. Yellow solution was observed.

**Test for alkaloids:** About 5 ml of plant extract was taken in a test tube evaporated to dryness and the residue was treated with 5 ml of 2% hydrochloric acid. The mixture was filtered through whatman paper no 1.

About 2-3 ml above filtrate was taken in a test tube and mixed with few drops of mayer's, wagner's and Dragendorff's reagent individually and the mixtures were observed for formation of orange brown precipitate.

**Test for tannins and phenolic compounds:** A small volume of plant extract was evaporated to dryness, and the resulting residue was re-dissolved in water. Upon adding 5% ferric chloride solution, the appearance of a deep blue color indicated the presence of phenolic compounds.

**Tests for carbohydrates:** To approximately 2 mL of plant extract, a few drops of 20% α-naphthol solution in ethyl alcohol were added. Then, about 1 mL of concentrated sulfuric acid was carefully poured along the side of the test tube. The formation of a reddish-violet ring at the interface of the two layers indicated the presence of carbohydrates.

**Test for glycosides:** Approximately 2 mL of plant extract was combined with chloroform. Then, 1–2 mL of acetic anhydride was added, followed by two drops of concentrated sulfuric acid carefully introduced along the side of the test tube. A color change was observed, initially turning red and then shifting to blue.

**Test for steroid:** Each plant extracts were evaporated to dryness and the remaining residue was sequentially extracted using petroleum ether and acetone. The portion of the residue that remained insoluble after extraction was then dissolved in chloroform. A few drops of acetic anhydride were added, followed by the careful addition of a few drops of concentrated sulfuric acid along the side of the test tube. The appearance of a color sequence starting with red, followed by blue and finally green indicated the presence of sterols in the extract.

**Test for proteins:** Around 3 mL of each plant extract was added to a test tube, then sodium hydroxide solution and a few drops of copper sulfate solution were introduced. The development of a violet or pink color in the mixture confirmed the presence of proteins.

**Test for terpenoids:** A 2.0 mL portion of each plant filtrate was mixed with 2.0 mL of chloroform. Then, 3 mL of concentrated sulfuric acid was carefully added to form a separate layer. The appearance of a reddish-brown coloration at the junction of the two layers was noted.

**Test for saponins:** 2 mL of each plant extract was vigorously shaken with 20 mL of water, and the mixture was examined for the sustained formation of foam.

### Determination of quantitative phytochemical composition

The quantitative phytochemical analysis of plant extract was carried out in the laboratory.

**Determination of total alkaloid content (TAC):** The total alkaloid content of the plant extract was quantitatively determined using the bromocresol green complexation method. Briefly, 1 mg of the plant extract was dissolved in dimethyl sulphoxide (DMSO), followed by acidification with 1 mL of 2 N hydrochloric acid. The mixture was filtered, and the clear filtrate was transferred to a separating funnel. Subsequently, 5 mL each of bromocresol green reagent and phosphate buffer were added to facilitate ion-pair complex formation. The reaction mixture was subjected to successive extractions with 1, 2, 3, and 4 mL aliquots of chloroform under vigorous shaking conditions. The combined chloroform extracts were collected in a 10 mL volumetric flask and the volume was adjusted with chloroform. A series of atropine reference standard solutions (20–100 µg/mL) were prepared following an identical procedure to generate the calibration curve. The absorbance of both standard and sample solutions was measured at 470 nm against a reagent blank using a UV-Visible spectrophotometer. The alkaloid content of the extract was calculated from the calibration curve and expressed as milligrams of atropine equivalents per gram of plant extract (mg AE/g)<sup>30</sup>.

**Determination of total tannin Content:** The tannin content of the sample extract was quantified using the Folin-Ciocalteu colorimetric method. An aliquot of 0.1 mL of the extract was transferred into a 10 mL volumetric flask containing 7.5 mL of distilled water, followed by the addition of 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% sodium carbonate solution. The final volume was adjusted to 10 mL with distilled water. The reaction mixture was thoroughly mixed and incubated at room temperature for 30 minutes to allow complete color development. A series of tannic acid standard solutions (20–100 µg/mL) were prepared using the same procedure to construct the calibration curve. The absorbance of both test and standard solutions was measured at 700 nm against a reagent blank using a UV-Visible spectrophotometer. All determinations were performed in triplicate,

and the tannin content was expressed as milligrams of gallic acid equivalents per gram of dried sample (mg GAE/g)<sup>31,32</sup>.

**Determination of total flavonoids content (TFC):** The total flavonoid content was determined using the aluminum chloride colorimetric method. In this assay, 1 mL of the plant extract and 4 mL of distilled water were added to a 10 mL volumetric flask. To this mixture, 0.30 mL of 5% sodium nitrite was added. After 5 minutes, 0.30 mL of 10% aluminum chloride was introduced, followed by the addition of 2 mL of 1M sodium hydroxide after another 5 minutes. The solution was then diluted to a final volume of 10 mL with distilled water. A series of quercetin standard solutions (20, 40, 60, 80, and 100 µg/mL) were prepared using the same procedure. The absorbance of both test and standard solutions was measured at 510 nm using a UV-Visible spectrophotometer, with the reagent blank as the reference. The total flavonoid content was expressed as milligrams of quercetin equivalent (QE) per gram of extract<sup>33</sup>.

**Determination of Total Phenolic Content (TPC):** The total phenolic content was estimated using the Folin-Ciocalteu assay method. In this procedure, 1 mL of plant extract was mixed with 9 mL of distilled water in a 25 mL volumetric flask. To this mixture, 1 mL of Folin-Ciocalteu phenol reagent was added and thoroughly mixed. After allowing the mixture to stand for 5 minutes, 10 mL of 7% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution was added. The final volume was adjusted to 25 mL with distilled water. A series of gallic acid standard solutions (20, 40, 60, 80, and 100 µg/mL) were prepared following the same steps. The mixtures were incubated at room temperature for 90 minutes, after which the absorbance of both test and standard solutions was measured at 550 nm using a UV/Visible spectrophotometer, with the reagent blank as reference. The total phenolic content was calculated and expressed in milligrams of gallic acid equivalent (GAE) per gram of extract<sup>33</sup>.

### Flow properties

The compaction characteristics, including **bulk density (g/ml)** and **tapped density (g/ml)**, of the Haritaki sample was determined using **standard analytical methods**. Additionally, the **flow behaviour** of the Ginger powder was assessed using **Carr's Compressibility Index** and the **Hausner Ratio** calculated according to the following formulas.

**Bulk density:** The bulk density (BD) of powdered drug was determined by pouring gently 10 gm of sample mixture through a glass funnel into a 100 ml graduated cylinder. The initial volumes occupied by the sample were recorded. The bulk density was calculated by using the following formula.

$$\text{Bulk density} = \frac{\text{Weight of the powder (g)}}{\text{Volume occupied by the powder (ml)}}$$

**Tapped density:** The tapped density (TD) of powdered drug was determined by pouring gently 10 gm of sample mixture through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from the height of 2 inches until a constant volume obtained and then the average value of all formulation reported. The final volume occupied by the sample after tapping was recorded and tapped density calculated by using the given formula.

$$\text{Tapped density} = \frac{\text{Weight of the powder (g)}}{\text{Tapped volume occupied by the powder (ml)}}$$

**Hausner ratio:** It is related to inter particle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner ratio greater than 1.6. It was calculated by using the given formula.

$$\text{Hausner Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

**Compressibility:** The Carr's compressibility gives a useful empirical guide. The compressibility of the polyherbal powder mixture was calculated by comparing the bulk density and tapped density. The percentage compressibility of all formulation was calculated using the formula<sup>34,35</sup>.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

## RESULTS AND DISCUSSION

### Organoleptic characteristics of *Terminalia chebula*

Organoleptic characteristics are fundamental criteria for the rapid identification and evaluation of the consumer acceptability of crude drugs. Sensory assessment based on visual appearance, colour, odour, taste, and texture (fracture) plays a crucial role in differentiating the sample. Such evaluation is a key component of powder analysis, as it supports the qualitative assessment of the morphological and sensory features of plant-derived powders. The macroscopical characteristics of the fruit pericarp of *Terminalia chebula* Retz. are summarized in Table 1, while representative photographs of the crude drug are depicted in Figure 1.

### Microscopical characters of powder fruit of *Terminalia chebula*

The powder microscopy of the fruit pericarp of *Terminalia chebula* Retz. revealed the presence of fragmented plant tissues and cellular debris of varying shapes and sizes. The microscopic field showed scattered dark-coloured particles and irregular fragments, characteristic of powdered crude drug material. These observations support the preliminary identification of the plant material. Several microphotographs showed **elongated and rectangular tissue fragments**, suggestive of broken parenchymatous or sclerenchymatous elements. These fragments appeared thick-walled in certain regions, indicating the presence of supporting tissues commonly encountered in fruit pericarp material. The fragments were mostly pale to light brown in colour. The powder further exhibits **parenchymatous cells**, often containing brownish contents attributable to tannins. **Vascular elements**, including spiral and pitted xylem vessels, are occasionally seen as broken fragments. **Epidermal cells** with wavy anticlinal walls may be present in surface view. In addition, the powder shows the presence of **simple starch grains**, which are mostly oval to round in shape, occurring singly or in small groups. Photo documentation of Haritaki powder microscopy are shown in Figure 2.

### Physicochemical Parameters of *Terminalia chebula* Retz.

Physicochemical evaluation of *Terminalia chebula* fruit powder was carried out to establish quality, purity, and identity of the crude drug in accordance with standard pharmacopoeial guidelines. Parameters such as loss on drying, total ash, acid-insoluble ash, water-soluble ash, extractive values, and pH were determined, as these indices are critical for assessing moisture content, inorganic matter, and the presence of extraneous materials. The loss on drying value reflects the extent of moisture and volatile components, which is an important factor influencing stability, microbial growth, and shelf life of the raw material. Low moisture content indicates better storage stability and reduced risk of degradation. The total ash value represents the total amount of inorganic residues remaining after incineration and provides an estimate of both physiological ash derived from the plant tissue and non-physiological ash arising from external contamination. Acid-insoluble ash, which mainly indicates siliceous matter such as sand and soil, was found to be minimal, suggesting proper cleaning and handling of the crude drug. Water-soluble ash further aided in

evaluating the proportion of inorganic salts soluble in water, thereby serving as an indicator of the authenticity and purity of the sample. Obtained values were compared with standard values mentioned in *API*. All the physicochemical tests were performed in triplicate and results are presented in Table 2.

#### Fluorescence analysis of *Terminalia chebula* Retz.

The powdered samples prepared from shade-dried fruit pericarp of *Terminalia chebula* were evaluated for their physicochemical characteristics, with particular emphasis on fluorescence behaviour. The untreated powder served as the control, and fluorescence responses were subsequently observed following treatment with various routine laboratory reagents under both visible and ultraviolet (UV) light. The analysis revealed pronounced variations in coloration when the treated samples were examined under visible as well as UV illumination. Such

fluorescence characteristics serve as a valuable diagnostic parameter for assessing the authenticity and quality of crude powdered drugs obtained from traditional medicinal plants available in the market. The fluorescence profiles of the powders of *Terminalia chebula* are systematically presented in Table 3. These characteristic fluorescence reactions may therefore be employed as reliable marker tests for the identification and authentication of the crude powdered drug of *Terminalia chebula*<sup>36</sup>.

The findings revealed significant color changes in the reaction mixtures when exposed to both visible and ultraviolet (UV) light. This distinct response serves as a valuable criterion for determining the authenticity of commercially available crude powdered drugs derived from traditional medicinal plants. From the result it was observed that the same reagent produced different color reactions under visible and UV light. Additionally, the use of different reagents resulted in varied colorations.

**Table 1. Organoleptic characterization of *Terminalia chebula***

Herb Name	Parameters						
	Plant part	Shape	Size	Colour	Taste	Odour	Texture
<i>Terminalia chebula</i>	fruit preicarp	Obovoid or ellipsoid	1.6-2.6 cm wide 2-4.6 cm long	Dark brown	Astringent	Characteristic	Hard and rough

**Table 2. Physico-chemical characters of *Terminalia chebula* Retz.**

S. No.	Physicochemical parameters	Result
1.	Foreign matter (%)	0.73± 0.03
2.	Total ash value (% w/w)	3.18±0.12
3.	Acid insoluble ash (% w/w)	2.78±0.66
4.	Alcohol soluble extractive (% w/w)	42.6±1.20
5.	Water soluble extractive (% w/w)	67.32±0.83
6.	Petroleum ether soluble extractive (% w/w)	18.79±0.41
7.	Loss on drying (Moisture content) (% w/w)	6.37±0.53

**Table 3. Fluorescence analysis of *Terminalia chebula* Rosc.**

S. No.	Chemical treatment	Visible light	UV light	
			254nm	366 nm
1.	Powder as such	Greyish yellow	Yellowish brown	Orange
2.	Powder + H <sub>2</sub> O	Light yellow	Yellowish grey	Caramel brown
3.	Powder + Conc. HNO <sub>3</sub>	Reddish brown	Brown	Dark brown
4.	Powder + 1N HCl	Orange	Golden yellow	Light brown
5.	Powder + 50 % H <sub>2</sub> SO <sub>4</sub>	Brown	Green	Creamish brown
6.	Powder + NH <sub>3</sub>	Dark brown	Fluorescent Light green	Light brown
7.	Powder + 1N NaOH in methanol	Light golden yellow	Greyish yellow	Dark yellow
8.	Powder + Glacial acetic acid	Brownish yellow	Creamish yellow	Green
9.	Powder + FeCl <sub>3</sub> solution	Dark brown	Orange red	Orangish green



**Figure 1.** Photo documentation of crude and powdered herb *Terminalia chebula*

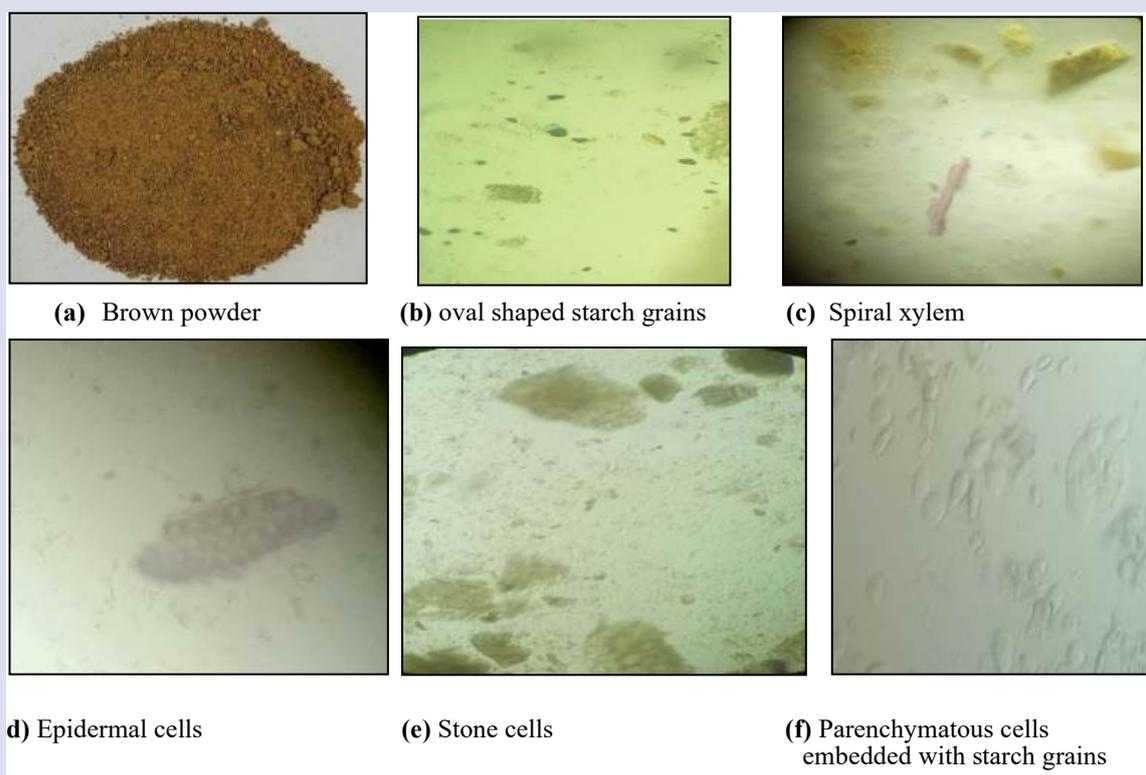


Figure 2. Powder microscopy of *Terminalia chebula*

Table 4. Qualitative Phytochemical Analysis of Haritaki fruit extract

S. No.	Phytochemical Components	Result
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Phenols	+
5.	Tannins	+
6.	Carbohydrates	+
7.	Steroids	-
8.	Saponins	-
9.	Amino acid and proteins	-
10.	Terpenoids	+

Table 5. Quantitative Phytochemical Analysis of Haritaki fruit extract

S. No.	Phytochemicals	Quantitative values (mg/100g)
1.	Total alkaloid content	7.52 ± 0.41
2.	Total flavonoids content	15.35 ± 0.31
3.	Total phenolic content	488.76 ± 1.07
4.	Total tannin content	7.45 ± 0.07

Table 6. Flow property of powdered *Terminalia chebula*.

Parameters	<i>Terminalia chebula</i>
Bulk density (g/ml)	0.71
Tapped density (g/ml)	0.87
Hausner's ratio	1.22
Carr's index (%)	18.00

#### Phytochemical screening of *Terminalia chebula* Rosc.

**Qualitative Phytochemical Analysis:** Phytochemical analysis of plants generally offers initial insights into the potential presence of bioactive compounds like flavonoids, tannins, alkaloids, saponins, terpenoids,

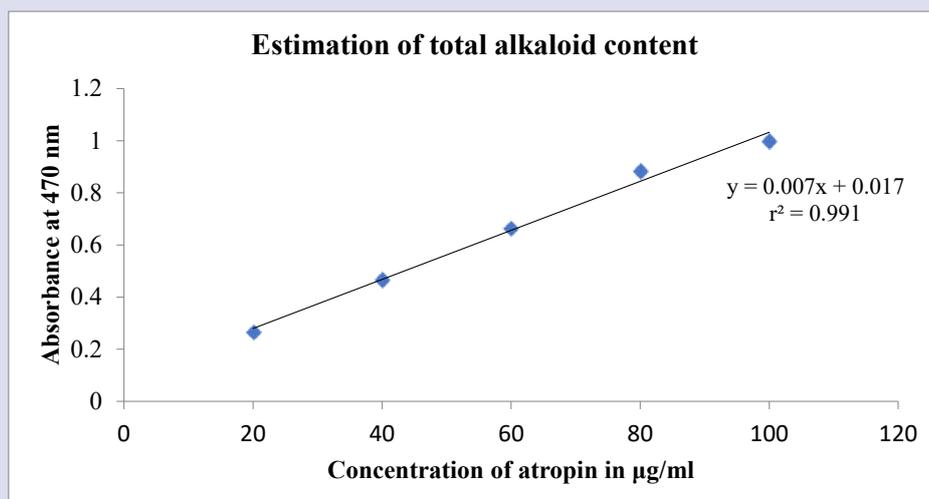
and others. Medicinal plants are recognized as important reservoirs of bioactive phytochemicals that modulate physiological functions and contribute to disease prevention<sup>37</sup>.

The phytochemical screening of the aqueous diluted extract of Haritaki indicated the presence of alkaloids, phenolic compounds (including phenols and tannins), flavonoids, carbohydrates, terpenoids and glycoside shown in Table 4.

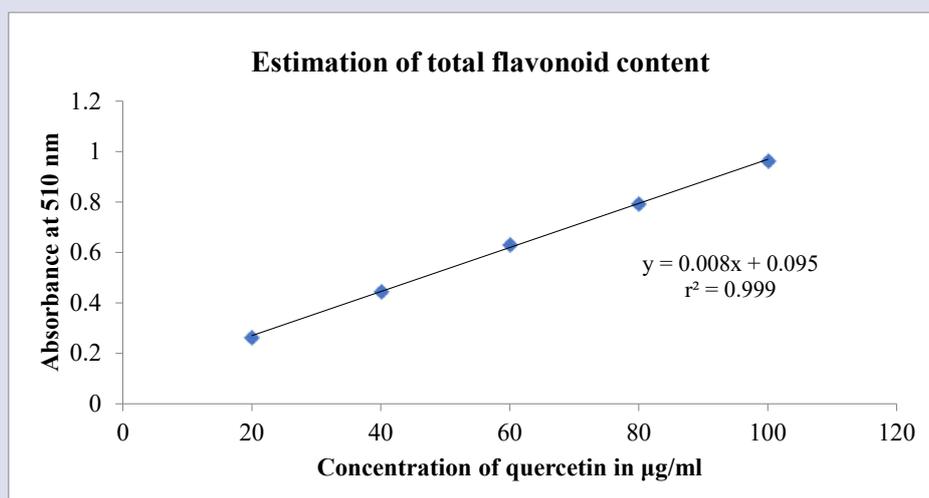
**Quantitative Phytochemical Analysis of Haritaki fruit extracts:** Identifying the phytoconstituents in a polyherbal formulation is crucial, as the diverse phytochemical it contains such as flavonoids, terpenoids, saponins, alkaloids, and phenolic compounds exhibit a wide range of pharmacological properties. Phytochemical constituents assist in linking the presence and concentration of certain compounds to their biological effects. In order to quantitatively evaluate the content of these therapeutically potent groups of phytochemical, UV-Vis spectroscopic methods were employed<sup>38</sup>.

The content of key classes of phytoconstituents, including total flavonoids, total phenolics, total alkaloids, total saponins, and total tannin, was quantified. To determine the levels of these phytochemical groups, regression equations were derived using calibrant samples containing specific representative markers from each of the above mentioned categories quercetin for total flavonoids; gallic acid for total phenolic; atropine in the estimation of total alkaloids; gallic acid for total tannins were used.

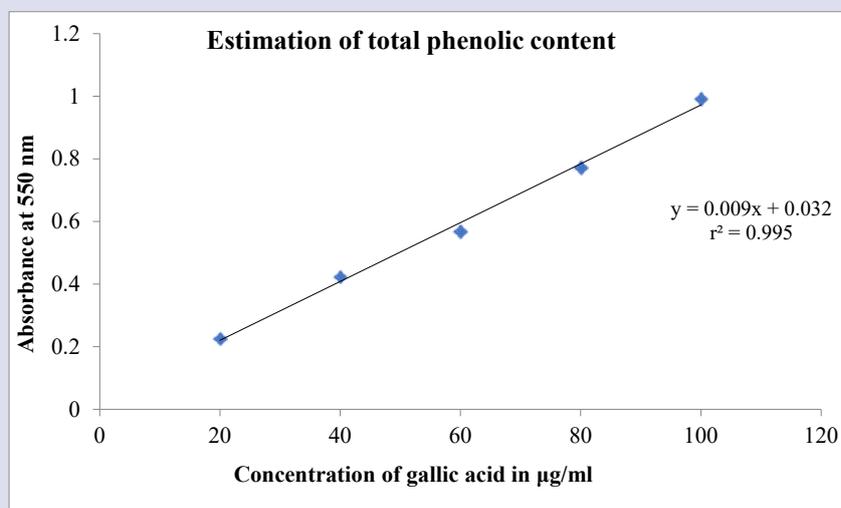
To evaluate the stability of the formulation, the levels of key phytoconstituents were measured. Table 5 shows variations in the quantitative values of Haritaki phytochemicals analysed. Total alkaloid content of Haritaki was determined by dimethyl sulfoxide (DMSO) method. Atropine was used for standard calibration curve. The total alkaloid contents of Haritaki was determined from regression equation of atropine on standard graph ( $Y=0.007x+0.017$ ,  $r^2=0.991$ ) (Figure 3).



**Figure 3.** Calibration curve of Atropine at 470 nm



**Figure 4.** Calibration curve of Quercetin at 510 nm



**Figure 5.** Calibration curve of Gallic acid at 550 nm.

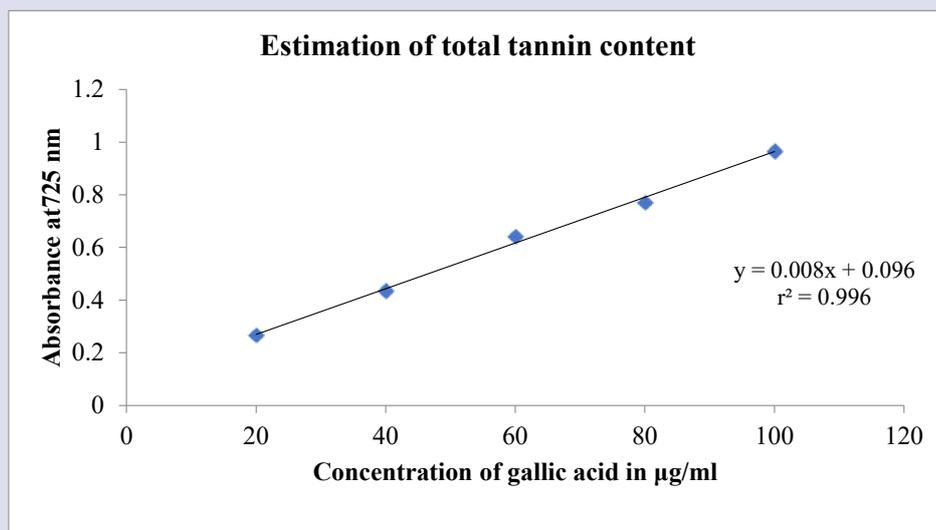


Figure 6. Calibration curve of Gallic acid at 725 nm.

The total alkaloid content of Haritaki was found to be  $7.52 \pm 0.41$  µg/mg, in term of Atropine Equivalent (AE).

Total flavonoid content of Haritaki was determined by aluminium chloride colorimetric method. Quercetin was used for standard calibration curve. The total flavonoids contents of Haritaki was determined from regression equation of Quercetin on standard graph ( $Y=0.008x+0.095$ ,  $r^2=0.999$ ) (Figure 4). The total alkaloid content of Haritaki was found to be  $15.35 \pm 0.31$  µg/mg, in terms of Quercetin Equivalent (QE).

Total phenolic content of Haritaki was determined by Folin-Ciocalteu assay method. Gallic acid was used for standard calibration curve. The total phenolic contents of Haritaki was determined from regression equation of Gallic acid on standard graph ( $Y=0.009x+0.032$ ,  $r^2=0.995$ ) (Figure 5). The total phenolic content of Haritaki was found to be  $488.76 \pm 1.07$  µg/mg, in terms of Gallic acid Equivalent (GAE).

Total tannin content of Haritaki was determined by Folin-Ciocalteu assay method. Gallic acid was used for standard calibration curve. The total tannin contents of Haritaki was determined from regression equation of Gallic acid on standard graph ( $Y=0.008x+0.096$ ,  $r^2=0.996$ ) (Figure 6). The total tannin content of Haritaki was found to be  $7.45 \pm 0.07$  µg/mg, in terms of Gallic acid Equivalent (GAE).

### Flow properties of Haritaki powder

The flow characteristics of pharmaceutical powders are critically important in the pharmaceutical industry. Powder flow behaviour plays a vital role in numerous unit operations, including blending, compression, filling, transportation, and scale-up processes. The flow properties of the herbal powder were evaluated to assess its suitability for handling, processing, and formulation development. Parameters such as bulk density, tapped density, angle of repose, Carr's compressibility index, and Hausner's ratio were determined using standard pharmacopeial methods. The results indicated that the powder exhibited acceptable flow characteristics, reflecting appropriate particle size distribution and interparticulate interactions. Good flow behavior is essential for ensuring uniform mixing, accurate dosing, and reproducibility during pharmaceutical processing, particularly in the development of solid dosage forms. The observed flow properties suggest that the herbal powder is suitable for further formulation and scale-up studies. Parameters such as the angle of repose, Hausner's ratio, and Carr's index help evaluate a powder's flowability and its compressibility potential. Data pertaining to the flow properties of

Haritaki powder presented in Table 6 elucidate that the powder had a Carr's compressibility of 18.00 per cent and Hausner ratio of 1.22 with a bulk density and tapped density as 0.71 g/ml and 0.87 g/ml respectively. These value indicating **fair to passable flow property** of powdered *Terminalia chebula*.

### CONCLUSION

The present study demonstrates that the standardization and identification of *Terminalia chebula* Rosc. through organoleptic evaluation and physicochemical analysis serve as essential and effective parameters for determining the authenticity of the test sample. The present investigation provides comprehensive phytochemical and microscopic characterization of the selected herbal plant, establishing its identity and therapeutic potential. Qualitative phytochemical screening confirmed the presence of diverse bioactive constituents, including primary metabolites and pharmacologically significant secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, tannins, glycosides, and triterpenoids, which collectively support the plant's traditional medicinal use. Microscopic evaluation revealed distinct diagnostic features, including characteristic tissues, cell inclusions, and anatomical structures that are valuable for authentication and quality control. Together, these phytochemical and microscopic findings contribute to the standardization of the plant material and provide a scientific foundation for its safe utilization and further pharmacological and phytochemical investigations. The quality of the drug ensures its therapeutic effectiveness. Moreover, the findings of phytochemical screening significantly contribute to the accurate identification and authentication of *Terminalia chebula* Rosc. Moreover, these analytical parameters for quality assurance also demonstrate the potential effectiveness of *Terminalia chebula* Rosc. in managing various health ailments. The results obtained from the present study will aid in the identification, standardization, and quality evaluation of different samples of *Terminalia chebula* Rosc. Based on the findings of the present investigation, the results of organoleptic evaluation indicated that the taste and odour of the plant sample can influence its medicinal applications. Fluorescence analysis proved to be an important tool for assessing the quality and purity of the sample. Observations under UV and visible light revealed that the sample exhibited different colours at various wavelengths, which can be attributed to the presence of diverse chemical constituents within the extract. Phytochemical screening of different solvent extracts confirmed the presence of multiple phytoconstituents. Among these,

the aqueous extract demonstrated the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, and terpenoids. Quantitative analysis further revealed that the aqueous extract of *Terminalia chebula* contains several bioactive molecules in varying concentration.

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