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Rispa Johns

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Anuranjini NA

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Joseph Thomas K

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Dr. Niranjana Sajikumar

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Dr. Sajikumar S

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Corresponding Author:**Rispa Johns**

Department of Research and Development, Dhathri Life Sciences Pvt. Ltd., Muvattupuzha, Ernakulam, Kerala, India

Pharmacognostical evaluation, phytochemical characterization, TLC profiling of ingredients used in a Polyherbal formulation: Amruthotharam Kashayam

Rispa Johns, Anuranjini NA, Joseph Thomas K, Niranjana Sajikumar and Sajikumar S

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Abstract

This study was undertaken to establish comprehensive pharmacognostic standards, microscopic characteristics, and the thin-layer chromatographic (TLC) profile of the ingredients used in *Amruthotharam Kashayam*, a traditional polyherbal formulation. To authenticate the formulation and assess its suitability for medicinal use, key physicochemical parameters such as total ash, acid-insoluble ash, and water and alcohol extractive values were determined for the whole plant materials. Detailed macroscopic and microscopic evaluations, as well as TLC analyses of both methanolic and hydro-methanolic extracts, were conducted. Anatomical studies focused on the stem, rhizome, and fruit rind. The pharmacognostic, microscopic, and TLC profiles generated in this study provide crucial baseline data for the identification, standardization, and quality control of the ingredients in *Amruthotharam Kashayam*. These findings substantiate its traditional therapeutic use and offer a foundation for future phytochemical and pharmacological investigations.

Keywords: Antimalarial drug, antiplasmodial, cell lines, malaria, neurotoxicity

1. Introduction

Ayurveda, derived from the Sanskrit terms Ayus (life) and Veda (knowledge), translates to "the science of life" and is traditionally regarded as an extension of the Vedic corpus. According to Ayurvedic principles, health is defined by the balanced state of doshas (biological energies), dhatus (tissue elements), malas (waste products), and agni (digestive fire). The three primary doshas—vata, pitta, and kapha—govern physiological and psychological functions, while the dhatus are classified into seven distinct tissue types that develop from the embryonic stage, sustain the body through adulthood, and gradually diminish with aging. As a natural consequence of metabolic activity, tissue cells undergo continuous turnover, wherein cellular degradation is counterbalanced by regeneration, facilitated by nutrients obtained from food, water, air, and sunlight through processes of digestion and metabolism. Oxidative stress resulting from free radical activity is mitigated by antioxidants, which are conceptualized in Ayurveda as ojas—the vital essence (tejas) derived from all tissue elements. Ayurvedic therapies aim to enhance cellular vitality and longevity by augmenting ojas, thereby promoting both qualitative and quantitative tissue regeneration. Ayurveda is derived from two words, 'Ayus' and 'Veda,' meaning 'the science of life.' Traditionally, it is considered a supplement to the Vedas. In Ayurveda, a healthy person is one who maintains equilibrium of doshas, dhatus, malas, and agni. The three doshas are vayu, pitta, and kapha. Dhatus or tissue elements are categorized into seven groups. These tissues grow from the embryonic stage through adulthood and begin to decline during old age. As part of the living process, some tissue cells are destroyed continuously. These are replenished by food, drink, wind, and sun rays through digestion and metabolism. The action of free radicals can be kept in check by the intake of antioxidants. In Ayurveda, these antioxidants are called ojas, which are considered the essence (tejas) of all the tissue elements. When these antioxidants are supplemented through various therapies, the cells continue to grow both in quality and quantity (1).

The Ayurvedic Materia Medica encompasses an extensive heritage of herbal medicine, detailing the therapeutic attributes of more than 600 plant species (20). A fundamental principle in Ayurvedic practice involves identifying an individual's prakriti (constitutional type) prior to treatment initiation. The selection of medicinal plants is tailored not only to

the patient's inherent constitution but also to the specific doshic imbalances present. Ayurveda also posits a profound relationship between physiological ailments and an individual's metaphysical or spiritual state (21). Traditional Indian medicine often favors polyherbal formulations—combinations of multiple plant extracts—over single-herb preparations, as they are believed to produce synergistic effects. Herbal interventions are a cornerstone of Ayurvedic therapeutics, with their use extensively recorded in classical scriptures such as the Vedas and Samhitas. These botanicals are typically administered in various dosage forms, most commonly as compound formulations (22, 23). Due to the often limited bioavailability of raw plant materials, traditional processing methods are employed to enhance their digestibility and therapeutic efficacy. The mode of administration is considered critical, as it can markedly influence the herb's pharmacodynamic action. The Ayurvedic *Materia Medica* embodies a vast tradition of herbal practices, featuring the medicinal properties of over 600 plants (20). In Ayurveda, determining an individual's constitutional type is essential before initiating treatment; the choice of herbs depends on both the patient's body type and the specific imbalance of doshas they are experiencing. Ayurveda emphasizes a clear connection between health issues and a person's metaphysical state (21). Within Indian traditional medicine, combined plant extracts and formulations are preferred over single herbs. Herbal remedies are a vital therapeutic element, documented in classical texts such as the Vedas and Samhitas. Typically, Ayurvedic herbs are available in various dosage forms, predominantly as polyherbal formulations (22, 23). These herbs are processed to improve their absorption, as they are often not easily digestible in their natural state, and the way a herb is consumed can significantly influence its effectiveness.

Ayurvedic pharmacology employs a diverse array of dosage forms derived from herbal sources, encompassing fresh juice (*svarasa*), herbal paste (*kalka*), powdered herbs (*churna*), decoctions (*kwatha*), distilled herbal waters (*arka*), hot infusions (*phanta*), cold infusions (*hima*), herbal confections such as jams and jellies (*paka*, *leha*, *avaleha*), fermented preparations like medicated wines (*arishta*, *asava*), tinctures, resin-based preparations such as *guggulu* (extracted from *Commiphora mukul*), alkaline extracts (*ksara*), and lipid-based carriers including medicated ghee (*siddha ghrita*) and medicated oils (*siddha taila*). In addition, Ayurvedic therapeutics incorporates mineral-based medicines as outlined in *Rasasastra* (24). Among these, *kwatha* (decoction) is a particularly important formulation widely used for internal administration in the treatment of various ailments. The standard method for preparing *kwatha* involves boiling one part by weight of coarsely ground plant material with sixteen parts by volume of water, reducing it to one-fourth of its original volume. For therapeutic purposes, especially internal use, the decoction is often further concentrated to one-eighth of the starting volume (25).

The human inclination toward herbal medicine is ancient, rooted in a deep interdependence between the plant and animal kingdoms. During the Vedic period in India, medicinal plants played a crucial role in healthcare practices. Early Vedic texts primarily describe the use of single herbs for the treatment of

common ailments. However, with the evolution of Ayurvedic pharmaceuticals, polyherbal formulations became increasingly prominent. The classical Ayurvedic scholar Sushruta introduced the concept of *Ganas*—specific groupings of medicinal herbs, each designed to address a range of indications based on their collective therapeutic properties (2). Ayurvedic pharmacology utilizes herbal remedies in multiple forms, including fresh juice (*svarasa*), herbal paste (*kalka*), herbal powder (*churna*), decoction (*kwatha*), distilled waters (*arka*), hot infusion (*phanta*), cold infusion (*hima*), herbal jams and jellies (*paka*, *leha*, *avaleha*), medicated wines (*arishta*, *asava*), tinctures, *guggul* (the resin extract of *Commiphora mukul*), alkaline extracts (*ksara*), medicated ghee (*siddha ghrita*), and medicated oils (*siddha taila*), along with minerals (*rasasastra*) (24). Among these forms, *kwatha* (decoction) is a significant preparation used in Ayurveda for various health conditions. It typically involves taking one part of coarsely ground herb (dry material) by weight and adding 16 parts of water by volume, which is then reduced to one-fourth of the original water volume. For internal medicinal use, *kwatha* is specifically directed to be concentrated to one-eighth of the original volume (25).

Human passion for herbal medicine dates back to times immemorial. As we all know, the survival of the animal kingdom is dependent on the plant kingdom. Plants and herbs have played a major role in pharmacotherapy during the Vedic period in India. Vedic literature indicates mainly about the utility of single herbs in the management of common diseases. Poly-herbal formulations have dominated the Ayurvedic pharmaceuticals subsequently. Susruta mentioned the *Ganas*, which are groups of herbs, each possessing multiple herbs with various indications (2).

Ayurveda, often referred to as the "Ancient Science of Life," is considered to have originated over 5000 years ago in India and remains one of the most prominent traditional systems of medicine globally. It is founded on the philosophical concept that all matter in the universe, including the human body, is composed of five fundamental elements (*pancha mahabhutas*). Within the human body, these elements manifest as three primary bio-energetic forces known as doshas—*vata*, *pitta*, and *kapha*. Although these doshas typically exist in a state of equilibrium, one dosha generally predominates in each individual, a constitution referred to as the person's *prakriti* (3).

Ayurvedic medicine predominantly utilizes natural herbs; however, raw herbs are seldom administered in their unprocessed form. Instead, they are transformed into specific dosage forms to enhance efficacy and bioavailability. Ancient Ayurvedic scholars developed techniques to optimize the extraction of water-soluble phytoconstituents by boiling herbs in water—a process that yields a potent herbal extract. This preparation is known as *kwatha* or *kashayam*, referring to a decoction derived from a single plant or a combination of multiple herbs.

Amruthotharam Kashayam is a classical Ayurvedic decoction formulated primarily to address imbalances associated with *kapha* dosha. It is traditionally indicated in the management of a wide range of conditions, including fever, early stages of rheumatoid arthritis, rheumatic fever, constipation, edema, jaundice, gouty arthritis, respiratory ailments (such as dyspnea

and nasal congestion), hepatic disorders, dermatological conditions, and certain viral infections. Its therapeutic applications are rooted in centuries of empirical use and are supported by traditional Ayurvedic texts. Ayurveda, the Ancient Science of Life, are believed to have been prevalent for 5000 years in India. It is one of the most renowned systems of medicine in the world. Ayurveda is based on the hypothesis that everything in the universe is composed of five basic elements. They exist in the human body in combined forms like vata, pitta and kapha. It is believed that they are in harmony with each other, but in every human being one of them is dominating which, in turn is called as prakruthi of that person (3). Ayurveda uses natural herbs as medicine, but the raw herbs cannot be consumed directly. It needs to be converted into a suitable dosage form. The ancient masters sought a method to extract the maximum amount of water-soluble herbal actives, so they boiled herbs with water. This process generated the highest possible extract. Such a dosage form is called Kwath or Kashayam, which refers to a water decoction or extract made from a single herb or a group of herbs.

Amruthotharam Kashayam is an Ayurvedic medicinal preparation in the form of decoction designed specifically for vitiating Kapha Doshas. Used in treating fever, early stages of rheumatoid arthritis, rheumatic fever, constipation, edema, jaundice, gouty arthritis, breathing difficulties, liver disorders, cold, running nose, skin diseases, viral infections, etc.

Ingredients

- 6 parts (Amruta / Guduchi) - *Tinospora cordifolia* (Willd.) Hook. f. and Thoms.)
- 4 parts (Kaduka/Haritaki) - *Terminalia chebula* Retz.
- 2 parts (Nagara/Ginger) - *Zingiber officinale* Zingiber officinale Rosc.)

Amruthotharam Kashayam is an Ayurvedic medicinal preparation in the form of a decoction, specifically designed for vitiating Kapha doshas. It acts on the digestive system, improving metabolism and digestion. The formulation contains Guduchi (*Tinospora cordifolia*), Haritaki (*Terminalia chebula*), and Shunthi (*Zingiber officinale*) in the ratio 6:4:2. Guduchi (*Tinospora cordifolia*) provides immunomodulatory action in the body, boosting the immune system. Haritaki (*Terminalia chebula*) has a mild laxative effect, promoting regular bowel movements and relieving constipation. Shunthi (*Zingiber officinale*) enhances the absorption and bioavailability of nutrients and other medicines, improving digestion and overall nutrient uptake. This formulation is commonly used in treating various conditions, including fever, early stages of rheumatoid arthritis, rheumatic fever, edema, jaundice, gouty arthritis, breathing difficulties, liver disorders, cold, running nose, skin diseases, and viral infections.

Amruthu (*Tinospora cordifolia* (Willd.) Hook. f. and Thoms.): It is one of the most extensively used and non-controversial herbs in Ayurvedic medicine. It consists of the dried, matured stems of *Tinospora cordifolia* (Willd.) Miers., a perennial climber found throughout tropical India. The drug is usually collected during the summer, preferably in the month of May, though it is also used in its fresh form (10).

Guduchi has been part of traditional medicine for centuries and is mentioned in Ayurvedic texts for its therapeutic properties. Its leaves are classified under the *Tikta-saka varga* (bitter group), which are said to be effective in treating conditions like *kushta* (skin diseases), *meha* (urinary disorders), *jwara* (fever), *svasa* (asthma), *kasa* (cough), and *aruchi* (loss of appetite). It has been indicated for several ailments, such as *kamala* (jaundice), *jwara* (fever), and *vatarakta* (gout) (16). In India, Guduchi is widely used as a tonic, alterative, antiperiodic, and diuretic. It is found throughout most parts of tropical India and has been valued for its wide range of therapeutic benefits. Major chemical constituents- Tinosporin, columbin, chasmanthin, palmarin, berberine, tinosporon, tinosporic acid, tinosporol, giloin, giloinisin, beta sitosterol etc. The bitter principles present in the drug show antiperiodic, antispasmodic, anti-inflammatory and antipyretic properties (9).

Kaduka (*Terminalia chebula* Retz.): The drug consists of the pericarp (outer covering) of the dried fruit of *Terminalia chebula* Retz. It contains at least 5% chebulagic acid and not less than 12.5% chebulinic acid, calculated on a dried weight basis. The fruit has a shiny external surface with longitudinal ridges, and its color ranges from yellowish-brown to light black. It has an astringent taste, with a slight bitterness. (11). Haritaki is one of the most important and commonly used herbs in folk, household, and traditional medicine. It is a key ingredient in Triphala (the three myrobalans), a renowned Ayurvedic formulation. Haritaki is primarily indicated for conditions like constipation, respiratory problems, and certain skin diseases. Bhavamisra describes 7 varieties of Haritaki Viz., -Vijaya, Rohini, Putana, Amrta, Abhaya, Jivanti, Cetaki (17). A number of glycosides have been isolated from *haritaki*. Other constituents include a coumarin conjugated with gallic acids called chebulin, as well as other phenolic compounds including ellagic acid, chebulinic acid, gallic acid, ethyl gallate, and tannic acid. Chebulic acid is a phenolic acid compound isolated from the ripe fruit.

Chukku (*Zingiber officinale* Rosc.)

Ginger (*Zingiber officinale*) has been known in China since as early as 400 BC and holds a prominent place in Ayurveda. It is one of the most reputed and extensively used drugs in Ayurvedic medicine, having been employed in indigenous systems of medicine for a long period. The great Ayurvedic scholars—Charaka, Sushruta, and Vagbhata—have all mentioned its therapeutic uses for various ailments such as Udarroga (abdominal disorders), Shotha (swelling), Karnasulah (ear pain), and in the treatment of Kapha and Vata disorders. It is Pungent, hot, cordial, aphrodisiac and easy to digest. It cures vomiting, ascites, dyspnoea, cough, anorexia, fever, swelling, colic, piles. It promotes digestive power, cleanses the throat and tongue and dispels cardiac disorders (4).

Ginger is the most frequent home remedy for coughs, colds, and indigestion. Its therapeutic properties have been well-documented (5). During pregnancy, it can help with nausea and vomiting (7). Its antioxidant effect is documented. By inhibiting calcium channels, it lowers blood pressure (8). It is

an erect, perennial, aromatic herb with a horizontal tuberous branched rootstock. The fresh form of ginger is called Ardraka, while the dried form is known as Sunthi. Green ginger contains several important vitamins, including: thiamine, riboflavin, niacin, and Vit. C. In addition to vitamins, ginger contains small amounts of sugars such as glucose, fructose, and sucrose, with the principal carbohydrate being starch.

2. Materials and Methods

2.1. Plant materials

Amruthotharam Kashayam was prepared from three ingredients, viz., Amruthu (*Tinospora cordifolia* (Willd.) Hook. f. and Thoms.), Kadukka (*Terminalia chebula* Retz.) and Chukku (*Zingiber officinale* Rosc.). All ingredients were collected from local market in Thrissur are washed with water, shade dried and powdered, were subjected to pharmacognostic analysis. The parameters included physicochemical analysis, Morphological study, microscopic evaluation, preliminary phytochemical analysis, Quantitative analysis, TLC analysis.

2.2. Macroscopical analysis

Morphological Study was carried out using simple hand lance. Color, shape and structure of the ingredient were studied.

2.3. Microscopical analysis

C.S of the materials was taken and stained using safranin, observed under Trinocular microscope. Analyzed for the cell arrangements and cell structure. This can be done as per the Ayurvedic pharmacopoeia of India (12).

2.4. Physico-chemical analysis

All physico-chemical parameters such as loss on drying, total ash, acid insoluble ash, water and alcohol soluble extractives were carried out as per the Ayurvedic pharmacopoeia of India.

2.5. Preliminary phytochemical screening

- **2.5.1. Tests for alkaloids**
- **2.5.1.1 Dragendorff's Test:** 2 ml of the extract was treated with a few drops of Dragendorff's reagent (solution of potassium bismuth iodide). Formation of orange brown precipitate indicates the presence of alkaloids.
- **2.5.1.2: Mayer's Test** 2 ml of the extract was treated with a few drops of the Mayer's reagent (Potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.
- **2.5.2 Test for flavonoids**
- **2.5.2.1: Shinoda Test** Crude extract was mixed with a few small pieces of Magnesium ribbon for a minute and drops of con. Hcl was added drop wise into this solution. Development of pink scarlet color or light red color after a few minutes indicates the presence of flavonoids.
- **2.5.2.2 Lead acetate Test:** Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow Colour or yellow creamy precipitate indicates the presence of flavonoids.
- **2.5.2.3 Alkaline reagent Test:** The extract was mixed with 2% NaOH solution. Intense yellow coloration which
- Loses the intensity on the addition of dilute acid indicates the presence of flavonoids.

The characteristic pleasant aromatic odor of ginger is attributed to an essential oil, which can be extracted from the rhizome through steam distillation. This oil is responsible for the spice's distinctive fragrance and therapeutic properties (18). Major chemical constituents- alpha curcumene, Beta-D curcumene, d-borneol, citral, d-camphene, citronellol, geraniol, gingerol, alpha & beta Zingiberenes, Zingiberol, zingerone, gingerols (19).

2.5.3 Test for tannins

2.5.3.1 Ferric chloride Test: 2 ml of freshly prepared ferric chloride solution was added to 2 ml of the concentrated extract. Formation of dark blue or green or black colour indicates the presence of tannins.

2.5.4 Test for Saponins

2.5.4.1 Froth test: 2 ml of the extract was mixed with 20 ml of distilled water in a graduated test tube and shaken well for 10 minutes. Formation of 1 cm thick froth indicates that the sample contains saponins.

2.5.5 Test for sterols

2.5.5.1 Liebermann-Burchard Test: 2 ml of the extract was mixed with a few drops of acetic unhydride. It was boiled and cooled and concentrated sulphuric acid was added along the side of the test tube carefully. A brown ring at the junction of two layers and upper layer turning green indicated the presence of sterols.

2.5.6 Test for carbohydrates

2.5.6.1 Molisch's test: 2 ml of the extract was taken in a test tube and a few ml of Molisch's reagent was added along the sides. The formation of violet ring at the junction indicates the presence of carbohydrates.

- **2.5.6.2 Fehling's test:** 1 ml each of Fehling's solution A and B were mixed and boiled for one minute. An equal volume of the extract was added and then boiled in a water bath for 5 minutes. Formation of reddish brown colour indicates the presence of reducing sugar.
- **2.5.6.3 Iodine test:** 1 or 2 drops iodine solution was added to 1ml of the extract. The formation of dark blue color indicates the presence of carbohydrates.

2.5.7 Test for proteins and Amino acids

- **2.5.7.1 Ninhydrin test:** 3 ml of the extract was boiled with 3 drops of 5% Ninhydrin solution. Formation of blue or violet coloration indicates the presence of amino acids.
- **2.5.7.2 Xanthoproteic test:** The extract was treated with a few drops of concentrated nitric acid. The formation of yellow color indicates the presence of proteins.

2.6 Quantitative analysis

2.6.1. Total Tannin

Defat 2 g of sample with 25 ml petroleum ether for 12 hours. Boil the marc for 2 hours with 300 ml distilled water. Cool, dilute up to 500 ml and filter.

Titration A

Measure 25 ml of this infusion into 2L porcelain dish; Add 20 ml Indigo solution and 750 ml distilled water. Titrate it with 0.1 N potassium permanganate solution, 1 ml at a time, until

solution changes to green. Thereafter add drop wise until solution becomes golden yellow color:

Titration B

- Titrate mixture of 20 ml indigo solution and 750 ml distilled water drop wise till golden yellow
- Calculate the difference between two titrations in ml.
- Each ml of 0.1 N potassium permanganate solutions is equal to 0.004157 g of total tannins (13).

Calculation

$$\text{Total tannins (\%)} = \frac{(A - B) \times N. \text{ of P. P} \times 0.004157}{\text{sample weight} \times N. \text{ of P. P} \times 25} \times 100 \times 500$$

N- Normality; P.P- Potassium permanganate

2.6.2. Total Polysaccharide

Weighed accurately about 1 g to 2 g of powder in a conical flask. 100ml distilled water was added and left overnight after shaking well for two hours. Next day filtered and concentrated up to 10 ml. Then 50 ml 90% methanol was added gently with constant stirring and left it for half an hour. Filtered the solution and dried the residue on filter paper at 80 degree Celsius till constant weight (14).

Calculation was carried out as given below

$$\text{Total polysaccharides (\%)} = \frac{\text{Wt. of the residue in g}}{\text{Wt. of the sample in g}} \times 100$$

2.6.3. Total Saponins

Take 5 g sample in 90% methanol (25 ml) by refluxing for half an hour and filter. Extract the residue two more times by taking 25 ml methanol. Combine the methanolic extract and distill of the solvent. Reflex the soft extract left after distillation of methanol, with petroleum ether (25ml) for half an hour. Cool and remove the solvent by decantation. Now treat the same soft extract successively with 25 ml chloroform and 25 ml ethyl acetate for 30 minutes and pour solvents after cooling, keeping the soft extract in the same flask. Dissolve the soft extract (after three extractions cited above) in 25 ml 90% methanol. Filter and concentrate to 5 ml. add the above extract drop by drop with constant stirring to 25 ml acetone taken in a pre-weighed beaker (W1), in order to precipitate the saponins. The precipitates are evaporated and dried to constant weight (W2).

Calculation

$$\text{Total Saponins (\%)} = \frac{\text{Wt. of the residue in g}}{\text{Wt. of the sample in g}} \times 100$$

2.7 TLC comparative study

Reflux 1g of coarsely powdered substance under examination with 25 ml Methanol for 15 min. cool and filter. Wash the residue with 10 ml of methanol. Combine all the filtrates and concentrate to 10 ml. To find out the probable number of compounds present in them suitable solvents are used for each

herb. For *Terminalia chebula* use a mixture of ethyl acetate, formic acid and toluene and methanol in the ratio 6:2:2:1. For *Zingiber officinale* use a mixture of hexane and diethyl ether in the ratio 30:70. For *Tinospora cordifolia* use a mixture of chloroform and methanol in the ratio 85:15 (15).

3. Result

3.1 Macroscopical analysis

Terminalia chebula Retz.

Intact fruit yellowish-brown, ovoid, 20-35 mm long, 13-25 mm wide, wrinkled and ribbed longitudinally, pericarp fibrous, 3-4 mm thick, non-adherent to the seed, taste, astringent.

Zingiber officinale Roxb.

Rhizome, laterally compressed bearing short, flattish, ovate, oblique, branches on upper side each having at its apex a depressed scar, pieces about 5-15 cm long, 1.5-6.5 cm wide (usually 3-4 cm) and 1-1.5 cm thick, externally buff coloured showing longitudinal striations and occasional loose fibres, fracture short, smooth, transverse surface exhibiting narrow cortex (about one-third of radius), a well-marked endodermis and a wide stele showing numerous scattered fibro-vascular bundles and yellow secreting cells, odour agreeable and aromatic, taste, agreeable and pungent.

Tinospora cordifolia (Willd.) Miers.

Drug occurs in pieces of varying thickness ranging from 0.6-5 cm in diameter, young stems green with smooth surfaces and swelling at nodes.

Older ones show a light brown surface marked with warty protuberances due to circular lenticels; transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues, taste bitter.

3.2 Microscopical analysis

Tinospora cordifolia (Willd.) Miers

The transverse section of the stem reveals a complex arrangement of tissues that contribute to its structure and function. The outermost layer consists of cork, which differentiates into two distinct zones: the outer zone, made up of thick-walled, brownish, and compressed cells, and the inner zone, characterized by thin-walled, colorless, tangentially arranged cells in 3-4 rows. The cork is broken at certain points due to the opening of lenticels. Beneath the cork lies a secondary cortex, composed of 5 or more rows of cells, with the outer rows being smaller than the inner ones. Within the openings of the lenticels, groups of sclereids (2-10 cells) are found in the secondary cortex region.

The outer zone of the cortex consists of 3-5 rows of irregularly arranged, tangentially elongated chlorenchymatous cells. Toward the inner side of the cortex, the cells are polygonal in shape and filled with abundant starch grains. Several secretory cells are scattered throughout the cortex.

The vascular zone comprises 10-12 or more wedge-shaped strips of xylem, which are externally surrounded by semi-circular strips of phloem, alternating with wide medullary rays. The phloem consists of sieve tubes, companion cells, and

phloem parenchyma, with polygonal or tangentially elongated cells. Some of these cells contain crystals of calcium oxalate.

The cambium layer is composed of one to two layers of tangentially elongated cells within each vascular bundle, responsible for the formation of new xylem and phloem. The secondary xylem elements are thick-walled, lignified, and include cylindrical vessels bearing bordered pits on their walls for efficient water transport.

The medullary rays, which extend radially from the center, are 15-20 or more cells wide. These rays contain cells with varying shapes, such as rounded, hemispherical, oblong, or ovoid, and some exhibit faintly marked concentric striations.

This arrangement of tissues allows the plant to efficiently transport water, nutrients, and food, while also providing structural support through sclereids and lignified xylem.

***Terminalia chebula* Retz.**

The transverse section of the pericarp reveals a detailed structure with distinct tissue layers. The epicarp consists of a single layer of epidermal cells, with the inner tangential and upper portions of the radial walls being thickened. Beneath the epicarp lies the **mesocarp**, which contains 2-3 layers of collenchyma, followed by a broad zone of parenchyma. Within this parenchyma, fibers and sclereids are arranged in groups, and vascular bundles are scattered throughout. The fibers exhibit peg-like outgrowths and have simple pitted walls. The sclereids, mostly elongated, vary in shape and size. Tannins and raphides are present in the parenchyma, contributing to the tissue's chemical composition. The endocarp is composed of thick-walled sclereids, which vary in shape and size but are predominantly elongated. Examination of the epidermal surface reveals polygonal cells, all uniformly thick-walled. Many of these cells are divided by a thin septum. Starch grains are abundant throughout the mesocarp, appearing as simple, rounded or oval shapes, and measure 2-7 μm in diameter.

This detailed arrangement of tissues within the pericarp contributes to the mechanical and protective functions of the fruit, while the starch grains stored in the mesocarp serve as an important reserve for the plant.

***Zingiber officinale* Roxb.**

The transverse section of the rhizome reveals a distinct tissue arrangement. The cortex is composed of isodiametric, thin-walled parenchyma cells with scattered vascular strands. Numerous idioblasts, measuring about 40-80 μm in diameter, contain a yellowish to reddish-brown oleo-resin. The endodermis is slightly thick-walled and free from starch. Immediately inside the endodermis, there is a row of nearly continuous collateral vascular bundles, typically lacking fibers.

The stele consists of thin-walled parenchyma cells arranged radially around several scattered collateral vascular bundles. Each bundle contains a few unligified, reticulate or spiral vessels, up to about 70 μm in diameter. The phloem of these bundles includes unligified, thin-walled, septate fibers that are up to 30 μm wide and 600 μm long, with small, oblique slit-like pits. Scattered idioblasts, similar to those in the cortex, are associated with the vascular bundles. These idioblasts, measuring 8-20 μm wide and up to 130 μm long, contain dark reddish-brown contents and are arranged in single or axial rows, adjacent to the vessels. Both the parenchyma of the cortex and stele are packed with flattened, rectangular, and ovate starch grains. These starch grains are mostly 5-15 μm to 30-60 μm long, about 25 μm wide and 7 μm thick, marked by five transverse striations. This well-organized tissue structure supports the plant's storage and transport functions while contributing to its mechanical strength.

3.3 Physico-chemical

Table 1: *Tinospora cordifolia*

Sl. No.	Test parameters	Limit	Result
1	Foreign matter	NMT 2%	1.1%
2	Total ash	NMT 16%	10%
3	Acid-insoluble ash	NMT 3%	1.6%
4	Alcohol-soluble extractive	NLT 3%	5%
5	Water-soluble extractive	NLT 11%	15%

Table 2: *Terminalia chebula*

Sl. No.	Test parameters	Limit	Result
1	Foreign matter	NMT 1%	0.7%
2	Total ash	NMT 5%	3.5%
3	Acid-insoluble ash	NMT 5%	4%
4	Alcohol-soluble extractive	NLT 40%	48%
5	Water-soluble extractive	NLT 60%	65%

Table 3: *Zingiber officinale*

Sl. No.	Test parameters	Limit	Result
1	Foreign matter	NMT 1%	0.6%
2	Total ash	NMT 6%	4%
3	Acid-insoluble ash	NMT 1.5%	1%
4	Alcohol-soluble extractive	NLT 3%	6%
5	Water-soluble extractive	NLT 10%	13%

3.4 Preliminary phytochemical analysis

Aqueous and aqueous: methanol extracts of three herbs were subjected to preliminary phytochemical analysis and the result of various phytochemical constituent depicted in Table I.

Fig 4: Preliminary phytochemical screening

Sl.No.	Class of compound	<i>Terminalia chebula</i>		<i>Zingiber officinalis</i>		<i>Tinospora cordifolia</i>	
		Water extract	Water:MeOH extract	Water:MeOH extract	Methanol extract	Water:MeOH extract	Methanol extract
1	Alkaloids	+	+	+	+	+	+
2	Flavonoids	-	+	+	+	-	-
3	Tannins	+++	+++	-	-	-	-
4	Saponin	+	+	-	-	-	-

5	Sterols	+	+	+	+	+	+
6	Proteins	-	+	-	-	-	-
7	Carbohydrates		-	+	-	+	-
8	Starch	+	-	+	+	-	-
9	Triterpenoids	-	-	-	-	+	+
10	Amino acid	-	-	-	-	-	-

MeOH-Methanol; (+) - Indicates the presence the respective phytochemical; (-) Indicates the absence of the respective phytochemical.

3.5 Quantitative analysis

Table 5: Quantitative Analysis of Phytochemical Content in *Tinospora cordifolia*, *Terminalia chebula*, and *Zingiber officinalis*

Sl. No.	Tests	<i>Tinospora cordifolia</i>	<i>Terminalia chebula</i>	<i>Zingiber officinalis</i>
1	Total Tannins (%)	0.415	11.639	Nil
2	Total Saponins (%)	2.91	52.82	50.29
3	Total Polysachrides (%)	3.05	Nil	3.45
4	Total Bitters (%)	2.6	3.02	Nil

3.6 TLC Studies using silica gel plate and Rf value calculated (Table II & Pict. II)

Table-III

Sl. No.	Identification/ Comparative TLC Study	Visualization	<i>Terminalia chebula</i> (Rf value)	<i>Zingiber officinale</i> (Rf value)	<i>Tinospora cordifolia</i> (Rf value)
1.	Tannins	FeCl ₃ reagent	0.05,0.16,0.26,0.37,0.67,0.81	0.1,0.37	Nil
		Long wave	0.6,0.11,0.81	0.37,0.88	0.48
		Short wave	0.93	Nil	0.91
2.	Phenolic compounds	Folin ciocalteau reagent	0.15	0.42,0.52,0.8	Nil
		Long wave	0.21,0.28,0.33,0.56	0.23,0.28,0.38,0.48,0.53,0.8,0.83	0.88,0.93
		Short wave	Nil	0.8,0.91	0.82,0.87,0.93
3.	Glycosides	Long wave	Nil	Nil	Nil
		Short wave	0.08,0.31,0.42,0.81	0.16,0.35,0.48,0.65,0.76,0.87	Nil
4.	Flavonoids	Long wave	0.037,0.07,0.137	0.26,0.28,0.35,0.41,0.48,0.75,0.78,0.85	Nil
		Short wave	Nil	0.2,0.37,0.75,0.85	0.81,0.87,0.92
5.	Alkaloids	Long wave	0.56	0.11,0.23,0.17,0.43,0.46,0.62,0.65,0.97	0.160.21
		Short wave	Nil	0.11,0.93	0.91,0.97

Macroscopical analysis

Picture I



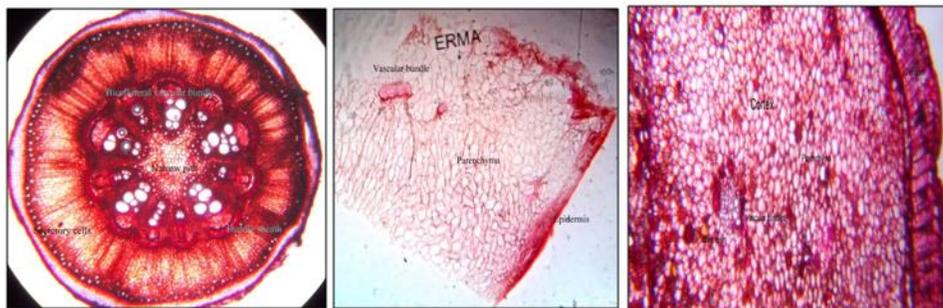
Tinospora cordifolia (Willd.)



Tinospora cordifolia (Willd.)

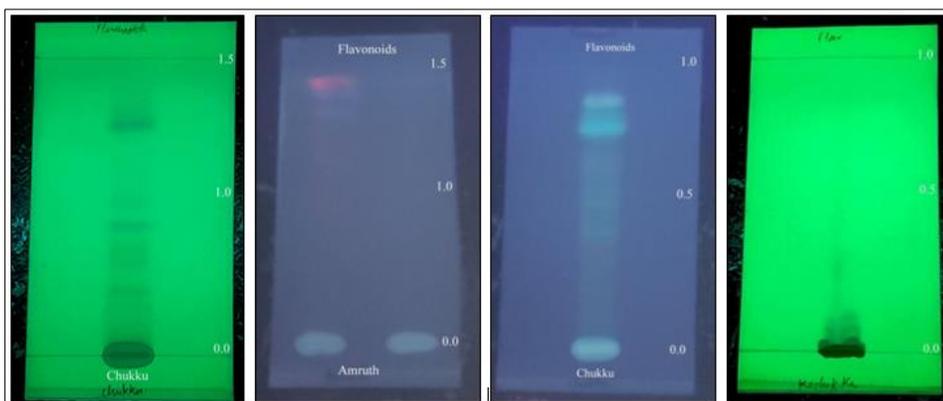


Tinospora cordifolia (Willd.) Miers.



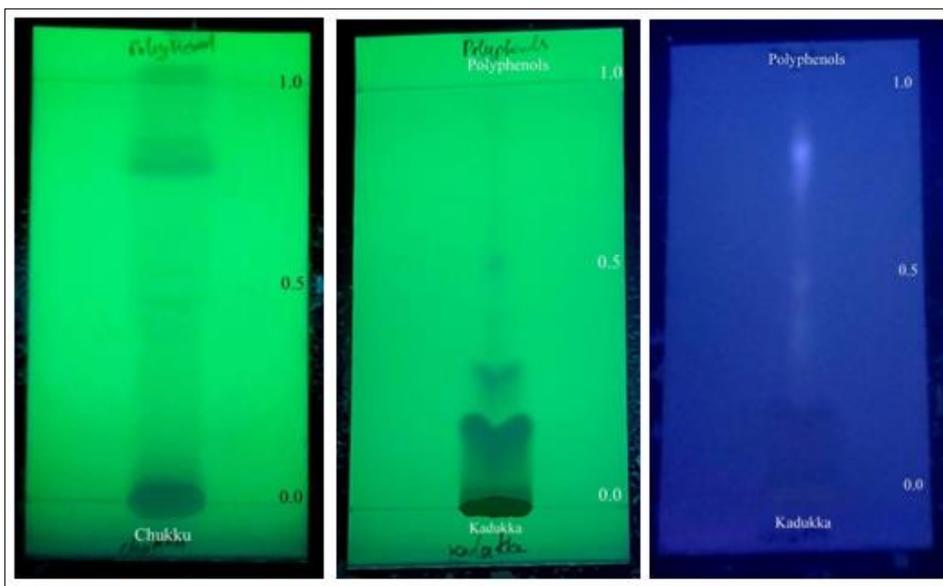
(1) *Tinospora cordifolia*, Stem C.S, (2) *Terminalia chebula*, Fruit rind C.S (3) *Zingiber officinale*, Rhizome C.S

Picture- III: Flavonoids



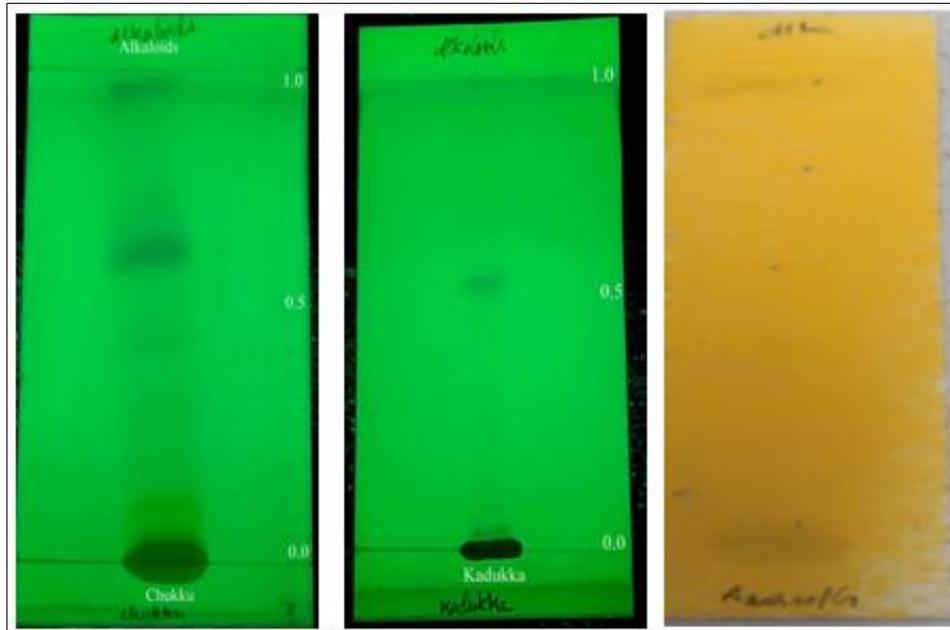
(1) *Tinospora cordifolia*- SW (2) *Terminalia chebula*-L.W (3) *Zingiber officinale*-S.W (4) *Zingiber officinale*-L.W

Phenolic compounds



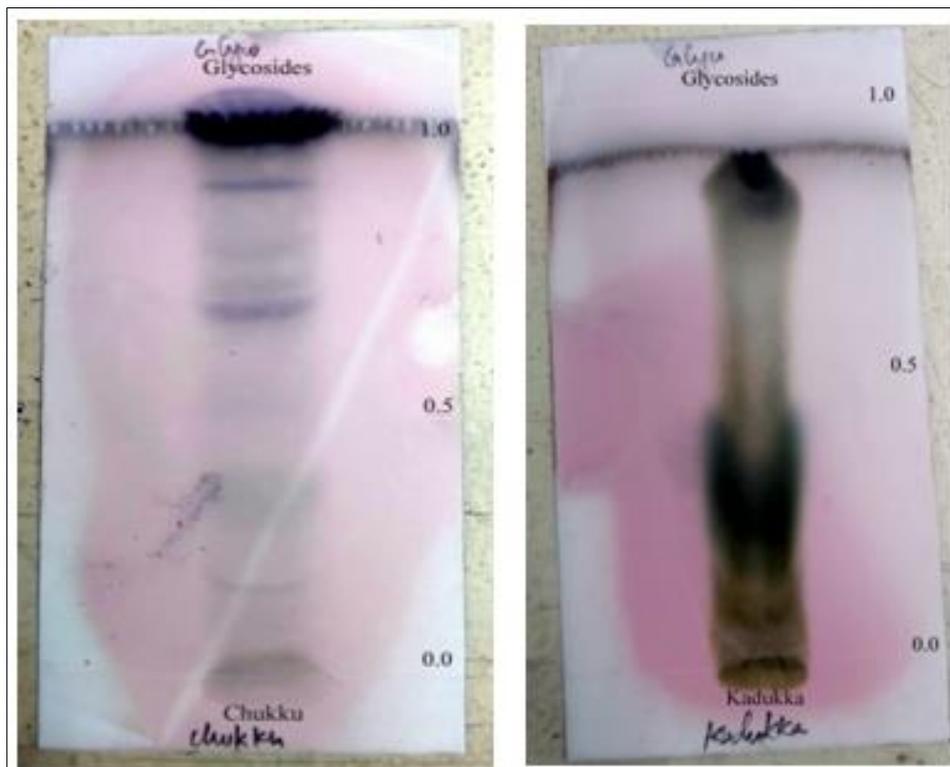
(1) *Zingiber officinale*-L.W (2) *Terminalia chebula*-L.W (3) *Terminalia chebula*-S.W

Alkaloids



(1) *Zingiber officinale*-L.W, (2) *Terminalia chebula*-L.W (3) *Tinospora cordifolia*- After Derivatization

Glycolic acid



(1) *Zingiber officinale*- After Derivatization (2) *Terminalia chebula*- After Derivatization

Tannins



(1) *Terminalia chebula*- After Derivatization (2) *Tinospora cordifolia*- S.W (3) *Zingiber officinale*- L. W

4. Discussion and Conclusion

The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in the present era. All the Raw materials used here were standardized using different methods. Authenticity of raw material has to be established for the efficacy of the product. Macroscopic and Microscopic analysis shows the authenticity of the ingredients. Physico-chemical parameters were within the limits which prove that there are no other impurities and inorganic matters were present. Qualitative and Quantitative study reveals the authenticity of the raw materials as per the TLC profile and Qualitative and Quantitative studies.

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