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Pharmacognostic Authentication and Phytochemical Profiling Coupled with Flowability Studies of *Zingiber officinale* for Quality Control

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ABSTRACT

Introduction: Medicinal plant extracts and other natural products have continued to find useful applications in nanomedicines due to their interesting biological properties. *Clerodendrum thomsoniae* (CT) is a plant used in traditional medicine to treat stress- and inflammation-related diseases, including jaundice, diabetes, and cancer. **Objectives:** This study, therefore, evaluated CT extract-based silver nanoparticles (Ag NPs) for their antioxidant and anti-inflammatory potential. **Materials and Methods:** The nanoparticles were prepared using green synthesis methods. They were characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). Antioxidant study was based on NO, H2O2, superoxide, and hydroxyl radical scavenging spectrophotometric methods. The *in vitro* anti-inflammatory test was based on a protein (egg albumin) denaturation assay. **Results:** Results showed CT-Ag NPs ranged from spherical to cubic shapes. The UV absorption peak at 427 nm suggests CT-Ag NP formation. The presence of elemental Ag (96.04 %) by EDX analysis suggests the conversion of metallic silver into elemental silver. The crystallinity of the nanoparticles was shown on the X-ray diffractogram as a sharp peak at 38.12° [reflection index (111)] with an average particle size of 47 nm. CT-Ag NPs showed dose-dependent hydroxyl and nitric oxide radical scavenging activities with 67.63 ± 0.78 % and 58.48 ± 1.20 %, respectively, at 200 µg/mL. It showed a notable anti-inflammatory effect by inhibiting protein denaturation with an IC50 of 53.58 ± 17.78 µg/mL. **Conclusions**: It can be deduced from this study that CT-Ag NPs show promise as antioxidant and anti-inflammatory agents.

Keywords: *Clerodendrum thomsoniae;* silver nanoparticles; antioxidant; anti-inflammatory

INTRODUCTION

In recent years, the Indian herbal industry has witnessed a substantial expansion, marked by the introduction of numerous new herbal pharmaceutical and cosmetic products. However, the increasing presence of inadequate, adulterated, and counterfeit herbal materials has compromised the assurance of product quality and purity. Since the efficacy, safety, and therapeutic potential of herbal formulations are directly linked to their quality, it is imperative to ensure accurate identification, standardization, and purity assessment of herbal medicines. Herbs are widely used to treat both chronic and acute illnesses, including major health issues such as heart disease, prostate disorders, depression, inflammation, and a weakened immune system. Across the world, people rely on herbs to manage various diseases, and numerous studies have confirmed their effectiveness. One of the main advantages of herbal medicines is that they generally do not cause side effects and promote long-term overall well-being. However, a few risks are also linked to the use of herbal remedies. The use of herbs marked the beginning of pharmacological treatment for diseases. Traditional healing practices across the world have long incorporated herbs as a key part of their remedies. A major advantage of these herbal supplements is that they are derived from natural sources. Since they originate from different types of foods, the body can more easily assimilate and balance them within the system. As a result, the body efficiently absorbs the necessary nutrients without experiencing the side effects often caused by chemical-based medicines[1].

In recent times, ensuring the safety, quality, and effectiveness of medicinal plants and herbal medicines has become an important concern for both developed and developing nations. Through proper standardization and evaluation of bioactive compounds derived from plants, herbal medicines can pave the way for a new era in healthcare for treating various human diseases. Moreover, understanding traditional knowledge and the use of medicinal plants can significantly contribute to the exploration and discovery of valuable natural plant resources. Medicinal herbs hold great promise for the future, as there are nearly half a million plant species worldwide, many of which remain unexplored in medical research. Ongoing and future studies on their medicinal properties may prove valuable in disease treatment. The evolution of herbal medicine began alongside advancements in chemistry, particularly with the isolation, purification, and identification of various plant-based compounds[2].

Ginger (*Zingiber officinale* Roscoe) is an herbaceous perennial plant belonging to the family ***Zingiberaceae***, believed to have originated in **Southeast Asia**. Its aromatic and pungent rhizome is widely used as a **spice, flavouring agent, food ingredient, and medicine**. The term *Zingiber* comes from the Greek word *zingiberis*, which traces back to

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the Sanskrit term *Singabera*. Cultivated for centuries, ginger is thought to be native to **southern China, Southeast Asia, and India.** As a major tropical horticultural crop, it plays a vital role in both **culinary and medicinal applications**. Ginger is commonly used in several forms such as **young or mature fresh ginger, dried ginger, ginger oil, oleoresin, paste, emulsion, and dry-soluble extract.** It is particularly rich in **secondary metabolites like oleoresins**, which are responsible for its characteristic **pungency and flavour.** Dried ginger rhizomes are **irregularly shaped,** often **branched or palmate,** with a color that ranges from **dark yellow or light brown to pale buff**. They contain roughly **2% essential oil,** whose main constituent is **zingiberene**, while **zinerone** is responsible for the spice's pungent flavor. The essential oil extracted from the rhizomes is widely utilized in both the **food** and **perfume** industries[3].

The health-promoting effects of ginger are largely due to its **bioactive constituents**, including **phenolic compounds, flavonoids, terpenes**, and various **volatile components** found in its essential oils. Ginger has been shown to help **treat or prevent numerous ailments,** such as **nausea and vomiting following chemotherapy, high blood pressure (antihypertensive effect),** and **diabetes** by lowering **blood glucose levels**. It also exhibits **anti-hyperlipidemic** and other therapeutic properties. Moreover, ginger is extensively used to **prevent chronic diseases** because of its **antioxidant potential,** which helps **neutralize reactive oxygen species** in the body[4].

The **chemical composition of ginger** varies depending on several factors such as its **variety, cultivation methods,** and **storage conditions**. The primary bioactive constituents in **fresh ginger rhizomes** are **gingerols**, with **[5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one]** being the most dominant compound in this group. In its powdered form, ginger typically contains **3–6% fatty oil, 9% protein, 60–70% carbohydrates, 3–8% crude fiber, about 8% ash, 9–12% moisture,** and **2–3% volatile oil.**

The **volatile oil** mainly consists of **mono- and sesquiterpenes**, along with compounds such as **camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terpineol, borneol, geraniol, limonene,** and **linalool.** Among these**, α-zingiberene (30–70%)** and **β-sesquiphellandrene** are the major components. **Shogaol,** a dehydrated derivative of gingerol, contributes to the **pungency of dried ginger powder**[5]**.**

The main **bioactive components** found in **ginger oil** are the **sesquiterpenes bisabolene, zingiberene,** and **zingiberol** whose concentrations vary depending on the **growing conditions.** In **laboratory studies on animals, gingerols** have been shown to enhance **gastrointestinal motility** and exhibit **analgesic, sedative, antipyretic** and **antibacterial** properties**.**

Shogaols, another key group of compounds, display a wide range of **beneficial effects**, including **anticarcinogenic, antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anti-obesity, anti-ulcer** and **antiallergenic** activities. Moreover, research indicates that **shogaols possess stronger biological activity than gingerols.** Experimental studies have also shown that **ginger oil can prevent skin cancer in mice,** while research conducted at the **University of Michigan** revealed that **gingerols are capable of destroying ovarian cancer cells**[6]**.**

Ginger is most commonly used to **relieve nausea**, but it also serves as an **anti-inflammatory agent,** a **natural pain reliever,** a **warming remedy,** and a **cholesterol-lowering herb. Randomized controlled trials** have confirmed its effectiveness in preventing nausea, while **case studies** indicate potential benefits in managing **migraines** and **inflammatory arthritis,** although no large-scale clinical trials have yet been conducted. **Animal research** suggests that ginger may have **thermogenic properties,** but these effects have not been tested in humans.

Furthermore, there is **insufficient evidence** to support the use of ginger as a **cholesterol-lowering supplement.** Since ginger has a long history of use as a food ingredient, it is generally considered **safe for consumption and supplementation.** However, because of its potential impact on **platelet aggregation** and **thromboxane synthesis** observed in laboratory studies, **caution is advised** for individuals taking **blood-thinning medications** or those **undergoing surgery**[7]**.**

Zingiber officinale is widely recognized for its **anti-nausea properties**, but it is also used in the treatment of **migraines** and various **inflammatory conditions** such as **hepatitis, esophagitis,** and **gastritis**. These disorders are often triggered by **infectious agents** like bacteria, viruses, and parasites, as well as by **chemical and physical factors** such as **acid, heat, and cigarette smoke**, all of which are considered potential contributors to **human cancers**. Additionally, ginger serves as a **natural pain reliever and warming remedy,** and as an **herbal medicine**, it helps in **reducing cholesterol levels** in the body, offering multiple **health-promoting benefits**[8]**.**

MATERIAL AND METHODS

Collection of Sample

D**ried rhizome of** *Zingiber officinale* **Rosc.** 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/2019/3535-26-2) 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**[9]**.**

Powdered Microscopical Studies

For **powder microscopy**, different **slides were prepared** from **powdered crude herb** Zinger 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 glycerin on glass slides, and examined under 10X and 40X objectives using a Brightfield microscope equipped with an analyser. Photomicrographs of the observed 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[10,11,12].

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 **British Pharmacopoeia**. Additionally, **fluorescence analysis** of the **rhizome powder** was carried out by treating it with various **chemical reagents** and examining the results under both **ultraviolet** and **daylight** conditions[13].

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[14].

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

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 [15, 16].

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

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[15, 16].

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

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

Exactly 5 grams of the coarsely powdered, air-dried drug was taken and subjected to cold maceration using 100 mL of ethanol in a sealed flask for 6 hours, followed by standing undisturbed for 18 hours. The resulting mixture was then filtered using Whatman filter paper No. 1. From the filtrate, 25.0 mL was measured and evaporated to dryness in a pre-weighed beaker. The residue was further dried in an oven maintained at 110°C, and the ethanol-soluble extractive value was calculated as a percentage[17].

$$\text{Ethanol soluble extractive value (\%)} = \frac{[W1 - W2]}{W_3} \times 100$$

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

Precisely 5 grams of coarsely powdered, air-dried drug was subjected to cold maceration with 100 mL of petroleum ether in a sealed flask for 24 hours, with frequent shaking during the first 6 hours and then left undisturbed for the remaining 18 hours. The resulting mixture was filtered using Whatman filter paper No. 1. A 25.0 mL portion of the clear filtrate was taken and evaporated to dryness in a pre-weighed beaker, followed by drying in an oven set at 110°C. The ether-soluble extractive value was then calculated as a percentage[18].

Ether soluble extractive value (%) = $\frac{[W1 - W2]}{W_3} \times 100$

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

Accurately 5 grams of previously weighed, air-dried powdered drug was placed in a glass-stopper flask and macerated with 100 mL of chloroform water (1:99). The mixture was shaken frequently over a period of 6 hours and then left to stand undisturbed for 18 hours. It was then filtered promptly, taking care to prevent any loss of solvent. A 25 mL portion of the filtrate was evaporated to dryness in a tared flat-bottomed petridish, dried at 105°C, cooled in a desiccator, and weighed. The water-soluble extractive value was calculated as a percentage relative to the air-dried drug. The final result was expressed as the average of three separate determinations[14].

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

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 Zinger was conducted following a standard procedure. Approximately 1 gram of finely powdered, dried drug was treated with freshly prepared acidic and alkaline solutions, as well as various solvents. The powders were exposed to acids (10% HCl, conc. HCl, conc. H_2SO_4, and conc. HNO_3), alkaline reagents (1 N aqueous NaOH, 1 N alcoholic NaOH, and 5% KOH), and other chemical agents (5% iodine, 5% ferric chloride, acetone), along with distilled water. The treated samples were then examined for fluorescence characteristics under visible light, short-wave UV light (254 nm), and long-wave UV light (365 nm)[19, 20].

Phytochemical screening of the active ingredients

The qualitative and quantitative phytochemicals present in Ginger rhizomes were analysed as follows.

Qualitative Analysis of Phytochemicals

The plant extracts were screened for the presence of the phytochemicals like Alkaloids, Tannin, Flavonoids, Carbohydrates, Steroids, Saponin and Protein.

Preparation of plant extract by cold maceration

Initially, the **Ginger was thoroughly washed** several times with **tap water** to remove any dirt, followed by a final rinse with **distilled water**. It was then **cut into small pieces** and **sun-dried for 2–3 days.** The **dried Ginger** was **ground using a mortar and pestle** to obtain a **fine powder,** which was later used for extraction. For the extraction process, **20 grams of the dried Ginger powder** were **extracted with water for 8 hours.** After the completion of maceration residue was removed by filtration followed by the evaporation of solvent and extract was concentrated. After extraction it was kept in hot air oven at 50-60 to give solid extract[15].

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 (Rao *et al.*, 2016).

Determination of quantitative phytochemical composition

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

Determination of total alkaloid content (TAC)

To determine the total alkaloid content, 1 mg of plant extract was dissolved in dimethyl sulfoxide (DMSO), followed by the addition of 1 mL of 2 N hydrochloric acid. The mixture was then filtered. The resulting solution was transferred to a separating funnel, where 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The mixture was vigorously shaken with 1, 2, 3, and 4 mL portions of chloroform. The chloroform layers were collected and combined in a 10 mL volumetric flask, then diluted to volume with chloroform. Reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/mL) were prepared using the same procedure. Absorbance of both the test and standard solutions was measured at 470 nm using a UV/Visible spectrophotometer, with the reagent blank as reference. The total alkaloid content was expressed as milligrams of atropine equivalent (AE) per gram of extract[19, 21].

Determination of total tannin Content

Tannin content was estimated using the Folin-Ciocalteu method. For this, 0.1 mL of plant extract was added to a 10 mL volumetric flask containing 7.5 mL of distilled water, followed by 0.5 mL of Folin–Ciocalteu reagent and 1 mL of 35% sodium carbonate (Na_2CO_3) solution. The volume was then made up to 10 mL with distilled water. The mixture was thoroughly shaken and allowed to stand at room temperature for 30 minutes. A series of gallic acid standard solutions (20, 40, 60, 80, and 100 µg/mL) were prepared in the same way. The absorbance of both test and standard solutions was recorded at 725 nm using a UV/Visible spectrophotometer, with the blank as reference. The tannin content was calculated and expressed as milligrams of gallic acid equivalent (GAE) per gram of extract[22,23].

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[24,25].

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_2CO_3) 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[26,27].

Flow properties

The compaction characteristics, including **bulk density (g/ml)** and **tapped density (g/ml)**, of the Ginger 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[27,28].

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

RESULTS AND DISCUSSION

Organoleptic characteristics of *Zingiber officinale*

Organoleptic characteristics serve as essential parameters for the **quick identification** and **consumer acceptability** of crude drugs. **Sensory evaluation** through visual inspection, color, odor, taste, and texture (fracture) plays a vital role in distinguishing the sample. It is an important aspect of **powder analysis**, which helps in the **qualitative assessment** of the **morphological and sensory attributes** of plant powders. The observations for macroscopical analysis of rhizome of *Zingiber officinale* Rosc. is presented in Table 1 and photographs of crude herb is shown in Figure 1

Microscopical Characters of Powder Rhizome of *Zingiber officinale*

The **microscopic examination** of the powdered drug revealed the presence of **yellowish cellular material, oil globules, parenchymatous cell fragments, vessel elements with spiral thickening** and **fibers**. The

Table 1. Organoleptic characterization of *Zingiber officinale*

Herb Name	Parameters						
	Plant part	Shape	Size	Colour	Taste	Odour	Texture
Zingiber officinale	Rhizome	Nodule or Irregular	8.67-10.4 cm wide 2.5-6.5 cm long	Light brown	Pungent	Pleasant	Rough

Zingiber officinale* (Crude drug)** **Brownish powdered *Zingiber officinale

Figure 1. Photo documentation of crude and powdered herb *Zingiber officinale*

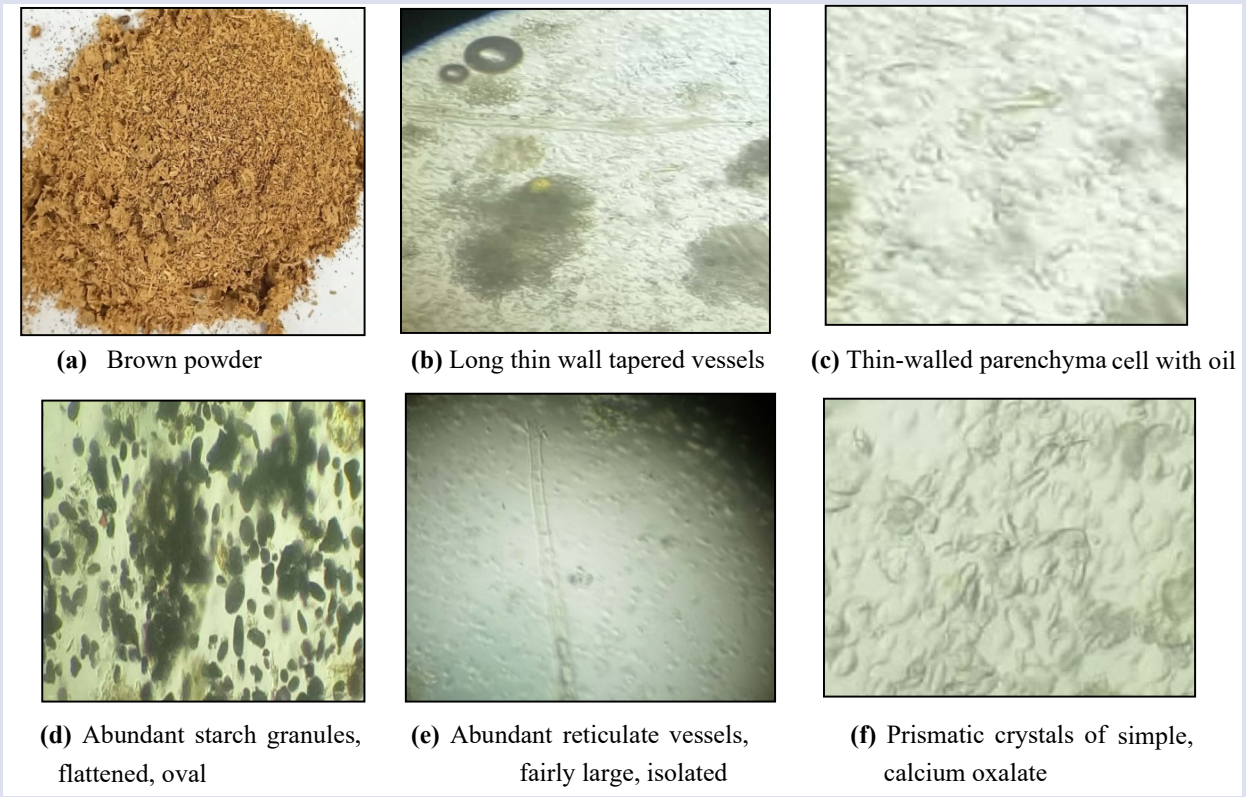

Figure 2. Powder microscopy of *Zingiber officinale*

starch grains were observed to be **simple, oval, oblong,** or **rounded** in shape (Figure 2). The **measurements** of various components, including **cells, starch grains, crystals** and **oil droplets** were recorded from the **rhizome of** *Zingiber officinale***.**

Physicochemical Parameters of *Zingiber officinale* Rosc.

The **ash values** and **alcohol- and water-soluble extractive values** of a powdered drug serve as vital indicators of its **purity, quality, and authenticity**. These parameters are fundamental and indispensable for the **standardization of herbal drugs**. The **total ash, acid-insoluble ash,** and **water-soluble ash values** provide insights into possible **adulteration with inorganic substances.** Similarly, the **water- and alcohol-soluble extractive values** represent the **quantity of extractable matter** present in a given solvent. Variations either low or high in extractive values may suggest **adulteration, addition of exhausted materials,** or **improper processing** during the **drying or storage** stages. Furthermore, the **moisture content** of the drug significantly affects its **quality and therapeutic efficacy,** as **excessive moisture** creates a suitable environment for **microbial growth**, leading to **spoilage and degradation** of the crude drug[13]. Physicochemical parameters of *Zingiber officinale* that is foreign material, total ash value, acid insoluble ash, extractive value, alcohol soluble extractive, water soluble extractive and loss in drying were quantitatively analyzed as per the standard procedure. The 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 *Zingiber officinale* Rosc.

The **fluorescent coloration** observed is **unique to each type of compound** and **different plant materials** exhibit **distinct colors** when treated with various **chemical reagents and solvents[29]. Fluorescence** is a significant characteristic exhibited by various **phytoconstituents** found in plant materials. Certain compounds display **visible fluorescence under daylight**, while others exhibit **fluorescence only under ultraviolet (UV) light**. Moreover, some non-fluorescent substances can be transformed into **fluorescent derivatives** when treated with specific **chemical reagents,** allowing for their qualitative assessment. Therefore, **fluorescence analysis** serves as an essential tool in the **pharmacognostic evaluation** of crude drugs. The **fluorescence study** of the **powdered Ginger** as presented in **Table 3** revealed **distinct color changes** upon treatment with various **chemical reagents**, indicating the presence of characteristic constituents[30].

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.

Phytochemical screening of *Zingiber officinale* 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. The information obtained from this analysis can play a crucial role in drug discovery and development, as the medicinal effects of plants in humans are often linked to these phytochemical components[31].

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

Quantitative Pyhtochemical Analysis of Ginger rhizome extracts: 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 ginger phytochemicals analysed. Total alkaloid content of Ginger was determined by dimethyl sulfoxide (DMSO) method. Atropine was used for standard calibration curve. The total alkaloid contents of Ginger was determined from regression equation of atropine on standard graph (Y=0.007x+0.017, r^2=0.991) (Figure 3). The total alkaloid content of *Ginger* was found to be 6.31 ± 0.17 µg/mg, in term of Atropine Equivalent (AE).

Total flavonoid content of Ginger was determined by aluminium chloride colorimetric method. Quercetin was used for standard calibration curve. The total flavonoids contents of Ginger 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 Ginger was found to be 20.34 ± 0.62 µg/mg, in terms of Quercetin Equivalent (QE).

Total phenolic content of Ginger was determined by Folin-Ciocalteu assay method. Gallic acid was used for standard calibration curve. The total phenolic contents of Ginger was determined from regression equation of Gallic acid on standard graph (Y=0.009x+0.032, r^2=0.995) (Figure 5). The total alkaloid content of Ginger was found to be 237.64 ± 1.88 µg/mg, in terms of Gallic acid Equivalent (GAE).

Total tannin content of Ginger was determined by Folin-Ciocalteu assay method. Gallic acid was used for standard calibration curve. The total tannin contents of Ginger was determined from regression equation of Gallic acid on standard graph (Y=0.008x+0.096, r^2=0.996) (Figure 6). The total alkaloid content of Ginger was found to be 8.23 ± 0.008 µg/mg, in terms of Gallic acid Equivalent (GAE).

Flow properties of Ginger powder

The flow characteristics of pharmaceutical powders are critically important in the pharmaceutical industry. Powder flow behavior plays a vital role in numerous unit operations, including blending, compression, filling, transportation, and scale-up processes. 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 ginger powder presented in Table 6 elucidate that the powder had a Carr compressibility of 15.00 per cent and Hausner ratio of 1.16 with a bulk density and tapped density as 0.59 g/ml and 0.69 g/ml respectively. These value indicating good and fair flow property of powdered *Zingiber officinale*.

CONCLUSION

The present study demonstrates that the standardization and identification of *Zingiber officinale* Rosc. through organoleptic evaluation and physicochemical analysis serve as essential and effective parameters for determining the authenticity of the test sample. The quality of the drug ensures its therapeutic effectiveness. Moreover, the findings of phytochemical screening significantly contribute to the accurate identification and authentication of *Zingiber officinale* Rosc. Moreover, these analytical parameters for quality assurance also demonstrate the potential effectiveness of *Zingiber officinale* Rosc. in managing various health ailments. The results obtained from the

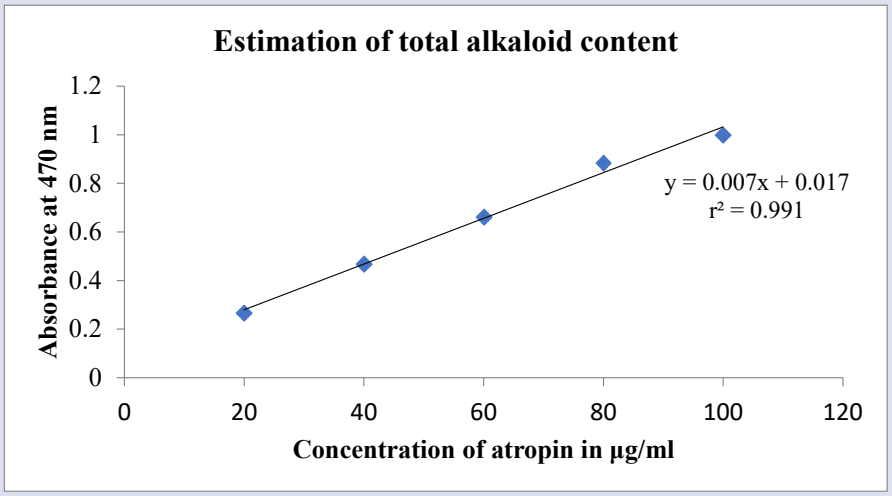

Figure 3. Calibration curve of Atropine at 470 nm

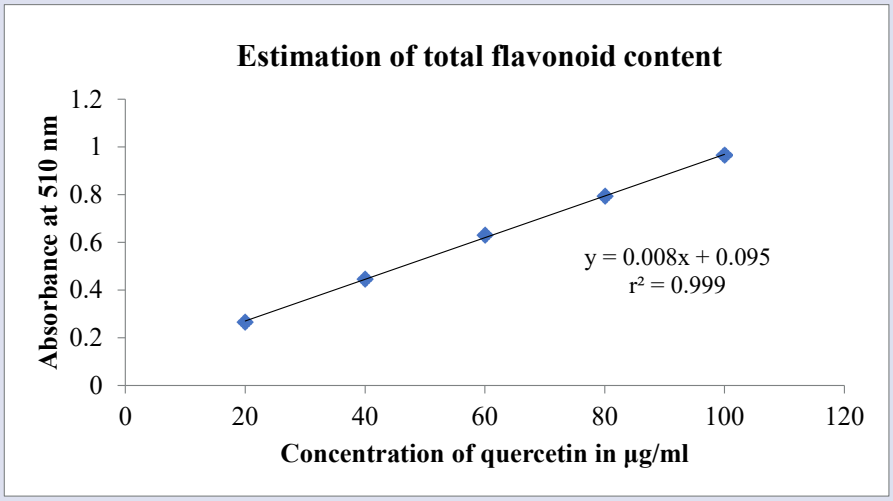

Figure 4. Calibration curve of Quercetin at 510 nm

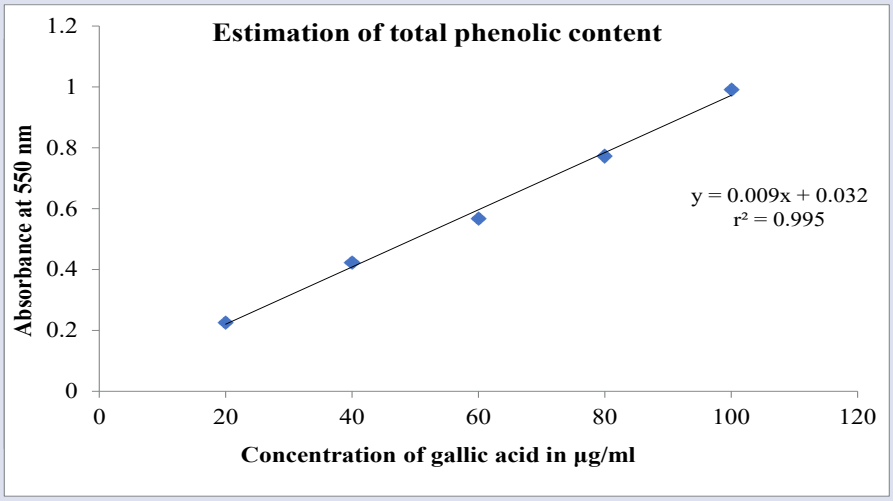

Figure 5. Calibration curve of Gallic acid at 550 nm.

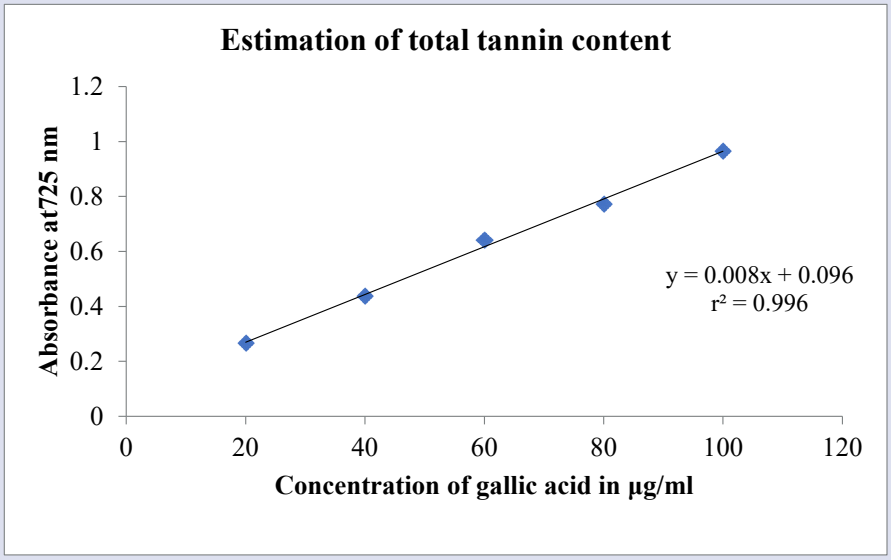

Figure 6. Calibration curve of Gallic acid at 725 nm.

Table 2. Physico-chemical Characters of *Zingiber officinale* Rosc.

S. No.	Physicochemical parameters	Result
1.	Foreign matter (%)	0.83 ± 0.08
2.	Total ash value (% w/w)	5.69±0.81
3.	Acid insoluble ash (% w/w)	1.05±0.37
4.	Alcohol soluble extractive (% w/w)	8.23±0.17
5.	Water soluble extractive (% w/w)	15.79±0.41
6.	Petroleum ether soluble extractive (% w/w)	3.51±0.73
7.	Loss on drying (Moisture content) (% w/w)	9.82±0.24

Table 3. Fluorescence analysis of *Zingiber officinale* Rosc.

S. No.	Chemical treatment	Visible light	UV light	
			254nm	366 nm
1.	Powder as such	Brown	Dark green	Caramel brown
2.	Powder + H_2O	Brown	Light green	Caramel brown
3.	Powder + Conc. HNO_3	Pinkish	Brown	Green
4.	Powder + 1N HCl	Light brown	Green	Dark brown
5.	Powder + 50 % H_2SO_4	Brown	Green	Creamish brown
6.	Powder + NH3	Dark brown	Light green	Brown
7.	Powder + 1N NaOH in methanol	Brown	Dark greenish	Blue
8.	Powder + Glacial acetic acid	Light brown	Green	Grey
9.	Powder + $FeCl_3$ solution	Light brown	Light green	Indigo

Table 4. Qualitative Phytochemical Analysis of Ginger rhizome 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 Ginger rhizome extract

S. No.	Phytochemicals	Quantitative values (mg/100g)
1.	Total alkaloid content	6.31 ± 0.17
2.	Total flavonoids content	20.34 ± 0.62
3.	Total phenolic content	237.64 ± 1.88
4.	Total tannin content	8.23±0.08

Table 6. Flow property of powdered *Zingiber officinale*

Parameters	Zingiber officinale
Bulk density (g/ml)	0.59
Tapped density (g/ml)	0.69
Hausner's ratio	1.16
Carr's index (%)	15.0

present study will aid in the identification, standardization, and quality evaluation of different samples of *Zingiber officinale* 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 *Zingiber officinale* contains several bioactive molecules in varying concentration.

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