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Research Article

Source-Dependent Variations in the Quality Attributes of Amurthashtaka Kwatha: A Comprehensive Pharmacognostic Evaluation

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Abstract

Amurthashtaka Kwatha is a classic Ayurvedic polyherbal remedy used to treat inflammatory diseases and fever. Comprehensive standardization is crucial as raw materials sources may affect formulation quality and consistency. The study evaluated the effect of raw material sourcing on the pharmacognostic characteristics of *Amurthashtaka Kwatha*. Five distinct sources, including natural habitats and Ayurvedic retail outlets, provided the constituent plant materials. Traditional Ayurvedic methods were used to prepare the formulations. Pharmacognostic evaluation comprised organoleptic, macroscopic, microscopic, powder microscopic, physicochemical, phytochemical, and chromatographic analyses. Physicochemical parameters included moisture content, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive value, and ethanol-soluble extractive value. Qualitative phytochemical screening was performed to identify major classes of secondary metabolites, while High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting was used to evaluate chromatographic profiles. Pharmacognostic testings confirmed the authenticity of all constituent plants and their diagnostic characteristics in the final formulation. Moisture percentages of the formulations varied from 11.25% to 13.07%, according to the physicochemical examination. Variability in phytochemical composition and inorganic content was shown by variations in ash and extractive values. Alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, phytosterols, cardiac glycosides, carbohydrates, and proteins were found in all formulations, according to qualitative phytochemical screening. While variations in band intensity, peak area, and chromatographic complexity were noted, HPTLC analysis showed common marker bands among batches. *Amurthashtaka Kwatha* prepared using raw materials from different sources retained comparable pharmacognostic characteristics and qualitative phytochemical composition despite variations in physicochemical properties and chromatographic fingerprints. The findings provide baseline quality parameters and emphasize the importance of raw material sourcing in Ayurvedic standardization.

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Introduction

Amurthashtaka Kwatha is one of the oldest and most widely used herbal decoctions in Ayurvedic medicine, renowned for its efficacy in treating fever associated with inflammation (1). This traditional remedy comprises eight medicinal plants, each offering unique therapeutic properties. The formulation includes bark of *Arista* (*Azadirachta indica* A. Juss.), rhizome of *Musta* (*Cyperus rotundus* L.), seeds of *Indrayava* (*Holarrhena pubescens* Wall. ex G. Don), rhizome of *Katukarohini* (*Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong), heartwood of *Chandana* (*Santalum album* L.), stem of *Amrita* (*Tinospora cordifolia* (Wild.) Hook.f. & Thomson), whole plant of *Patola* (*Trichosanthes cucumerina* L.), and rhizome of *Nagara* (*Zingiber officinale* Roscoe). Collectively, these botanical ingredients

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contribute to the therapeutic activity of the formulation in the management of inflammatory febrile conditions (1).

The increasing interest in natural-origin drugs, often referred to as "green medicines," stems from their perceived safety, affordability, and minimal side effects compared to synthetic drugs (2). However, challenges such as adulteration, substitution, and incorrect identification of plant materials pose significant threats to the quality and efficacy of herbal formulations. Adulteration, involving the replacement of original plant materials with inferior or foreign substances, compromises the therapeutic potential of these remedies (2).

Pharmacognosy plays a crucial role in the authentication, standardization, and quality evaluation of medicinal plant materials. Through the application of organoleptic, macroscopic, microscopic, physicochemical, phytochemical, and chromatographic techniques, pharmacognostic studies provide essential tools for the characterization and quality evaluation of medicinal plant materials and herbal formulations (3). These evaluations are essential for ensuring the reproducibility, safety, and therapeutic efficacy of herbal products. Microscopic characterization is particularly valuable for identifying crude drugs, even in powdered form, and is widely recommended by regulatory authorities for herbal drug evaluation (2).

Despite the widespread use of *Amurthashtaka Kwatha* in Ayurvedic practice, comprehensive quality evaluation and standardization data for the formulation remain limited. Although pharmacognostic and phytochemical characteristics of several individual constituent plants have been reported, comparative studies examining source-dependent variations in raw materials and their impact on the quality attributes of *Amurthashtaka Kwatha* are lacking. Furthermore, standardized pharmacognostic specifications for *Amurthashtaka Kwatha* have not been adequately established. This lack of comprehensive quality control data may affect the consistency, authenticity, and reproducibility of the formulation, highlighting the need for detailed standardization studies.

Therefore, the present study was undertaken to evaluate the organoleptic, macroscopic, microscopic, and powder microscopic characteristics of the eight constituent medicinal plants of *Amurthashtaka Kwatha* collected from five different sources. In addition, the physicochemical properties, phytochemical composition, and High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profiles of *Amurthashtaka Kwatha* prepared from these plant materials were evaluated. These analyses aimed to establish quality control parameters and pharmacognostic specifications for the standardization of the formulation.

Materials and Methods

Chemicals

Ethyl acetate (HPLC grade; Sigma-Aldrich, USA) and *n*-hexane (HPLC grade; Sigma-Aldrich, USA) were used for HPTLC analysis. All other chemicals and reagents used in the study were of analytical grade. These included safranin, absolute ethanol, dichloromethane, xylene, light green SF, clove oil, Canada balsam, glycerin, chloral hydrate, hydrochloric acid, sulfuric acid, glacial acetic acid, acetic anhydride, chloroform, aqueous ammonia, sodium hydroxide, sodium carbonate, ferric chloride, aluminium chloride, lead acetate, copper acetate, ninhydrin reagent, Folin–Ciocalteu reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, Fehling's solutions A and B, picric acid, tannic acid, vanillin, magnesium turnings, ammonium hydroxide, and olive oil.

Instrumentation

A microscope (Zeiss Axio, Carl Zeiss AG, Germany) was used to obtain photomicrographs. Blades, glass slides, and cover slips were used for specimen preparation. The instruments used for HPTLC analysis included a water bath (Modern Lab, India), rotary evaporator (Buchi, Switzerland), and an HPTLC system comprising a Linomat 5 sample applicator, twin-trough development chamber, UV inspection cabinet, and TLC Scanner 3 equipped with winCATS software (version 1.4.2) (CAMAG, Switzerland). Pre-coated silica gel 60 F254 TLC plates (20 × 10 cm, 0.2 mm thickness; Merck, Germany) were used as the stationary phase. General laboratory equipment included a hot air oven, orbital shaker, analytical balance, muffle furnace, mechanical grinder, sieves, desiccator, crucibles, watch glasses, ashless filter papers, Whatman filter papers, conical flasks, beakers, test tubes, droppers, and a fume hood.

Collection of plant materials

Plant samples were collected from their natural habitats and Ayurvedic retail outlets. Plants/plant parts which are unavailable in Sri Lanka as fresh plant or restricted in collection as fresh plant without permission (*N. scrophularriflora, H. pubescens* and *S. album*) were collected from the five different Ayurvedic shops in western province, Sri Lanka. The Plants/plant parts which are available in Sri Lanka (*T. cordifolia, Z. officinale, A. indica, T. curcumerina* and C. *rotundus*) were collected from three different Ayurvedic shops in Western province, Sri Lanka and two different natural habitats, Colombo and Matara Districts, Sri Lanka.

Identification and authentication of plant

Dried raw plant/plant parts collected from Ayurvedic shops were authenticated from Bandaranayke Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka. Fresh plants collected from natural habitats were authenticated from the National Herbarium, Royal Botanic Gardens, Peradeniya as herbarium specimens

Preparation of plant powder

The eight different plant materials collected from five different locations were washed thoroughly in running water, air dried and cut into small pieces. The dried plants were further dried in 40 ^{0}C in an oven until a constant weight was obtained and then grounded to course powder and stored in cleaned, labelled and air tighten bags.

Preparation of proportionate blend for *Amurthashtaka Kwatha*

Equal quantities of the eight constituent plant materials were accurately weighed and thoroughly mixed to obtain a homogeneous proportionate blend. The prepared blend was subsequently used for the preparation of *Amurthashtaka Kwatha* according to the traditional formulation procedure.

Preparation of *Amurthashtaka Kwatha*

Amurthashtaka Kwatha was prepared according to the traditional decoction method. Equal quantities (7.5 g each) of the eight plant materials (total 60 g) were mixed with 1.92 L of water and the mixture was boiled and reduced to one-eighth of the initial volume (approximately 240 mL), then filtered to obtain the decoction (1).

Organoleptic evaluation

Coarsely powdered plant samples and proportionate blend for *Amurthashtaka Kwatha* were subjected to organoleptic evaluation, assessing colour, odour, taste, texture, etc. The evaluation was conducted based on the sensory impressions observed by the human senses, following the guidelines set by the World Health Organization (WHO) (4).

Macroscopic study

The macroscopy of the authenticated plants was studied according to shape, size, colour, surface characteristics, texture and appearance of the cut surface through naked eye or magnifying lens according to WHO guidelines (4).

Microscopic analysis

The transverse sections (TS) of the herbs were studied to identify the arrangement of general and specialized tissues specific to each herb. TS of all plants were stained using Safranin and Light green SF, by counter stain permanent slide method. The free hand TS were first stained in concentrated safranin solution for 5 minutes, then sequentially dehydrated in 30% ethanol for 5 minutes, 50% ethanol for 2 minutes, 70% ethanol for 1 minute, 90% ethanol for 1 minute, and absolute ethanol for 1 minute. The dehydrated sections were then stained with counter stain light green solution for 3 minutes, followed by washing with absolute ethanol twice to remove excess colour. The sections were dipped in clove oil for 2 minutes to clear the sections. Once ready in clove oil, the slides were properly labelled and cleaned. The sections were then dipped in xylene to harden and neutral Canada balsam was used as the mounting medium. Each plant section was mounted in one drop of Canada balsam on a glass slide and covered with a cover slip. The prepared slides were examined using dissecting and compound light microscopes (Zeiss Axio microscope with a camera) and relevant photographs were taken (5, 6).

Powder microscopy

A rounded and moistened head pin was used to get equal volume of fine powder on a glass slide. The adhered powders in to moistened pin was mounted in 1 drop of safranin solution on a glass slide. To avoid air bubbles while placing the cover glass, the slide was held in the left hand and the cover glass with the right hand. The slide was tilted slightly to the left, while placing the edge of the cover glass, to form a line of the liquid along the edge and slowly dropped the cover glass. The excess liquid from the slide was removed with blotting paper without applying pressure. Glass slides after staining with safranin were observed carefully under the microscope. At least 3-4 slides were prepared for powder study (7).

Physico-chemical analysis

Physico-chemical parameters of the proportionate blend used for the preparation of *Amurthashtaka Kwatha* were evaluated using standard analytical methods. These included moisture content, ash values (total ash, acid-insoluble ash, and water-soluble ash), and extractive values in different solvents (water, ethanol, and dichloromethane).

Moisture content

Moisture content (loss on drying) of proportionate blend for *Amurthashtaka Kwatha* was determined using a standard gravimetric method. Approximately 2 g of the air-dried powdered sample was accurately weighed into a previously dried and tared weighing dish. The sample was dried in an oven at 105 °C. After drying, the sample was cooled in a desiccator and weighed. Drying and weighing were continued until a constant weight was obtained, indicated by a difference of not more than 5 mg between two consecutive weighings. The loss in weight was calculated and expressed as a percentage (% w/w) of the air-dried material (4,8). The moisture content was determined and calculated using the formula given below.

$$\text{Moisture } \% = \frac{W1 - W2}{W} \times 100$$

Where:
W_1 = Initial weight of sample + dish (g)
W_2 = Final weight of sample + dish after drying (g)
W = Weight of sample (g)

Total Ash

Total ash was determined by accurately weighing about 2 g of the air-dried powder sample into a previously ignited and tared crucible. The sample was evenly spread and incinerated by gradually increasing the temperature to 550 °C until a white ash was obtained, indicating the absence of carbon. The crucible was then cooled in a desiccator and weighed. If carbon-free ash was not obtained, the crucible was allowed to cool and the residue was moistened with approximately 2 mL of water or a saturated solution of ammonium nitrate. The sample was then dried on a water bath, followed by heating on a hot plate, and re-ignited to constant weight. The final residue was cooled in a desiccator for 30 minutes and weighed without delay (4, 8). The total ash content was determined and calculated using the formula given below.

$$\text{Total Ash } \% = \frac{W2 - W1}{W} \times 100$$

Where:
W_1 = Weight of empty crucible (g)
W_2 = Weight of crucible + ash (g)
W = Weight of sample (g)

Water-soluble ash

The total ash obtained above was boiled with 25.0 mL of distilled water, and the crucible was covered with a watch glass and gently boiled for 5 minutes. The watch glass was rinsed with 5 mL of distilled water, and the rinsing was added back to the crucible. The insoluble matter was collected on ashless filter paper and thoroughly washed with hot water. The water-insoluble residue retained on the filter paper was transferred to the original crucible and ignited to constant weight. The residue was then cooled in a desiccator for 30 minutes and weighed without delay (8, 9). The water-soluble ash content was calculated using the formula given below.

$$\text{Water} - \text{soluble ash } \% = \frac{W1 - W2}{W} \times 100$$

Where:
W_1 = Weight of total ash (g)
W_2 = Weight of water-insoluble ash (g)
W = Weight of sample (g)

Acid-insoluble ash

The acid-insoluble ash was determined by adding 25.0 mL of dilute hydrochloric acid (70 g/L) to the crucible containing the total ash. The crucible was covered with a watch glass and gently boiled for 5 minutes. The watch glass was then rinsed with 5 mL of hot water, and the rinsing was added to the crucible. The insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the acid-insoluble residue was transferred to the original crucible, dried on a hot plate, and ignited to constant weight. The residue was cooled in a desiccator for 30 minutes and weighed without delay (4, 8). The acid-insoluble ash content was calculated as percentage (% w/w) using the following formula:

$$\text{Acid} - \text{insoluble ash } \% = \frac{W2 - W1}{W} \times 100$$

Where:
W_1 = Weight of empty crucible (g)
W_2 = Weight of crucible + acid-insoluble ash (g)
W = Weight of sample (g)

Extractive values

Extractive value was determined by accurately weighing about 4.0 g of powdered air-dried sample into a glass-stoppered conical flask. The sample was macerated with 100 mL of different solvents such as water, ethanol and dichloromethane for 6 hours with frequent shaking and then allowed to stand for 18 hours. The mixture was rapidly filtered to avoid loss of solvent. An aliquot of 25 mL of the filtrate was transferred to a previously tared flat-bottomed dish and evaporated to dryness on a water bath. The residue was dried in an oven at 105 °C for 6 hours, cooled in a desiccator for 30 minutes, and weighed without delay (4, 8). The extractive value was calculated as percentage (% w/w) using the following formula:

$$\text{Extractive value } (\%) = \frac{(W2 - W1) \times V}{S \times V_a} \times 100$$

Where:
W1 = weight of evaporating dish (g)
W2 = weight of (extract +evaporating dish) (g)
S = Weight of air-dried sample (g)
V = Total volume of extract (mL)
V_a = Volume of aliquot taken (mL)

Qualitative screening of phytochemicals

Preliminary qualitative phytochemical screening was carried out to identify the major classes of bioactive secondary metabolites present in the *Amurthashtaka Kwatha*. The samples were screened for alkaloids, flavonoids, phenolic compounds, saponins, tannins, carbohydrates, proteins, cardiac glycosides, terpenoids, sterols and phytosterols using standard phytochemical tests.

Alkaloids

Alkaloids were qualitatively assessed using standard phytochemical tests, including Mayer's reagent test, picric acid test, tannic acid test and Wagner's reagent test.

(a) Mayer's Test: The formulation was acidified with 1% hydrochloric acid, filtered, and treated with Mayer's reagent. The formation of a cream or brownish-red/orange precipitate indicated the presence of alkaloids (9, 10).

(b) Picric acid Test: The formulation was treated with picric acid solution. The formation of a yellow crystalline precipitate indicated the presence of alkaloids (11).

(c) Tannic Acid Test: A few drops of tannic acid solution were added to 5 mL of the formulation and mixed thoroughly. The formation of a yellow crystalline precipitate indicates the presence of alkaloids (11).

(d) Wagner's test: Approximately 5 mL of the formulation was treated with a few drops of 1% hydrochloric acid and then filtered. A few drops of Wagner's reagent were added and mixed gently. The appearance of a reddish-brown or brown precipitate confirms the presence of alkaloids (12).

Flavonoids

(a) Ammonium test: A 3 mL of formulation was treated with 5 mL of 10% aqueous ammonia solution followed by sulfuric acid. The appearance of a yellow color that disappeared upon acid addition indicated the presence of flavonoids (13).

(b) Shinoda Test: A 3 mL of formulation was treated with magnesium turnings and 5 drops of concentrated hydrochloric acid. The development of a pink, red, or orange color indicated the presence of flavonoids (13).

(c) Alkaline Reagent Test: A 3 mL of formulation was treated with 10 drops of 20% sodium hydroxide solution followed by 10 drops of 20% hydrochloric acid. The appearance of a yellow color that disappeared upon acidification indicated the presence of flavonoids (13, 14).

(d) Aluminium Chloride Test: A 3 mL of formulation was treated with few drops of aluminium chloride solution. The formation of a yellow color indicated the presence of flavonoids (13).

(e) Sulfuric Acid Test: A 3 mL aliquot of the formulation was treated with a few drops of concentrated sulfuric acid added along the sides of the test tube. The development of an orange, red, or reddish-brown color indicated the presence of flavonoids (15).

Phenolics

(a) Folin–Ciocalteu test: A 1 mL aliquot of the formulation was treated with 10% diluted Folin–Ciocalteu reagent and 7.5% sodium carbonate and stand for 5-10 minutes. The development of a blue or bluish-green color indicated the presence of phenolic compounds (13).

(b) Ferric Chloride Test: A 2 mL aliquot of formulation was treated with 2 mL of 5% ferric chloride solution and mixed well. The formation of a blue, green, violet, or black color indicated the presence of phenolic compounds (13).

(c) Vanillin Test: A 2 mL aliquot of the formulation was taken and a few drops of 10% vanillin solution in ethyl alcohol were added, followed by the addition of concentrated hydrochloric acid. The appearance of a red color indicated the presence of phenolic compounds (11).

(d) Lead Acetate test: A 5 mL aliquot of the formulation was taken and few drops of lead acetate solution were added and mixed well. The formation of a yellow precipitate indicated the presence of phenolic compounds (11).

Saponins

(a) Froth test: A small amount of the formulation (0.5mL) was taken and diluted with 5mL distilled water. The mixture was shaken vigorously for a few minutes and then allowed to stand. The formation of a stable froth persisting for at least 10 minutes indicated the presence of saponins, while absence of persistent froth indicated a negative result (11).

(b) Emulsion test: A 5 mL aliquot of the formulation was taken and 2.5 mL of water was added. The mixture was shaken vigorously and allowed to stand for 10 minutes. The froth formed was then mixed with 3 drops of olive oil and shaken again vigorously. The formation of a stable emulsion and persistent froth indicated the presence of saponins in the extract, while absence of stable froth indicated a negative result (11).

Tannins

(a) Ferric chloride test: A 2 mL aliquot of formulation was boiled with 10 mL of distilled water and then filtered. To the filtrate, a few drops of 0.1% ferric chloride solution were added and mixed well. The formation of a blue-black coloration indicated the presence of tannins (9).

(b) Lead acetate test: A 5 mL aliquot of the formulation was taken and 3 drops of 10% lead acetate solution were added. The mixture was shaken thoroughly, and the formation of a yellow precipitate indicated the presence of tannins (11).

(c) Modified Lead acetate test: A 2 mL aliquot of the formulation was taken and diluted with 2 mL of distilled water. A small quantity (about 0.01 g) of lead acetate was then added, and the mixture was shaken well. The formation of a white or yellowish precipitate indicated the presence of tannins (16).

Carbohydrates

(a) Fehling's test: 1 mL of Fehling's A solution and 1 mL of Fehling's B solution were mixed and boiled for 1 minute. Then, 2 mL of the formulation was added to the mixture and heating was continued in a water bath for about 5 minutes. The formation of a brick-red precipitate indicated the presence of reducing sugars (13).

(b) Benedict's test: 2 mL of Benedict's reagent was mixed with 2 mL of the formulation and heated in a boiling water bath for about 5 minutes. The formation of a green, yellow, orange, or brick-red precipitate indicated the presence of reducing sugars depending on their concentration (13).

Proteins

(a) Ninhydrin test: A 3 mL aliquot of the formulation was taken and a few drops of 0.25% ninhydrin reagent were added. The mixture was then heated in a boiling water bath for about 10 minutes. The development of a purple or blue color indicated the presence of free amino acids or proteins (17).

(b) Xantoprotein test: A 3 mL aliquot of the formulation was taken and 1 mL of concentrated sulphuric acid was added. A white precipitate was initially formed, which turned yellow upon boiling. After cooling, 1 mL of ammonium hydroxide was added, resulting in the development of an orange color, indicating the presence of aromatic amino acids or proteins (17).

Cardiac Glycosides

(a) Keller–Killiani test: A 5 mL aliquot of the formulation was taken and 2 mL of glacial acetic acid was added, followed by the addition of 1 drop of 5% ferric chloride solution. Then, 1 mL of concentrated sulphuric acid was carefully added along the side of the test tube to form a separate layer. The formation of a blue or bluish-green color in the acetic acid layer indicated the presence of cardiac glycosides (17).

Terpenoids

(a) Salkowski test: A 5 mL aliquot of the formulation was mixed with 2 mL of chloroform. Then, 3 mL of concentrated sulphuric acid was carefully added along the side of the test tube to form a separate layer. The formation of a reddish-brown coloration at the interface indicated the presence of terpenoids (17).

(b) Monoterpenes (Vanillin–sulphuric acid test): A 2 mL aliquot of the formulation was taken and a few drops of 10% vanillin solution in ethanol were added, followed by the addition of a few drops of concentrated sulphuric acid. The mixture was shaken well, and the development of a red color indicated the presence of monoterpenes (18).

(c) Diterpenes (Copper Acetate Test): The formulation was first dissolved in water, after 3–4 drops of copper acetate solution were added. The mixture was then observed for any color change. The formation of an emerald green color indicated a positive result, confirming the presence of diterpenes in the sample (19).

(d) Sesquiterpenes: A 2 mL aliquot of the formulation was taken and 1 mL of concentrated sulphuric acid was added and mixed well. The development of a reddish-brown color indicated the presence of sesquiterpenes (11).

Steroids

(a) Lieberman Burchard test: Two hundred milligrams of *Amurthashtaka Kwatha* powder was extracted with 10 mL of chloroform and then filtered to obtain the filtrate. Two milliliters of the filtrate were taken, to which 2 mL of acetic anhydride and 2 mL of concentrated H_2SO_4 were added and mixed well. The formation of a dark bluish-green color indicates the presence of steroids (11).

(b) About 0.5 g of *Amurthashtaka Kwatha* powder was extracted with ethanol. Then, 2 mL of the extract were taken, and 2 mL of acetic anhydride followed by 2 mL of concentrated H_2SO_4 were added and mixed well. The appearance of a color change from violet to blue or green indicates the presence of steroids (11).

Phytosterols

(a) Salkowski's test: the aqueous extract was first mixed with chloroform were carefully added to the filtrate, after which the mixture was shaken and allowed to stand. The development of a golden yellow coloration confirmed the presence of phytosterols (19).

High Performance Thin Layer Chromatography

Five batches of *Amurthashtaka Kwatha* were prepared and concentrated for analysis. Samples were applied as bands on silica gel 60 F_{254} TLC plates using a CAMAG Linomat 5 applicator and developed in a CAMAG twin-trough chamber using *n*-hexane: ethyl acetate (5:5, v/v) as the mobile phase. Chromatograms were visualized under UV light at 254 and 366 nm and scanned using a CAMAG TLC Scanner 3 with winCATS software. Constituents were characterized and compared based on Rf values, peak areas, and relative peak area percentages to evaluate fingerprint consistency among batches.

Statistical Treatment

Quantitative data obtained from the physicochemical analyses were expressed as mean ± standard error of Mean (SEM). Statistical analysis was performed using IBM SPSS Statistics software (Version 25, IBM Corp., Armonk, NY, USA). Differences among *Amurthashtaka Kwatha* powders prepared using raw materials procured from different sources were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

Results

Macroscopic characteristics of raw plant materials of *Amurthashtaka Kwatha*

The macroscopic characteristics of the official parts of eight raw plant materials used in *Amurthashtaka Kwatha* were meticulously examined and documented. The macroscopic features of official parts are shown in Figure 1 below.

Azadirachta indica: *Azadirachta indica* A. Juss., commonly known as "Neem," is a highly valued medicinal plant belonging to the family Meliaceae. It is known as "*Kohomba*" in Sinhala, "*Nimbaka*" in Sanskrit, and "*Veppai*" in Tamil (20). The bark of *A. indica* is channelled, tough, fibrous, brownish grey with a rough scaly surface. The outer bark exhibited prominent longitudinal and

transverse wrinkles. Internally bark is yellowish, laminated and coarsely fibrous.

***Cyperus rotundus*:** *Cyperus rotundus* L., commonly known as nut-grass, is a grass-like weed that belongs to the family Cyperaceae. This plant features dark green, glabrous culms rising from underground tubers. It is known as "*Kalanduru*" in Sinhala, "*Musta*" in Sanskrit, and "*Muttakkacu*" in Tamil (21). The rhizomes are elongate and slender, a hallmark of this species. They bear hard, ovoid, tunicate structures that are black and fragrant, measuring between 0.8 to 2.5 cm in diameter. The inner surface of these rhizomes is white, and they exhibit a mealy texture upon fracture, indicating their fibrous nature and potential for ease of processing. The rhizomes also feature wiry root fibres, covered with flexuous root hairs, which aid in nutrient absorption and anchoring the plant in its environment.

***Holarrhena pubescens*:** *Holarrhena pubescens* Wall. ex G. Don, commonly known as "Indrajav" or "Coneru" in English, "*Vatsaka*" in Sanskrit, "*Kelinda Haal*" in Sinhala, and "*Veppalai*" in Tamil, is a shrub belonging to the family Apocynaceae (22). These seeds are numerous and exhibit a distinct linear-oblong shape. They are characterized by their tips, which are adorned with a spreading, deciduous coma, and crowned with a tuft of long, silky hairs, facilitating their dispersion.

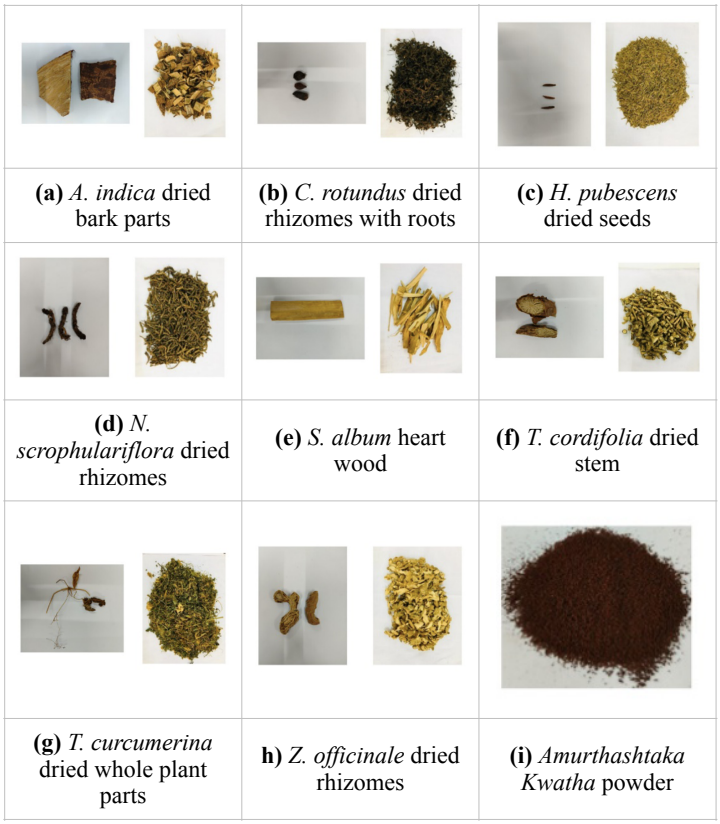
***Neopicrorhiza scrophulariiflora*:** *Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong, belongs to the family Scrophulariaceae. Commonly referred to as "Karu" or "Picroliv" in English, it is known as "*Katukarosana*" in Sinhala, "*Katuka*" in Sanskrit, and "Katukarojini" in Tamil (23). This perennial herb features greyish-brown cylindrical rhizomes that are distinctly bitter. These rhizomes are approximately as thick as a fifth finger, ranging from 2.5 to 5 cm in length. They are woody in texture and covered with withered leaf bases, rooting at the jointed nodes, which is characteristic of the species. The withered leaf bases remain attached, and the jointed nodes are clearly visible, confirming the identity of the rhizomes.

***Santalum album*:** *Santalum album* L., known as "White sandalwood" in English, "*Srigandha*" in Sanskrit, "*Sudu Handun*" in Sinhala, and "*Sandanam*" in Tamil, belongs the family Santalaceae (24). *S. album* is a small tree that typically reaches a height of 6-10 meters. It is characterized by its numerous, opposite, slender, and drooping branches. The heartwood is the most valued part of the tree, notable for its brownish- yellow color, hardness, and distinctive aromatic smell, which are essential attributes contributing to its therapeutic efficacy.

***Tinospora cordifolia*:** *Tinospora cordifolia* (Willd.) Hook.f. & Thoms., is a large, deciduous, glabrous climbing shrub belonging to the family Menispermaceae. Commonly known as "Heart-leaved moonseed" in English, "*Guduchi*" in Sanskrit, "*Rasakinda*" in Sinhala, and "*Seenthil*" in Tamil (25). *T. cordifolia* is a large, glabrous, deciduous climbing shrub known for its therapeutic benefits. The plant is characterized by its terete, slender stem, which is closely warty and sparsely lenticellate. The young stems are green with smooth surfaces and noticeable swellings at the nodes, indicating active growth regions. As the stems mature, they develop a light brown surface marked with protuberances due to circular lenticels, which are indicative of the plant's age and vitality. The dried stem of *T. cordifolia* retains its distinctive wart surface and lenticels, maintaining the light brown colour, which helps in authenticating the plant material used in Ayurvedic formulations.

***Trichosanthes cucumerina*:** *Trichosanthes cucumerina* L., is a well-known dioecious, annual climber belonging to the family Cucurbitaceae. Commonly called "Wild gourd" in English, it is known as "*Dummalla*" in Sinhala, "*Bhibhitaki*" in Sanskrit, and "*Kattuppeypputal*" in Tamil (26). *T. cucumerina* is a climbing plant with tendrils that enable it to scale supports, contributing to its distinctive growth habit. The leaves of *T. cucumerina* are simple, alternate, and range from 5 to 12 cm in length and 6.5 to 11 cm in width, sometimes broader. They have a very cordate shape with an excavated sinus at the base, giving them a heart-like appearance. The leaves are more or less deeply palmately 3-, 5-, or 7- lobed, usually about halfway down, with lobes that are either rounded or subacute. The leaf margins are distantly denticulate, and the surface is glabrous or nearly so above, while the underside is more or less pubescent. The flowers of *T. cucumerina* are white, regular, and unisexual, with the plant being monoecious, meaning it has both male and female flowers on the same plant. The male flowers are arranged in long-stalked racemes, while the female flowers are often solitary and short-stalked, occurring in the same axils as the males. The fruit of *T. cucumerina* is ovoid-fusiform, tapering at both ends with a long sharp beak. It measures 3.7 to 5 cm in length and 2 to 2.3 cm in diameter. The fruit is initially striped with white and later turns scarlet as it matures. The pericarp is thin, and the seeds inside are oblong, glabrous, about 1 cm long, and surrounded by a red pulp.

Figure 1: The macroscopic views of constituent plant parts of *Amurthashtaka Kwatha*



(a) *A. indica* dried bark parts	**(b)** *C. rotundus* dried rhizomes with roots	**(c)** *H. pubescens* dried seeds
(d) *N. scrophulariflora* dried rhizomes	**(e)** *S. album* heart wood	**(f)** *T. cordifolia* dried stem
(g) *T. curcumerina* dried whole plant parts	**h)** *Z. officinale* dried rhizomes	**(i)** *Amurthashtaka Kwatha* powder

***Zingiber officinale*:** *Zingiber officinale* Roscoe, commonly known as "Ginger" in English, "*Inguru*" in Sinhala, "*Nagara*" in Sanskrit, and "*Inji*" in Tamil, pertains to the Zingiberaceae family (27). The dried rhizome of *Z. officinale* generally occurs in pieces that are about 5-15 cm long, 1.5-6.5 cm wide, and 1-1.5 cm thick. Externally, the rhizome is buff-coloured, displaying longitudinal striations and occasional loose fibres. When broken, the fracture is

short and smooth, indicative of its internal fibrous structure. Young rhizomes are fresh and juicy, typically pale yellow to light brown in colour, with a distinctive spicy aroma. They are covered with a thin skin that can be easily peeled away. Dried rhizomes, on the other hand, are harder and darker in colour, with the buff-coloured surface displaying the characteristic longitudinal striations and occasional loose fibres. The internal structure remains fibrous, and the fracture upon breaking is smooth, revealing the dried ginger's dense nature.

Organoleptic characteristics of *Amurthashtaka Kwatha* powder and its raw herbal materials for five samples

The organoleptic characteristics of *Amurthashtaka Kwatha* powder and its constituent raw herbal materials were systematically evaluated to ensure the quality, authenticity, and efficacy of this traditional Ayurvedic formulation. Organoleptic analysis, which involves the assessment of sensory attributes such as colour, odour, taste, and texture, plays a crucial role in the preliminary identification and quality control of herbal medicines. The results are represented in Table 1 below.

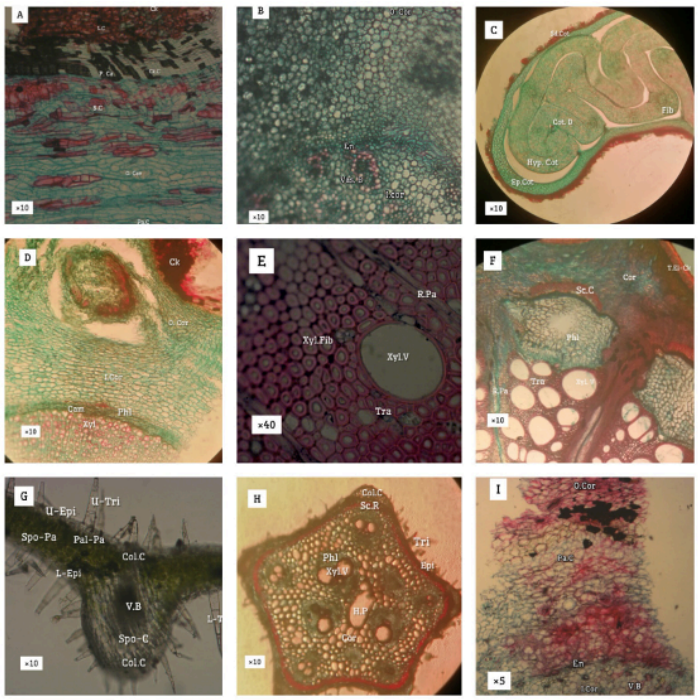
Table 1: Organoleptic properties of *Amurthashtaka Kwatha* powder and its raw herbal materials for five samples

Plant/mixture	Sample No.	Colour	Odour	Taste	Texture
Bark of *Azadirachta indica*	S1	Reddish brown	Slight	Intensely bitter	Coarse
	S2	Reddish brown	Slight	Intensely bitter	Coarse
	S3	Reddish brown	Slight	Intensely bitter	Coarse
	S4	Reddish brown	Slight	Intensely bitter	Coarse
	S5	Reddish brown	Slight	Intensely bitter	Coarse
Rhizome of *Cyperus rotundus*	S1	Blackish brown	Strong, pleasant, characteristic and aromatic	Slight bitter	Coarse
	S2	Blackish brown	Strong, pleasant, characteristic and aromatic	Slight bitter	Coarse
	S3	Blackish brown	Strong, pleasant, characteristic and aromatic	Slight bitter	Coarse
	S4	Blackish brown	Strong, pleasant, characteristic and aromatic	Slight bitter	Coarse
	S5	Blackish brown	Strong, pleasant, characteristic and aromatic	Slight bitter	Coarse
Seeds of *Holarrhena pubescens*	S1	Brown	Slight characteristic	Bitter characteristic	Medium fine
	S2	Brown	Slight characteristic	Bitter characteristic	Medium fine
	S3	Yellowish brown	Slight characteristic	Bitter characteristic	Medium fine
	S4	Pale yellowish brown	Slight characteristic	Bitter characteristic	Medium fine
	S5	Pale yellowish brown	Slight characteristic	Bitter characteristic	Medium fine
Rhizome of *Neopicrorhiza scrophulariflora*	S1	Blackish brown	Slight characteristic	Intensely bitter	Medium fine
	S2	Blackish brown	Slight characteristic	Intensely bitter	Medium fine
	S3	Blackish brown	Slight characteristic	Intensely bitter	Medium fine
	S4	Blackish brown	Slight characteristic	Intensely bitter	Medium fine
	S5	Blackish brown	Slight characteristic	Intensely bitter	Medium fine
Heartwood of *Santalum album*	S1	Brownish yellow	Pleasant characteristic aromatic	Slight	Coarse
	S2	Brownish yellow	Pleasant characteristic aromatic	Slight	Coarse
	S3	Brownish yellow	Pleasant characteristic aromatic	Slight	Coarse
	S4	Brownish yellow	Pleasant characteristic aromatic	Slight	Coarse
	S5	Brownish yellow	Pleasant characteristic aromatic	Slight	Coarse
Stem of *Tinospora cordifolia*	S1	Yellowish Brown	Slight	Bitter punget	Coarse
	S2	Yellowish Brown	Slight	Bitter punget	Coarse
	S3	Yellowish Brown	Slight	Slight bitter punget	Coarse
	S4	Pale brownish huff	Slight	Bitter, pungent astringent	Coarse
	S5	Greyish brown	Slight	Bitter punget	Coarse
Whole plant of *Trichosanthes cucumerina*	S1	Greenish brown	Slight characteristic	Bitter	Medium fine
	S2	Greenish brown	Characteristic	Slight bitter	Medium fine
	S3	Greenish brown	Characteristic	Bitter	Medium fine
	S4	Greyish brown	Slight characteristic	Bitter	Medium fine
	S5	Greenish brown	Slight characteristic	Internse Bitter	Medium fine
Rhizome of *Zingiber officinale*	S1	Brownish yellow	Pleasant characteristic aromatic	Characteristic & pungent	Starchy fine
	S2	Brownish yellow	Pleasant characteristic aromatic	Characteristic & pungent	Starchy fine
	S3	Brownish yellow	Pleasant characteristic aromatic	Characteristic & pungent	Starchy fine
	S4	Pale yellow	Pleasant characteristic aromatic	Characteristic & pungent	Starchy fine
	S5	Brownish yellow	Pleasant characteristic aromatic	Characteristic & pungent	Starchy fine
***Amurthashtaka Kwatha* powder**	S1	Dark brown	Slight aromatic	Intensely bitter	Coarse
	S2	Dark brown	Slight aromatic	Intensely bitter	Coarse
	S3	Dark brown	Slight aromatic	Intensely bitter	Coarse
	S4	Light brown	Slight aromatic	Intensely bitter	Coarse
	S5	Brown	Slight aromatic	Intensely bitter	Coarse

Microscopic characteristics of raw herbal materials of *Amurthashtaka Kwatha*

In this section, we present the microscopic characteristics of the key ingredients in *Amurthashtaka Kwatha*, including their unique cellular patterns, presence of specific tissues, and other diagnostic features. The findings are depicted in Figure 2 below.

Figure 2: Microscopic evaluation of constituent herbal materials of *Amurthashtaka Kwatha*



(A) *A. indica* T.S. in the bark**; Ck:** cork area **L.C:** lenticells in cork area **Ck.C:** tangentially elongated cork cells **P.Ca:** prismatic calcium oxalate crystals **S.C:** elongated stone cells **O.Cor:** outer part of cortex containing thin wall parenchyma cells **Pa.C:** parenchymal cells including starch grains **(B)** *C. rotundus* T.S. in the rhizome**; Ck:** cork area **O.Cor:** outer cortex **En:** endodermis **Vas.B:** vascular bundle **I.Cor:** inner cortex **(C)** *H. pubescens* T.S. in the seed; **Sd.Cot:** seed coat **Ep.Cot:** epicotyl **Hyp.Cot:** hypocotyl Cot.D**:** cotyledon **Fib:** cluster of fibers. **(D)** *N. scrophulariiflora* T.S. in the rhizome; **Ck:** cork cells **O.Cor:** outer cortex containing elongated parenchyma cells **I.Cor:** inner cortex containing typical parenchyma cells **Phl:** phloem area **Cam:** cambium layer **Xyl:** xylem area **(E)** *S. album* T.S. in the heartwood; **Xyl.Fib:** xylem fibers **Tra:** tracheid **Xyl.V:** xylem vessels **R.Pa:** ray parenchyma with starch grains **(F)** *T. cordifolia* T.S. in the stem; **T.El-Ck:** tangentially elongated cork cells **Cor:** cortex **Sc.C:** sclerenchyma cap **Phl:** phloem **Xyl.V:** xylem vessels **Tra:** tracheid **R.Pa:** ray parenchyma riched with starch grains **(G)** *T. cucumerina* T.S. in the leaf; **U-Tri:** segmented trichome in upper epidermis **U-Epi:** upper epidermis **Col.C:** collenchyma cell layer **Pal-Pa**; palisade parenchyma cells **Spo-Pa:** sponge parenchyma cells **V.B:** vascular bundle with xylem and phloem areas **L-Epi:** lower epidermis **L-Tri:** segmented trichome in lower epidermis. **(H)** *T. cucumerina* T.S. in the stem; **Tri:** trichome **Epi:** epidermis **Col.C:** collenchyma cells **Sc.R:** sclerenchyma ring **Xyl:** xylem vessels of vascular bundle **Cor:** cortex cells **H.P:** hollow pith **Phl:** phloem of vascular bundle **(I)** *Z. officinale* T.S. in the rhizome; **O.Cor:** outer cortex **Pa.C:** parenchyma cells **En:** endodermis **I.Cor:** inner cortex **V.B:** vascular bundle

Bark of *Azadirachta indica:* The microscopic examination of *A. indica* reveals several distinctive anatomical features that aid in its identification and quality assessment. The cork area contains lenticels **(L.C)**, which are visible as interruptions in the otherwise continuous layer of tangentially elongated cork cells. These cork cells **(Ck.C)** often contain prismatic calcium oxalate crystals **(P.Ca)**. The presence of elongated stone cells **(S.C)** is another characteristic feature, contributing to the structural integrity of the plant tissue. The outer part of the cortex is composed of thin-walled parenchyma cells **(Pa.C).** Within these parenchyma cells, both simple and compound starch grains can be identified. Moving inward, the cortex cells become densely packed with stone cells **(S.C)**. Clusters of stone cells **(S.C)** are interspersed within the cortex. These microscopic features collectively highlight the intricate structure and diverse cellular composition of *A. indica*, reinforcing its therapeutic potential and importance in traditional medicine.

Rhizome of *Cyperus rotundus*: The cork area **(Ck)** forms the outermost protective layer. Beneath the cork, the outer cortex **(O.Cor)** consists of parenchyma cells. Tannin-containing cells are dispersed within this region, indicating the presence of phenolic compounds. The endodermis **(En)**, a single layer of cells, acts as a selective barrier. The vascular bundle **(Vas.B)** is a crucial component, containing both xylem and phloem tissues. Surrounding the vascular bundle is the inner cortex **(I.Cor)**, which also consists of parenchyma cells.

Seeds of *Holarrhena pubescens*: The seed coat **(Sd.Cot)** forms the outer protective layer. Within the seed, the epicotyl **(Ep.Cot)** represents the embryonic stem situated above the point of attachment of the cotyledons. The hypocotyl **(Hyp.Cot)**, located between the epicotyl and the radicle, serves as a transition zone connecting the embryonic shoot and root systems. The cotyledons **(Cot.D)**, often two in number, are the primary source of nutrient reserves for the developing seedling upon germination. These structures are rich in starch and other storage compounds essential for initial growth and establishment. Surrounding the cotyledons is a cluster of fibers **(Fib)**.

Rhizome of *N. scrophulariflora*: The outermost layer consists of cork cells **(Ck),** calcium oxalate crystals are often observed. Beneath the cork layer lies the outer cortex **(O.Cor)**, characterized by elongated parenchyma cells. The inner cortex **(I.Cor)** contains typical parenchyma cells. The vascular system includes the phloem area **(Phl)** and the xylem area **(Xyl).** Between the phloem and xylem lies the cambium layer **(Cam)**, a meristematic tissue that produces secondary growth.

Heartwood of *Santalum album*: Xylem fibers **(Xyl.Fib)** are elongated cells that provide mechanical support and contribute to the strength and durability of the heartwood. Tracheids **(Tra)** are elongated cells with tapered ends. Xylem vessels **(Xyl.V)** are larger, tube-like structures. Ray parenchyma **(R.Pa)**, located between the xylem vessels and fibers, and consists of radially arranged cells that store starch grains.

Stem of *Tinospora cordifolia*: The outermost layer consists of tangentially elongated cork cells **(T.El-Ck),** prismatic calcium oxalate crystals are dispersed in it. Beneath the cork, the cortex **(Cor)** contains parenchyma cells. Within the cortex, sclerenchyma caps **(Sc.C)** are present. The vascular system is composed of the phloem **(Phl)** and the xylem vessels **(Xyl.V)**. Tracheids (**Tra**) and xylem fibers are found within the xylem. Ray parenchyma **(R.Pa)**, rich in starch grains.

Whole plant of *Trichosanthes cucumerina*: The transverse section (T.S.) of *T. cucumerina* leaf reveals a variety of anatomical features essential for its identification and functional roles. The upper epidermis **(U-Epi)** is the outermost layer of cells. This layer often contains segmented trichomes **(U-Tri)**, which are hair-like structures. Beneath the lower epidermis lies the collenchyma cell layer **(Col.C)**. The palisade parenchyma cell layer **(Pal-Pa)** is situated just below the collenchyma, consisting of tightly packed, elongated cells that are rich in chloroplasts. The sponge parenchyma cell layer **(Spo-Pa)**, located beneath the palisade layer, contains loosely arranged cells with large intercellular spaces. The vascular bundle **(V.B)** within the leaf contains both xylem and phloem areas. The lower epidermis **(L-Epi)**, similar to the upper epidermis and contains segmented trichomes **(L-Tri).**

The transverse section (T.S.) of the stem *T. cucumerina* reveals the outermost layer, the epidermis **(Epi)**. This layer is often accompanied by trichomes **(Tri)**, which are hair-like structures. Beneath the epidermis, the collenchyma cells **(Col.C)** are present. Surrounding the vascular bundle is a ring of sclerenchyma **(Sc.R)**, composed of thick-walled cells. The vascular bundle itself comprises xylem vessels **(Xyl)** and phloem **(Phl)**. The cortex cells **(Cor)** are located between the epidermis and the vascular bundle. At the centre of the stem lies the hollow pith **(H.P)**, a region composed of parenchyma cells.

Rhizome of *Zingiber officinale*: The outer cortex **(O.Cor)** forms the protective outer layer of the rhizome. Beneath the outer cortex, parenchyma cells **(Pa.C)** are present, additionally, parenchyma cells rich in starch grains. The endodermis **(En)**, a specialized layer of cells. Beyond the endodermis lies the inner cortex **(I.Cor)**, which also consists of parenchyma cells. The vascular bundle **(V.B)** within the rhizome includes both xylem and phloem tissues.

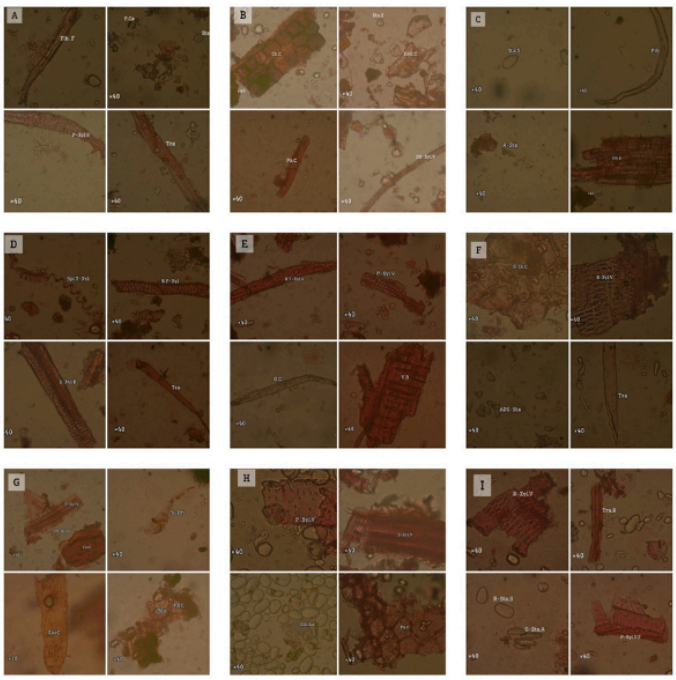
Powder Microscopic characteristics of *Amurthashtaka Kwatha* and its raw herbal materials:

The powder microscopic examination of *Amurthashtaka Kwatha* powder and its raw herbal materials involves observing the distinct cellular structures and diagnostic features present in the powdered form of each herbal component. By examining these characteristics, we can confirm the presence of specific herbs, detect any adulteration or contamination, and ensure consistency in the preparation of the Kwatha. The findings are depicted in Figure 3 below.

Bark of *Azadirachta indica*: Prismatic crystals of calcium oxalate **(P.Ca)**, are commonly present and serve as a diagnostic feature. Fiber fragments **(Fib.F)** indicate the presence of supportive tissues, Starch grains **(Sta.S)** are visible highlighting the storage of energy reserves within the cells. Tracheids **(Tra)**, showcasing the water-conducting cells that contribute to the bark's vascular system. Pitted xylem vessels **(P-Xyl.V)** further emphasize the efficient water transport mechanisms within the bark.

Rhizome of *Cyperus rotundus*: Cork cells **(Ck.C)** form the outer protective layer, preventing water loss and shielding the inner tissues. Endodermis cells **(End.C)** act as a selective barrier. Parenchyma cells **(Pa.C)** are seen, highlighting their role in storage and metabolic activities within the rhizome. Simple starch grains **(Sta.S)** are observed, indicating the storage of energy reserves within the cells. Finally, spirally arranged narrow xylem vessels **(SN-Xy.V)** highlight the specialized structure of vessels that facilitate efficient water conduction within the rhizome.

Figure 3: Powder microscopic evaluation of constituent plant materials of *Amurthashtaka Kwatha*



(A) Powder microscopic evaluation of *A. indica* bark; **P.Ca:** prismatic crystals of calcium oxalate **Fib.F:** fiber fragment **Tra:** tracheid **P-Xyl.V:** pitted xylem vessel **Sta.S:** Simple starch grain **(B)** Powder microscopic evaluation of *C. rotundus* rhizome; **Ck.C:** cork cells **End.C:** Fragment of endodermis **Pa.C:** parenchyma cells **Sta.S:** simple starch grains **SN-Xyl.V:** spirally arranged narrow xylem vessels. **(C)** Powder microscopic evaluation of *H. pubescens* seeds*;* **Fib.B:** a bundle of fibers **Sta.S:** simple starch grains **Fib:** fiber **A-Sta:** compound starch grains **(D)** Powder microscopic evaluation of *N. scrophulariiflora* rhizome*;* **Spi.T-Xyl:** spiral thickening of xylem vessels **Tra:** tracheid **B.P-Xyl:** fragmented bordered pitted xylem vessel **S-Xyl.B:** a bundle of spirally arranged xylem vessels. **(E)** Powder microscopic evaluation of *S. album* heartwood*;* **R.T-Xyl.V:** reticulately thickened xylem vessels **P-Xyl.V:** pitted xylem vessel fragment **O.C:** oil cannel **V.B:** vascular bundle with ray parenchyma **(F)** Powder microscopic evaluation of *T. cordifolia* stem*;* **S-Ck.C:** surface view of cork cells **ADS-Sta:** aggregated different shaped starch grains **Tra:** tracheid **R-Xyl.V:** reticulately arranged xylem vessel **(G)** Powder microscopic evaluation of *T. cucumerina* whole plant*;* **S-Xyl.V:** spirally arranged xylem vessels **ExoC:** part of exocarp of fruit **ST-Xyl.V:** spiral thickening of xylem vessels **Pa.C:** parenchymal cells include **(Sta.S)** starch grains **ST-Xyl.V:** spiral thickening of xylem vessels **S-Tri:** segmented trichome **(H)** Powder microscopic evaluation of *Z. officinale* rhizome*;* **DSS.Sta:** different sized and shaped simple starch grains **S.Xyl.V:** spirally arranged xylem vessels **P-Xyl.V:** pitted xylem vessel **Pa.C:** parenchyma cells **(I)** Powder microscopic evaluation of *Amurthashtaka Kwatha;* **Tra.B:** a bundle of tracheids **R-Xyl.V:** a part of reticulately arranged xylem vessel **R-Sta.S:** round shaped simple starch grain **O-Sta.A:** oval shaped aggregated starch grains **P-Xyl.V.F:** pitted xylem vessel fragment.

Seeds of *Holarrhena pubescens*: A bundle of fibers **(Fib.B)** further emphasizes the strength and durability of the seed coat. Simple starch grains **(Sta.S)** observed highlight the storage of energy reserves within the seed. Individual fibers **(Fib)** demonstrate the supportive role of these cells. Aggregated starch grains **(A-Sta)** observed indicate the accumulation of storage compounds, which are essential for the seed's germination and early growth.

Rhizome of *Neopicrorhiza scrophulariiflora*: Spiral thickening of xylem vessels **(Spi.T-Xyl)** illustrates the specialized structure of the xylem, which enhances its efficiency in water transport. Tracheids **(Tra)** are essential for conducting water and minerals, showcasing their elongated structure. The bordered pitted xylem vessel **(B.P-Xyl)** highlights the intricate pattern of pits that facilitate the lateral movement of water between vessels. A bundle of spirally arranged xylem vessels **(Spi-Xyl.B)** showcases the organized structure of the xylem, ensuring efficient water conduction throughout the plant.

Heartwood of *Santalum album*: Reticulately thickened xylem vessels **(R.T-Xyl.V)** highlight the specialized structural adaptations that enhance the efficiency of water transport within the wood. Pitted xylem vessel fragments **(P-Xyl.V)** showcase the intricate network of pits that facilitate lateral water movement between vessels, enhancing the overall functionality of the vascular system. Oil canals **(O.C)** observed are characteristic of *S. album*, containing the essential oils that contribute to the heartwood's distinctive aroma and therapeutic properties. The vascular bundle with ray parenchyma **(V.B)** highlights the organization of vascular tissues and the presence of parenchyma cells, which aid in the storage and transport of nutrients.

Stem of *Tinospora cordifolia*: The surface view of cork cells **(S-Ck.C)** shows the outer protective layer, which helps in reducing water loss and shielding the internal tissues. Aggregated different shaped starch grains **(ADS-Sta)** indicate the presence of stored energy reserves, essential for the plant's metabolism and growth. Tracheids **(Tra)** are observed, playing a key role in water conduction and providing structural support to the stem. The reticulately arranged xylem vessels **(R-Xyl.V)** further emphasize the specialized structure that aids in the efficient transport of water and nutrients.

Whole plant of *Trichosanthes cucumerina*: Spirally arranged xylem vessels **(S-Xyl.V)** illustrate the specialized structure that enhances the efficiency of water transport within the plant. Part of the exocarp of fruit **(ExoC)** shows the outer protective layer of the fruit, which helps in reducing water loss and shielding the internal tissues. Spiral thickening of xylem vessels **(ST-Xyl.V)** further emphasizes the specialized adaptations that improve water conduction. Simple starch grains **(Sta.S** indicate the storage of energy reserves, which are essential for the plant's metabolism and growth. Fragments of secondary thickening **(Sc.T.F)** highlight the additional support and durability provided to the plant's structure. Parenchymal cells that include starch grains **(Pa.C)** are observed, indicating the role of these cells in storage and metabolic activities. The presence of segmented trichomes **(S-Tri)** showcases the plant's protective structures, which help reduce water loss and protect against herbivory.

Rhizome of *Zingiber officinale*: Different sized and shaped simple starch grains **(DSS.Sta)** indicate the storage of energy reserves, vital for the plant's growth and metabolic processes. Spirally arranged xylem vessels **(S-Xyl.V)** demonstrate the specialized adaptations that improve water conduction efficiency within the rhizome. Pitted xylem vessels **(P-Xyl.V)** illustrate the intricate network of pits that facilitate lateral water movement between vessels, enhancing the overall functionality of the vascular system. The presence of parenchyma cells **(Pa.C)** reflects their role in storage, photosynthesis, and wound repair.

***Amurthashtaka Kwatha* powder:** A bundle of tracheids **(Tra.B)** shows the elongated cells that play a crucial role in water conduction and structural support within the plant. Part of a reticulately arranged xylem vessel **(R-Xyl.V)** illustrates the intricate pattern that enhances the efficiency of water transport. Round-shaped simple starch grains **(R-Sta.S)** and oval- shaped aggregated starch grains **(O-Sta.A)** highlight the presence of stored energy reserves, essential for the plant's metabolism and growth. Pitted xylem vessel fragments **(P-Xyl.V.F)** showcase the pits that facilitate lateral water movement between vessels, enhancing the overall functionality of the vascular system. These microscopic features collectively illustrate the complex anatomical structure of *Amurthashtaka Kwatha* powder, ensuring its quality and confirming its identity. The presence of these specific structures supports the therapeutic properties of the formulation and underscores its significance in traditional medicine.

Physico-chemical analysis

The physicochemical characteristics of *Amurthashtaka Kwatha* powder samples, formulated by proportionate blending of the eight constituent powdered plant materials procured from five different sources, are presented in Table 2. For convenience, *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from the five procurement sources were designated as AK-S1, AK-S2, AK-S3, AK-S4, and AK-S5, corresponding to sources S1–S5, respectively.

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed significant variations in several physicochemical parameters among the formulations. Moisture content differed significantly between AK-S2 and AK-S3, AK-S2 and AK-S5, and AK-S3 and AK-S4 ($p < 0.05$), whereas the remaining comparisons were not statistically significant ($p > 0.05$). Significant differences in total ash content were observed between AK-S2 and both AK-S1 and AK-S5 ($p < 0.05$), while no significant differences were detected among the other formulations ($p > 0.05$). For water-soluble ash, AK-S5 differed significantly from AK-S1 and AK-S3 ($p < 0.05$), whereas the remaining pairwise comparisons showed no significant differences ($p > 0.05$). In contrast, AK-S2 exhibited significantly higher acid-insoluble ash values than all other formulations ($p < 0.05$). Water-soluble extractive values showed significant variation among most formulations. However, no statistically significant differences were observed between AK-S4 and AK-S5 or between AK-S1 and AK-S3 ($p > 0.05$), while all other pairwise comparisons were significant ($p < 0.05$). Ethanol-soluble extractive values were significantly higher in AK-S4 and AK-S5 than in the remaining formulations ($p < 0.05$). Significant differences were also observed in dichloromethane-soluble extractive values. AK-S2 exhibited significantly lower values than AK-S1, AK-S3, and AK-S5 ($p < 0.05$). Similarly, AK-S4 showed significantly lower dichloromethane-soluble extractive values compared with AK-S3 and AK-S5 ($p < 0.05$).

Qualitative screening of phytochemicals

The qualitative phytochemical profile of *Amurthashtaka Kwatha* samples prepared from constituent plant materials procured from five different sources is presented in Table 3. Phytochemical screening revealed the presence of alkaloids, flavonoids,

phenolics, tannins, saponins, carbohydrates, proteins, cardiac glycosides, terpenoids, steroids and phytosterols in all samples. No variation in the occurrence of the tested phytochemical groups was observed among the samples obtained from the different sources, indicating a consistent qualitative phytochemical composition across all *Amurthashtaka Kwatha* preparations. These phytoconstituents are responsible for pharmacological activities of *Amurthashtaka Kwatha.*

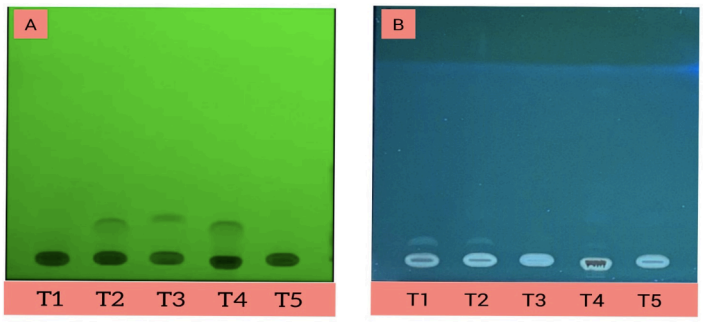
High Performance Thin Layer Chromatography

The HPTLC chromatograms of the five *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from different sources are presented in Figure 4. The corresponding densitometric chromatograms recorded at 254 nm and 366 nm are shown in Figures 5 and 6, respectively. The densitometric data, including Rf values, peak areas, and relative peak area percentages, are presented in Tables 4 and 5.

At 254 nm, HPTLC densitometric analysis revealed that most *Amurthashtaka Kwatha* formulations exhibited two dominant bands, whereas the formulation prepared using raw materials from Source 5 (AK-S5) showed only a single dominant band. The major bands were predominantly observed within the Rf ranges of 0.03–0.14, 0.11–0.19, and 0.64–0.75. Formulations AK-S2 and AK-S4 were characterized by a dominant band at Rf 0.03–0.14, while AK-S3 exhibited a major band within the Rf range of 0.11–0.19. In contrast, AK-S1 and AK-S5 showed dominant bands at higher Rf values, particularly within the range of 0.64–0.75. Notably, AK-S2 displayed a broad co-eluting peak spanning the Rf regions of 0.03–0.14 and 0.11–0.19, suggesting the presence of multiple closely migrating constituents within this chromatographic zone.

Under UV 366 nm detection, most formulations exhibited two dominant fluorescent bands, whereas AK-S1 and AK-S2 showed comparatively higher chromatographic complexity with 4 and 3 dominant bands, respectively. The principal fluorescent bands were observed within the Rf ranges of 0.09–0.16, 0.19–0.28, 0.40–0.45, and 0.64–0.75. Dominant bands at Rf 0.09–0.16 were detected in AK-S2 and AK-S4, while AK-S3 and AK-S5 were characterized by a prominent band within the Rf range of 0.64–0.75. In contrast, AK-S1 exhibited its most abundant fluorescent constituent at Rf 0.19–0.28. The recurrence of these dominant bands across different formulation batches indicates the presence of common phytochemical markers, while variations in band number and relative abundance reflect source-dependent differences in phytochemical composition.

Figure 4: HPTLC fingerprint profiles of five batches of *Amurthashtaka Kwatha*



(A) Chromatogram visualized under UV 254 nm and (B) chromatogram visualized under UV 366 nm. Tracks T1–T5 represent the five independently prepared batches of *Amurthashtaka Kwatha*

Table 2: Physicochemical parameters of *Amurthashtaka Kwatha* powder samples from five different sources

Parameter	*Amurthashtaka Kwatha* powder sample					Average%
	AK-S1	AK-S2	AK-S3	AK-S4	AK-S5	
Moisture Content %	12.39 ± 0.22	13.07 ± 0.07	11.25 ± 0.33	12.84 ± 0.37	11.51 ± 0.42	12.21 ± 0.36
Total Ash %	8.12 ± 0.21	6.55± 0.23	7.52 ± 0.38	7.34 ± 0.03	8.23 ± 0.18	7.55 ± 0.27
Water-Soluble Ash %	3.02 ± 0.33	2.13 ± 0.22	3.17 ± 0.43	2.71 ± 0.18	1.81 ± 0.08	2.57 ± 0.33
Acid-Insoluble Ash %	1.55 ± 0.18	3.50 ± 0.51	2.00 ± 0.06	1.38 ± 0.07	1.88 ± 0.05	2.06 ± 0.44
Water Soluble Extractives %	2.70 ± 0.21	3.50 ± 0.01	2.63 ± 0.12	4.28 ± 0.09	4.65 ± 0.21	3.55 ± 0.41
Ethanol Soluble Extractives %	2.66 ± 0.04	2.61 ± 0.01	2.84 ± 0.03	3.97 ± 0.15	4.02 ± 0.09	3.22 ± 0.32
Dichloromethane Soluble Extractives %	2.37 ± 0.26	1.68 ± 0.12	2.68 ± 0.08	1.95 ± 0.08	2.55 ± 0.06	2.25 ± 0.19

Table 3: Qualitative phytochemical profile of *Amurthashtaka Kwatha* prepared from constituent plant materials procured from five different sources

Phyto constituents	Test	*Amurthashtaka Kwatha* sample				
		AK-S1	AK-S2	AK-S3	AK-S4	AK-S5
Alkaloids	Mayer's test	+	+	+	+	+
	Picric acid test	++	++	++	+++	+++
	Tannic acid test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
Flavonoids	Ammonium test	+	+	+	+	+
	Shinoda test	++	++	++	++	++
	Alkaline reagent test	+	+	+	+	+
	Aluminium chloride test	+	+	+	+	+
	Sulfuric acid test	++	++	++	++	++
Phenolics	Folin–Ciocalteu test	+++	+++	+++	+++	+++
	Ferric chloride test	+	+	+	+	+
	Vanillin test	+	+	+	+	+
	Lead acetate test	+	+	+	+	+

Saponins	Froth test Emulsion test	+ +	++ +	+ +	++ +	++ +
Tannins	Ferric chloride test Lead acetate test Modified lead acetate test	++ + +	++ + +	++ + +	++ + +	++ + +
Carbohydrates	Fehling's test Benedict's test	++ ++	++ ++	++ ++	++ ++	++ ++
Proteins	Ninhydrin test Xantoprotein test	++ ++	++ ++	++ ++	+++ ++	+++ ++
Cardiac Glycosides	Keller–killiani test	+	+	+	+	+
Terpenoids	Salkowski test Monoterpenes (Vanillin–sulphuric acid test) Diterpenes (Copper acetate test) Sesquiterpenes (Sulphuric acid test)	+ + +++ +	+ + +++ +	+ + +++ +	+ + +++ +	+ + +++ +
Steroids	Lieberman Burchard test Acetic anhydride test	+ +	+ +	+ +	+ +	+ +
Phytosterols	Salkowski's test	+	+	+	+	+
-ve: negative, +: positive in low level, ++: positive in moderate level, +++: positive in high level						

Figure 5: Densitometric chromatograms of *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from five different sources (AK-S1 to AK-S5) under UV 254 nm detection

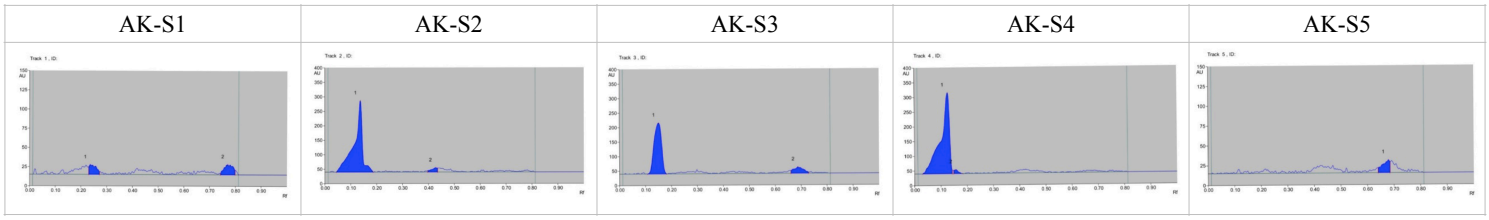


Figure 6: Densitometric chromatograms of *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from five different sources (AK-S1 to AK-S5) under UV 366 nm detection

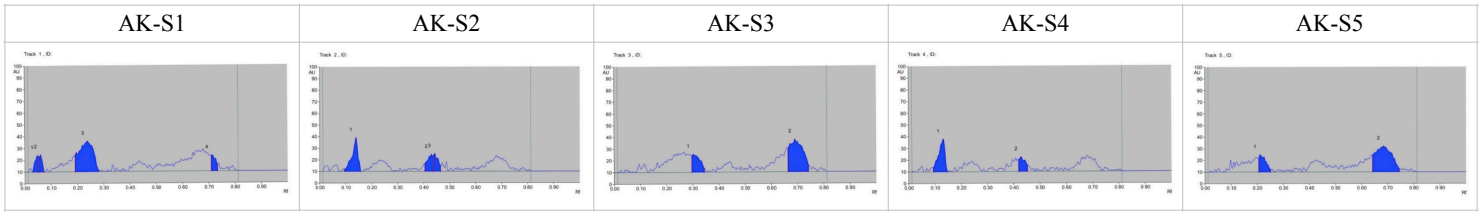


Table 4: Densitometric analysis of HPTLC chromatograms of *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from five different sources under UV 254 nm detection

Source	Total bands	Dominant Rf Bands	Area (AU)	Relative peak area %
AK-S1	2	0.23-0.27 0.74-0.80	336.4 440.9	43.27 56.73
AK-S2	2	0.03-0.19 0.40-0.45	7215.4 321.7	95.73 4.27
AK-S3	2	0.11-0.19 0.64-0.75	4719.0 763.8	86.07 13.93
AK-S4	2	0.03-0.14 0.11-0.19	8564.0 196.7	97.75 2.25
AK-S5	1	0.64-0.75	410.6	100.00

Table 5: Densitometric analysis of HPTLC chromatograms of *Amurthashtaka Kwatha* formulations prepared using constituent plant materials obtained from five different sources under UV 366 nm detection

Source	Total bands	Dominant Rf Bands	Area (AU)	Relative peak area %
AK-S1	4	0.03-0.05 0.05-0.07 0.19-0.28 0.71-0.73	178.7 163.3 1321.6 221.4	9.48 8.66 70.11 11.74
AK-S2	3	0.09-0.16 0.40-0.45 0.44-0.47	614.1 286.8 257.2	53.03 24.76 22.21
AK-S3	2	0.30-0.34 0.64-0.75	451.6 1333.0	25.30 74.70
AK-S4	2	0.09-0.16 0.40-0.45	635.4 277.4	69.61 30.39
AK-S5	2	0.19-0.28 0.64-0.75	364.3 1317.7	21.66 78.34

Discussion

The current study used organoleptic, macroscopic, microscopic, powder microscopic, physicochemical, phytochemical, and chromatographic tests to assess the impact of raw material sourcing on the quality attributes of *Amurthashtaka Kwatha*. All formulations maintained the distinctive characteristics of the traditional preparation while showing differences in specific physicochemical and chromatographic parameters, despite the fact that the constituent herbs were obtained from natural habitats and Ayurvedic retail outlets.

An essential method for verifying and evaluating the quality of herbal raw materials is pharmacognostic evaluation. The botanical legitimacy of the constituent herbs was confirmed by the macroscopic features that matched previous pharmacognostic descriptions. The primary diagnostic characteristics remained unchanged, despite slight differences in color and sensory characteristics between samples from various sources. Variations in plant maturity, harvesting techniques, post-harvest processing, environmental factors, and geographic origin can all contribute to these discrepancies. The preservation of distinctive morphological and organoleptic characteristics implies that the constituent herbs' basic identity and quality traits were preserved regardless of their origins (26, 28–41).

The authenticity of the raw materials was further confirmed by microscopic and powder microscopic tests. The corresponding plant materials exhibited diagnostic anatomical characteristics such as calcium oxalate crystals, stone cells, starch grains, fibers, trichomes, oil canals, tracheids, and distinctive xylem arteries. These features are crucial taxonomic indicators for identifying therapeutic plants and spotting adulteration. Such microscopic features persisted in the final powdered formulation, indicating that diagnostic markers were still detectable after blending and pulverization, supporting their use in regular *Amurthashtaka Kwatha* quality control and authentication.

The physicochemical characteristics showed quantifiable variations between the formulations. The range of moisture content was 11.25% to 13.07%, suggesting moderate levels of residual moisture. Elevated moisture levels may promote microbiological growth, enzymatic activity, and phytoconstituent breakdown during storage since moisture has a significant role in the stability of herbal products. In order to reduce moisture absorption and maintain product quality, proper storage conditions are therefore required. Ash values varied amongst the formulas as well. While acid-insoluble ash gives an estimate of siliceous materials like sand and soil, total ash shows the whole inorganic content of a sample. A higher percentage of earthy pollutants introduced during harvesting, handling, or processing may be indicated by the relatively higher acid-insoluble ash value found in AK-S2. Variations in the water-soluble ash values also point to differences in the formulations' water-soluble inorganic element concentrations.

Extractive values provide an indirect estimate of the quantity and nature of phytoconstituents extracted by solvents of varying polarity. Differences in water-, ethanol-, and dichloromethane-soluble extractive values indicate variability in the abundance of polar, moderately polar, and non-polar constituents among the samples. The comparatively higher water- and ethanol-soluble extractive values observed in AK-S4 and AK-S5 suggest a greater recovery of soluble phytoconstituents, which may partly explain the differences observed in their chromatographic profiles. Such variation is expected because environmental conditions, soil characteristics, climatic factors, harvesting season, plant age, and storage practices are known to influence secondary metabolite accumulation in medicinal plants.

All formulations contained alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, proteins, cardiac glycosides, terpenoids, steroids, and phytosterols, according to qualitative phytochemical screening. The consistent occurrence of these phytochemical classes suggests that the fundamental chemical composition of *Amurthashtaka Kwatha* was largely preserved regardless of the source of the raw materials. Many of these ingredients have been linked to antioxidant, anti-inflammatory, antibacterial, immunomodulatory, and antipyretic properties, all of which may add to the therapeutic benefits that have historically been ascribed to the mixture. However, qualitative screening does not provide information regarding constituent abundance, and therefore may not detect subtle chemical differences among formulations.

HPTLC fingerprinting was employed to assess chemical consistency and variation among the formulations. Despite variations in band number, intensity, and peak size, the chromatographic profiles obtained at 254 and 366 nm showed the presence of multiple common bands. Recurring chromatographic zones within comparable Rf ranges support the formulation's chemical identification and show the presence of common marker ingredients. AK-S1 and AK-S2 showed relatively higher chromatographic complexity than the other formulations at 366 nm, where the chromatograms showed a wider variety of fluorescent chemicals. Changes in the concentration of secondary metabolites resulting from environmental and post-harvest conditions influencing the source materials are probably responsible for variations in band intensity and peak area.

The use of aqueous decoctions as test materials may be the reason for the comparatively small number of chromatographic bands seen in the HPTLC fingerprints. While less polar and non-polar phytochemicals may be extracted inefficiently or remain undetected under the selected chromatographic conditions, water preferentially extracts polar compounds. Additionally, co-elution of chemically related chemicals within the complex polyherbal matrix may cause broad peaks to appear and lower chromatographic resolution. As a result, rather than representing the formulation's entire phytochemical makeup, the observed fingerprints most likely reflect its water-extractable component. In subsequent research, better separation and detection of a wider variety of contents may be made possible by the use of other extraction solvents or enhanced chromatographic settings.

Overall, the results show that the formulations retained similar pharmacognostic traits and qualitative phytochemical content despite differences in physicochemical properties and chromatographic fingerprints. While the observed variations demonstrate the impact of raw material quality on constituent abundance, the existence of common HPTLC marker bands across all batches validates the chemical identification of *Amurthashtaka Kwatha*. These results highlight the significance of thorough standardization methods that include pharmacognostic, physicochemical, and chromatographic analyses to guarantee the therapeutic reliability, quality, and consistency of Ayurvedic formulations.

Conclusion

The present study established comprehensive pharmacognostic profiles of *Amurthashtaka Kwatha* prepared using raw materials obtained from different sources. While all formulations retained

comparable pharmacognostic characteristics and qualitative phytochemical composition, variations in physicochemical properties and chromatographic fingerprints indicated differences in phytochemical abundance. These findings provide scientific evidence for the authenticity and quality of the formulation and highlight the importance of raw material sourcing in the standardization and quality control of *Amurthashtaka Kwatha*.

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