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Establishment of quality and safety markers for the identification of Amomum seed and Cinnamon leaf

Research Article

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Abstract

Introduction: A crucial and essential prerequisite is the standardization of crude drugs for the purpose of authenticating their effectiveness, safety, and quality. The Pharmacognostical evaluation, microscopy, HPTLC profiling, and safety assessment of *Amomum subulatum* Roxb seed and *Cinnamomum cassia* Blume leaf have been carried out. Methods: The evaluation was achieved by following standard World Health Organization (WHO) protocols. The outcomes have been collated and set up in a tabular format for HPTLC profiling and physicochemical evaluation of *Cinnamomum cassia* and *Amomum subulatum*. Both the drugs underwent for safety assessment by estimating aflatoxin (B1, B2, and G1, G2) using HPLC, and heavy metals (Lead, Mercury, Cadmium, and Arsenic) by applying atomic absorption spectrometer, whereas pesticidal residue were estimated by using recommended GC-MS method. Results: All the procedures repeated thrice and the average reading with standard deviation has been represented with figures and tabular form. The results of safety assessment have been compared with reference values and discussed, respectively whereas results of standardization are valuable for ensuring the scientific significance and quality of herbal drugs. The study demonstrated the microscopic cellular identification of both the plant species, TLC profiling is presented with chromatograms with respective R_f values of different separated components, which has been acceptable technique to phytochemical standardization of different extracts (polar or non-polar). Conclusion: In this way all collective work can be used remarkably for ensuring the quality and therapeutic efficacy of these drugs, whereas results of safety assessment are encouraging and suggestable to be used these both spices as phytopharmaceuticals.

Keywords: Herbal drugs, Pharmacognostical evaluation, Quality control, Standardization, *Amomum subulatum, Cinnamomum cassia*.

Introduction

During the COVID-19 epidemic, the globe came to appreciate the significance of conventional treatments, which has drawn researchers from all over the world to herbal remedies. Numerous medicinal plants clearly demonstrated enormous potential in healthcare for both curative and preventative purposes. Due to its viability, affordability, and dependability for the populace, herbal therapies have received support, suggestions, and encouragement from the World Health Organization (WHO). The most crucial component of their primary evaluation is the establishment of pharmacognostical standards for accurate identification and assurance of the excellence. To determine the identification and level of purity of such plant-based therapeutic materials, each and every traditional standardization parameter suggested by the WHO and pharmacopoeial guidelines is of utmost importance (1-2). Herbal medicines are becoming more acceptable around the globe, especially in industrialized nations, but the main necessity is standardization and ensuring quality, safety, and efficacy. The WHO also emphasizes the necessity of physicochemical and phytochemical evaluation of raw medicinal materials in herbal medicine (3). *Amomum subulatum Roxb.,* also recognized as large Cardamom has long been a popular spice that is used to flavor the native cuisine of the Eastern Himalayan region, mainly Nepal, Bhutan, and India. Sikkim State of India is the largest producer of cardamom. The drug's significant curative and preventive potential for a variety of illnesses has earned it widespread recognition in the Ayurvedic medical system. 1, 8-cineole is the primary chemical constituent of large cardamom seed oil. It is known as heel kalan and badi ilaichi in the Unani medical system. *Amomum*

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subulatum fruits have been used as an insecticide as well as a traditional remedy for indigestion, vomiting, biliousness, acne, gout, spasms, and cardiac arthymia (4). In South Asian countries, the cardamom fruits are typically employed as condiment in food and in herbal preparations to cure a variety of diseases. Being a legal substance, it is indicated for cough, lung congestion, pulmonary tuberculosis, and throat ailments in the Indian Ayurvedic Pharmacopoeia (5). Some reports suggested the anticancer potential of *A. subulatum* extract in induced apoptosis model, which has been employed in clinical practice and some scientific studies to treat cancer. The medication has also demonstrated evidence of its lipid-lowering, cardio-protective, and gastro-protective properties in stomach ulcers (5-6). *Cinnamomum cassia* Blume is also well-liked herbal drug, which has been extensively described in the treatment of dyspepsia, gastritis, and inflammatory disease (7-8). The chosen medications have been utilized in conventional treatment and are found in many Ayurvedic and Unani formulations. Ibn Sina, a Persian doctor, explored the use of herbal medicines for chronic illnesses and wrote a book describing 63 plants for their significance in heart problems with cardio-protection. Therefore, the current study is carried out by extracting a specific portion from the plants, specifically the seeds of *A. subulatum* and the leaves of *C. cassia*, and evaluating it for physicochemical parameters, phytochemical screening, TLC profiling, and safety profiling by performing quantitative estimation of aflatoxins, heavy metals, and pesticides.

Methodology

Experimental

The crude samples were procured from a supplier of Old Delhi, drug samples referenced as NISCAIR/ RHMD/Consult/-2008-09/1149/181/02/01-02, was verified by botanist Dr. H. B. Singh, Scientist F and Head Raw Material Herbarium and Museum, NISCAIR, New Delhi. The analysis performed by using only analytical-grade chemicals and solvents, all of which were supplied by Merck India.

Morphology of drugs

The physical attributes of drug samples, including their size, shape, flavor, color, and odor, were assessed.

Microscopy of drug samples

Preparation of specimens

The study's sample drugs were procured from a Delhi neighborhood market. Care was made to choose high-quality drugs, such as cassia leaves and amomum fruits. The necessary samples were cleaned up and preserved in the mixture of 5.0 mL formalin, 5.0 mL acetic acid, and 90 mL of 70% ethyl alcohol. The water was removed from the specimens using tertiary butyl alcohol at different concentrations while fixing for 24 hours (9). The specimens were gradually filled with paraffin wax (melting point 58–60 °C) until the t-butyl acrylate (TBA) solution was super saturated. The specimens were molded into paraffin blocks.

Sectioning

The paraffin-embedded samples were segmented using a Rotary Microtome. The parts were preserved at a thickness of between 10 and 12µm. According to tradition, the sections were dewaxed (10). The pieces were colored with toluidine blue stain (11). Toluidine blue can trigger extraordinarily potent cytochemical reactions because it is a polychromatic stain. The color changed the color of the cellulose walls to pink, the lignified cells to blue, the suberin to dark green, the mucilage to violet, and the protein bodies to blue. The slices were additionally stained with safranin, fast green, and IKI (iodine-potassium iodide) to look for the presence of starch. For macerated/cleared material, a temporary preparation mounted in glycerine was created. After staining, powdered components from various parts were cleaned with sodium hydroxide and mounted in glycerine medium. Research and measurement were done on several cell components.

Photomicrographs

To observe tissue descriptions at the microscopic level, micrographs were employed. Photographs were taken using a Nikon lab photo microscopic equipment (Nikon, Canada). We used the light field for our usual observations. Starch grains, lignified cells, and crystals were all examined using polarized light. When lit by polarized light, these structures stood out on a black backdrop because they are birefringent. The scale-bars indicate the magnifications of the figures. The numerous anatomical details are carefully described in the drawings (12).

Physicochemical evaluation of drugs

The physicochemical evaluation was conducted in accordance with the Indian Pharmacopoeia (IP), 1996, and WHO recommendations (13-14).

Determination of Foreign Matter

Samples that had been weighed (100 gm) were laid out in a thin layer on filter paper. By utilizing both a lens (6x) and inspection with the unaided eye, the foreign object was found three times. After being removed from the samples, all foreign matter was weighed to determine its percentage presence.

Loss on drying (LOD)

LOD quantifies the amount of moisture and volatile substances that are present in a certain sample. The LOD content of the powdered sample (1.0-2.0 gram), which was dried at 105°C for two hours while measurements were taken every 20 minutes, was measured using an infrared torsion balance moisture meter (Adair Dutt, Kolkata, India). The drying process was repeated until the results were consistent with one another or the variation of two readings observed less than 0.25%.

Moisture content

Karl Fischer method is a popular technique used to calculate the amount of water in a wide range of products. Methanol, a primary alcohol, served as the

solvent, and pyridine, served as a buffer agent in Karl Fischer titration. The water factor, which provides information about the amount of moisture contained in methanol and Karl Fischer reagent, was determined as the first step in determining the moisture content. A precisely weighed sample of about 2.0 grams was added to the methanol once the water factor had been established, and a titration using the Karl Fischer reagent was then performed using an automatic titrator to identify the end point. This is how the computation was done:

Water factor = Weight of the water taken (mg) / Burette reading

% moisture content = [burette reading X Water factor / Weight of the sample (mg)] X 100

Ash values of drug samples

Total Ash

The method outlined in the WHO recommendations was used to calculate the ash values. To determine the ash value, the powdered drug (1.0 gm) was burned until become carbon-free in a silicon crucible at a temperature no higher than 450°C. To determine the complete ash content, it was cooled and weighed. Sample's ash values were computed and compiled.

Acid insoluble ash

The total amount of ash found was heated in 25 mL of diluted hydrochloric acid (6.0 N) for five minutes. The insoluble material was gathered on filter paper without ash, washed with hot water, and burned to a constant weight at a temperature no greater than 450°C. The acid-insoluble ash was then estimated after determining the weight.

Water soluble ash

It was determined as amount insoluble material was remained on ash-free filter paper, rinsed using hot water, then burned for 15 min up to 450°C. The weight of the undissolved material was deducted from the amount of the total ash. The weight variation describes the water-soluble ash.

Determination of pH

pH of 1% solution

The precisely weighed powdered sample 1.0 gm was added in 100 mL of water and shacked. A standardized glass electrode pH meter (pH 1500, Eutech Instrument, Singapore) has been applied to determine the pH of prepared samples.

pH of 10% solution

The precisely weighed powdered sample 10.0 gm was incorporated in 100 mL of water and stirred. A standardized glass electrode (pH 1500, Eutech Instrument, Singapore) was used to measure the pH of the filtrate.

Extractive value of drug samples

Drug sample extraction values were calculated using the technique outlined in I P, 1996.

Successive extraction

Using the Soxhlet device and a series of solvents including petroleum ether, chloroform, acetone, methanol, and water, the known amount of both dry drug powders was extracted. Continue extraction until the solvent became colourless during siphoning. The obtained extract was concentrated on a water bath by being evaporated until dry, and the resulting residue was weighed. In similar circumstances, the process was performed three times. The extractive values were quantitatively estimated as a percentage of the weight of the air-dried drug sample used.

Extractive values (Ether, alcohol and water-soluble extractives)

Air-dried samples that were coarsely powdered and weighed 4.0 gm were refluxed in petroleum ether for an hour. The resultant extract was thoroughly agitated and quickly filtered. The 25.0 mL extract to tarred container and dried off over a water bath. The resulting residue was dried and immediately weighed. Water and ethanol underwent the same extraction process under the same conditions. To ensure reproducibility, the entire process was tripled.

Preliminary phytochemical screening

As per the method described by Ahmad (2007), phytochemical investigations were carried out by performing various qualitative chemical tests (15), such as tests for alkaloids, glycosides, tannins, carbohydrates, saponins, proteins and amino acids, resins, lipids/fats, phenolic compounds, test for acidic compounds, and for flavonoids in methanolic extract of drug samples.

TLC/HPTLC chemoprofiling

The solvent systems for the separation of the various drug extracts were developed by TLC in order to create the HPTLC fingerprints. The solvent system chosen for HPTLC was the one with the most and best resolved spots. The samples were applied using a Linomat V (HPTLC sample applicator) to precoated silica gel 60 F_{254} plates (E. Merck, 0.20 mm thickness), and then developed through optimized solvent systems. Following spectral analysis, the plates were examined at 254 and 366 nm wavelengths. The HPTLC plates were photographed using the Reprostar Chromatography Documentation Apparatus. Following the spraying of the visualizing reagent, developed plates were studied in visible spectrum.

Linomat V sample applicator was used to apply samples to pre-coated plate. Each sample was applied in triplicate, with a track width of 5.0 mm consisting 1.3 cm distance between each track. Further, plates were incorporated in a development chamber that was saturated with the specific mobile phase; the solvents were allowed to run up to 80 mm. It was removed from chromatographic chamber and air-dried plates underwent scanning at 254, 366 nm, whereas by spraying anisaldehyde reagent, the plates were also studied under visible spectrum.

Safety evaluation of drug samples

Heavy or toxic metal analysis

The dried powdered samples underwent a heavy metal estimation to check the lead, cadmium, mercury, and arsenic content. It has done by burning 1.0 g of powdered medication in a silica crucible at 600°C till ash formed while utilizing an atomic absorption spectrometer. Acqua regia was used to dissolve the ash. It was carefully heated to allow for thorough digestion. The volume was made up to 100 mL. In a similar manner, standard dilutions of known concentrations of each element were created in ascending order, and all standards and samples were then examined for their absorption. A standard calibration plot was obtained by putting absorbance against concentration and determined the presence of each metal in samples.

Aflatoxins determination

Sample preparation for aflatoxin

For the purpose of determining the presence of aflatoxins, the AOAC's standard technique of analysis was used (16). The methanolic acidic extract of drug samples was transferred to separating funnel and added NaCl and hexane. The addition of dichloromethane to the aqueous layer and separated. Dichloromethane extract was collected by repeating the process two or three times, and then up to 2.0 to 3.0 mL of it was evaporated. This extract was run to a silica gel column followed by washing with 9:1 benzene-to-acetic-acid and 3:1 ether-to-hexane. Finally, dichloromethane containing 10 %, v/v acetone was used for elute aflatoxin. Dried aflatoxins were passed nitrogen gas after being concentrated up to 5.0 mL.

Derivatization

The dried extracts were incorporated to hexane (200 µL) and trifluoroacetic acid (50 µL). Following an accurate 30 second vortex in a vortex mixture, this solution was left to stand for exactly five minutes. Finally, this solution received 1.95 mL of a 9:1 acetonitrile and water combination.

Derivatization of standards

The standard aflatoxin B1, G1, B2, and G2 at known concentrations (20 ppb, 40 ppb, and 80 ppb) were obtained and derivatized in the same way as samples.

HPLC analysis

The analysis was achieved by using water (70 parts), acetonitrile (17 parts), and methanol (17 parts) as the solvent system under fluorescence detection (Waters, USA) and by injecting 20 µL of the derivatized sample (drug extracts and standards) into HPLC column (C18; 15 cm x 0.46 cm) through 1.0 mL min^{-1} flow. Aflatoxin peak concentrations in medication samples were compared to standards' peak concentrations (B1, G1, B2, and G2) and were then assessed.

Pesticide determination

Sample preparation for pesticides

50 mg of each sample were dissolved in methanol, and then 1.0 gram of sodium oxalate, 100 mL mixture of diethyl ether and petroleum ether (50: 50) were mixed and shook for a minute. After adding the organic layer into a separator funnel containing 600 mL of saturated solution of sodium chloride. After removing the polar fraction, the process was carried out two or three more times. Following the application of the sodium sulfate solution to the organic layer, the mixture was collected and allowed to evaporate up to 2 to 5 mL. After mixing this concentrated solution with acetonitrile and petroleum ether (30 mL each), run over the column and eluted with diethyl ether. Rotavapor (Buchi, R-215, Switzerland) was used to condense the solution up to 5.0 mL and analysed in GC-MS (16).

Results

Morphology

The dried *Amomum* fruit is brownish-black in colour, has a potent aromatic scent, and tastes spicy. It measures between 1.5 and 2.5 cm long and 1.2 and 1.8 cm in wide. It has seeds that are 0.4 cm long with 0.3 cm width, irregular ovoid with three flattened faces, and brown to dark brown in colour. They have a fragrant scent and a spicy, pungent flavour (Fig. 1A).

The cassia leaves are yellowish green, oblong-lanceolate to lanceolate in shape, and 7.5 - 10 cm long (Fig. 1B).

Figure 1: Dried *Amomum* fruits (A) and dried leaves of *Cinnamon caesia* (B) representing the morphological characters

Microscopy

Cassia Leaf

Anatomy of the leaf

The leaf is made up of an even, smooth lamina and a robust midrib. Wide, short adaxial bud formed by midrib that is somewhat elevated. The thick and wide flattened section is noticeably projected from the abaxial part. 730 mm thick is the midrib. The abaxial portion is 450 m wide and 200 m thick. Broad schlerenchyma zones with thick lignified walls can be found in the adaxial and abaxial parts. The schlerenchyma region's inner cells are parenchymatous and heavily tannified (Fig. 2).

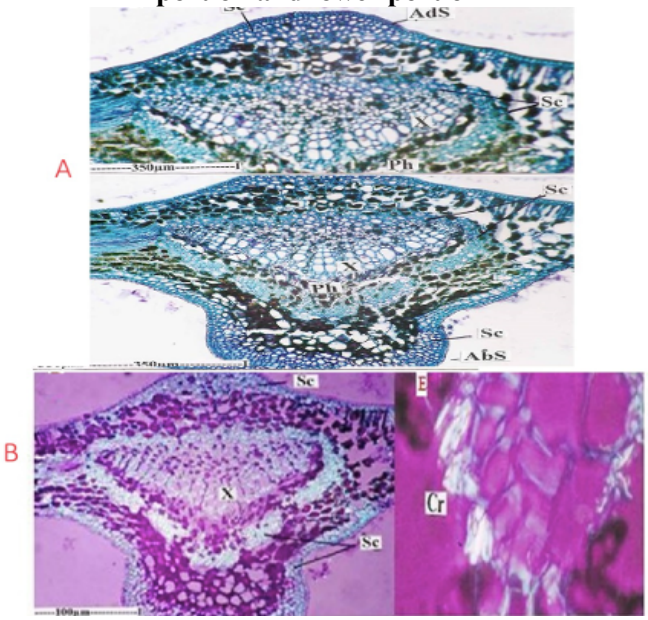
The lower portion of the xylem in the vascular strand has a thin coating of phloem covering a vast shallow area of xylem elements. The xylem components are arranged in brief parallel rows. They have wide, thin walls. Adaxial plates and schlerenchyma layers make up the vascular strand. The fibers that round the vascular

strands contain sporadic calcium oxalate spherocrystals (Fig. 2).

Lamina

Lamina thickness is 400 m. It differentiates into an adaxial zone of palisade cells and an abaxial spongy parenchyma and is clearly dorsiventral. The palisade zone is made up of a single band of 100 m-tall, thin, cylindrical palisade cells that have been vertically extended. Wide air chambers are formed by the tiny, strongly lobed, and loosely organised spongy parenchyma cells. The lateral vein's vascular strands are noticeable and have thick bundle sheath fibers that are vertically prolonged. In the palisade zone, wide, circular secretory cavities are frequent. The cuticle of the adaxial epidermal cells is smooth, and the cells have very thick walls. Abaxial epidermal cells (Fig. 2) have a dome shape and are notably pappilate.

Figure 2: TS of *C. cassia* through midrib - upper portion and lower portion



(A) (Abs.- Abaxial side; Ads.- adaxial side; Ph.-phloem; Sc.-sclerenchyma; X- xylem), TS of midrib-viewed under polarized light to show the lignifications of the xylem and fibres (B). Crystals located in the bundle sheath fibres (under polarized light) (Cr. Crystals; Sc. Sclerenchyla sheath; X- Xylem elements)

Amomum Seed

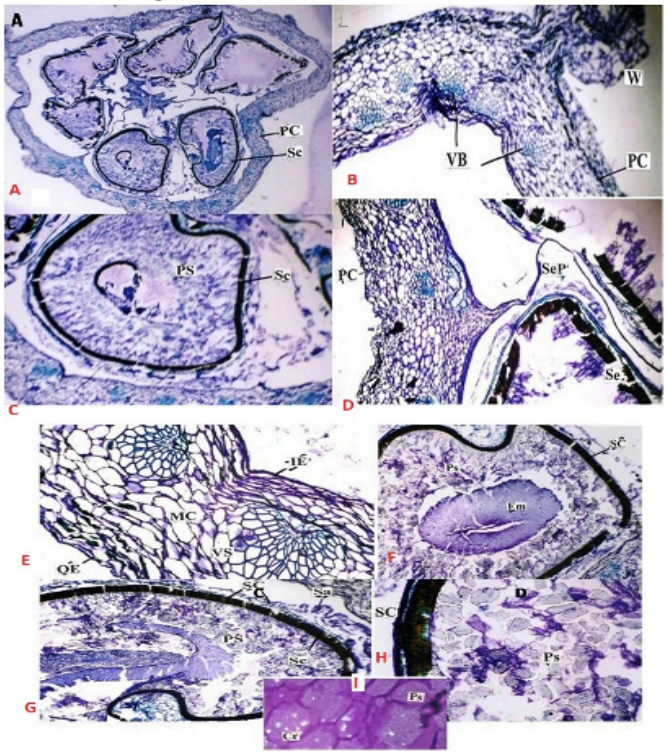
Structure of the fruit

The fruit is a capsule with three chambers that is tricarpellary syncarpous. Two seeds are placed on the center axile of each chamber. The fruit features a few small ridges and three shallow furrows. The cutise interior of the fruit is home to six relatively big seeds (Fig. 3).

Fruit wall/pericarp

On the outside of ridges, the pericarp may be seen to have three quite long and thick wings. The pericarp has a thickness of 80 µm. It was associated with a narrow inside epidermal portion with tiny cellular structures along lean external epidermal layer. There are 15 to 20 layers of stretched, compact, thin-walled parenchyma cells between the epidermal layers. The fruit's mesocarp is represented by the thin section. All over the fruit wall, there are prominent, circular or wedge-shaped vascular strands that range in thickness from 200 to 300 µm. They are made up of lignified wide, angular, compact xylem components. Phloem components are broken down and invisible. The inner epidermis, which is made up of a thin layer of crushed cells, is the pericarp's innermost layer (Fig. 3). The seed coat is thick, and the perisperms are tightly packed. The outer, broad, undulating parenchymatous sarcotesta and the inner, dense, deeply pigmented sclerotic sclerotesta make up the seed coat (testa). The sclerotests have palisade-like, thin, columnar cells. The walls are lignified and thick. The sclerotic layer thickness is 70 µm. Masses of the perisperm are dispersed and ill-organized. The big embryo is situated in the middle of the perisperm. Most of the perisperm's cells include starch granules with minute calcium oxalate structures. The prismatic crystals have dispersed throughout perisperm cells (Fig. 3).

Figure 3: TS of Fruit- entire view



(A), A portion of the pericarp and seed-enlarged (B), A portion of the fruit with seed enlarged (C) and Pericarp and wing portion of the fruit (D), Showing more clear picture of pericarp with vascular strands (E), Sectional view of the seed with centrally placed embryo A portion of the seed (F-G) Seed coat and perisperm- enlarged (H) and Crystals in the perisperm as seen under the polarised light (I) (Abbrebiations in figures: Pc-Pericarp; Se-seed; VB-vascular bundles of the pericarp; W-wing portion of the pericarp; Ps- perisperm, Cr-Crystals; Em-embryo, SC-Seed coat; IE-inner epidermis; Mc- mesocarp; OE-outer epidermis; ; Sc-sclerenchyma layer; VS-vascular strand)

Physicochemical evaluation

To ensuring the quality herbs as well as its chemical and physical characteristics, the water content must be determined. The determination of water is important to check the herb quality and the basic principle of Karl Fischer's titration, is based on the reaction between iodine and sulphur dioxide under water medium. He found that the water content can be measured using this reaction in a water free environment with a higher amount of sulfur dioxide. The cassia leaves lost 6.81 % and amomim seed lost 8.79 % of its original weight after drying, and 5.26 and 6.73 % moisture were estimated in both the drugs, respectively. Inorganic compounds like carbonates, silicates, oxalates, and phosphates can be identified using total ash. Organic material is lost during heating as CO2, leaving the inorganic components left. Ash value is a crucial element of a medicine, and using this parameter, we may ensure the quality and purity of given herbal sample along with degree of adulteration. The ash values of various medications vary significantly, yet they typically do so within very specific bounds for the same substance. High acid insoluble ash content and the presence of silica are signs of earthly component contamination. By analyzing an aqueous dissolved portion of total ash, the amount of elements other than organic matter has been detected. It was found that the cassia and amomum had 3.78 and 3.20 % w/ w of total ash. While the proportions of ash that were solubilized in water and undissolved in acid were found to be 1.12 and 2.38 % in cassia leaves whereas 1.45 and 0.73% in amomum seeds, respectively. The drugs were demonstrated to have a slight acidic nature when the P^H of the 1.0 and 10.0% solutions were found as 6.53 and 5.57 for cassia leaves and 5.23 and 5.97 for amomum seeds, respectively. A drug's extract yield in a given solvent is frequently a rough indicator of how much of a certain ingredient is present in the drug. In order to obtain accurate and trustworthy values, the medications have to extract using various solvents in terms of polarity. Petroleum ether, alcoholic, and aqueous extractives are typically considered when determining a drug's standard. Oily constituents and resins are come in the non-polar extract, but when it is heated to a 105 °C, the volatile substances are volatilized away, leaving resin, coloring material, and fixed oil. Although alcohol may almost always dissolve a compound, it is typically employed to calculate the extractive index for medicines that contain glycosides, resins, alkaloids, etc. For medications that primarily consist of water-soluble ingredients, water is employed. When sequential extraction was done, the methanol extract produced the highest yield, while petroleum ether produced the lowest yield. Methanolic extract had the highest extract yield in soluble extraction, too. Cassia leaf and amomum seed physicochemical properties were all tested in triplicate and the findings are shown in Table 1 with standard deviation.

Phytochemical Screening

Cassia leaf

The standard qualitative chemical tests have been performed to detect different primary and secondary metabolites on both the samples for the phytochemical screening. Chemical tests observations of the methanolic extract of cassia leaves showed that it contained terpenoids, carbohydrates, amino acids, phenolic and flavonoidal chemicals.

***Amomum* Seed**

By adopting similar methodology, tests were also performed for the phytochemical investigations of amomum seed extract. Terpenoids, tannins, saponins, proteins, amino acids, and phenolic substances were detected by the chemical testing.

TLC/HPTLC profiling

***Cassia* leaf**

For the various extracts, various solvent systems were tested using a hit-and-miss methodology. The optimum resolution of components has been achieved in the mobile phase hexane: Chloroform (3: 1, v/v), hexane: Ethyl acetate (19: 1, v/v), and hexane: Ethyl acetate (4: 1, v/v) for petroleum ether, chloroform and methanol extract, respectively. Chromatograms have been created using the samples and the appropriate solvents. When the TLC plate of petroleum ether extract has been sprayed with anisaldehyde reagent for visualizing the spots, the results were favorable. Anisaldehyde sulphuric acid was poured onto the produced, air-dried chromatogram, and it was then heated to 110°C for 10 minutes (until spots became visible). The optimal wavelength (450 nm for petroleum ether) was chosen after scanning the chromatograms at several visible wavelengths based on peak absorbance maxima. The findings of scanning the methanolic extract chromatograms at 366 nm were good (Fig. 4A & B). Petroleum ether and methanol extract's HPTLC fingerprints revealed the presence of 8, and 10 components, respectively (Table 2).

***Amomum* Seed**

For the various extracts, various solvent systems were tested using a hit-and-miss methodology. In solvent solutions, effective constituent separation was achieved. For the methanol and petroleum ether extract, the ratios were ethyl acetate: toluene: methanol (5: 5: 0.5, v/v/v) and formic acid: ethyl acetate: toluene (1: 4: 5, v/v/v), respectively. Chromatograms were created using the samples and the appropriate solvents. Both extract's chromatograms performed better after being used anisaldehyde reagent. Anisaldehyde sulphuric acid was poured onto the produced, air-dried chromatograms and they were then heated to 110°C for 10 minutes (until spots became visible). The optimal wavelength 530 and 560 nm were found to be suitable to petroleum ether and methanol extract based on peak's resolution and intensity after the scanning of chromatograms at various visible wavelengths (Fig. 4C & D). The HPTLC fingerprints data with respect to sample, solvent system,

wavelength used and numbers of spots were depicted in Table 2.

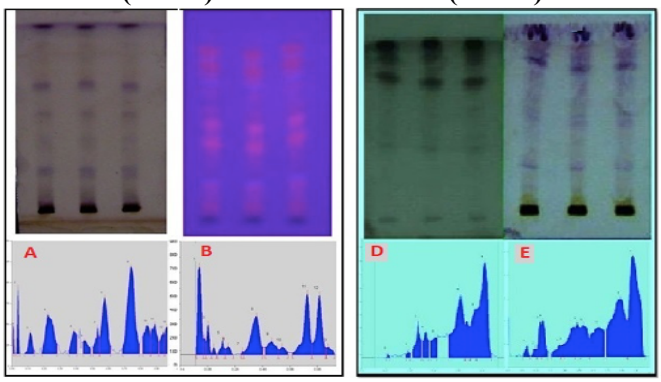
Safety evaluation

A precise scientific definition of the raw material serves as the foundation for the quality standards for herbal medications. Physical constants should be studied to standardize the crude drug, and adulterations must be verified for identification and quality. The study of pesticides, toxic metals and aflatoxins are the crucial factors for safety.

Test for heavy or toxic metal analysis

One should be aware that when using herbs as a form of treatment for various illnesses, they may also have hazardous side effects due to the presence of heavy metals and other contaminants. Controlling the amount of impurities in pharmaceutical raw materials is crucial for these reasons. Atomic absorption spectrometry is frequently advised among the several techniques reported in the literature for the trace measurement of the aforementioned metals in plant material (17-19). Products made from ayurvedic herbal medicine that contain heavy metals are widely accessible, reasonably priced, and recommended for both adults and children in most South Asian countries. Lead, mercury, and arsenic were present in some of the most widely used Ayurvedic herbal medicines. Reports of heavy metals content in phyto-pharmaceuticals are beyond the limit have been discussed by researchers (14). Therefore, Lead, cadmium, arsenic, and mercury were tested in amomum seed and Cassia leaf powder and were determined to be within acceptable limits (Fig. 5 A, B, C and Table 3).

Figure 4: Developed plates and HPTLC chromatograms of petroleum ether and methanol extracts of cassia leaf (A & B) and amomum seed (C & D)



Aflatoxins

Aflatoxins are mycotoxins made by some Aspergillus fungi, which can be found on various foods and therapeutic herbs. Aflatoxin toxins fall into one of four categories: B1, B2, G1, or G2. An extra unsaturation in aflatoxins B1 and G1 causes the formation of reactive epoxide during hepatic metabolism, making them more hazardous than aflatoxins B2 and G2. These have the potential to severely harm the human body's key organs' structural and functional integrity. According to research, aflatoxins B1 may be a cause of human hepatocellular carcinoma (20-21). When food or medicinal plants are produced or stored, certain environmental factors, such as excessive moisture and temperature, encourage the chances of Aspergillus and aflatoxins infectivity. Different samples of amomum and cassia were examined for the presence of aflatoxins after being subjected to an HPLC analysis for aflatoxins. Aflatoxin is not present in the samples of almond and cassia, according to the chromatograms of mixed standard aflatoxins and samples (Fig. 5 E, F, and G).

Pesticides

Since it has been demonstrated that pesticides can cause a number of health risks in humans, including cancer (22), neurological conditions (23), and male infertility (24), many nations have established their limits for pesticides in therapeutic herbs. Some reports demonstrating many estimation techniques for identifying pesticides in therapeutic plants (25). Gas chromatography is an appropriate method for this (26); therefore, pesticide estimate by GC-MS was done. After comparing the chromatograms and retention times of the standard sample and the 31 pesticides (including 12 organophosphorus insecticides, 19 organochlorine and pyrethroid insecticides), it was demonstrated that the amomum and cassia have not been found to have any of these.

Figure 5-Calibration plot of different standard metals (ABC and D), HPLC chromatograms of mixed standard aflatoxins showing peaks of B1, B2 and G1, G2 (E) along with chromatograms of Amomum seed (F) and cassia leaf (G) samples

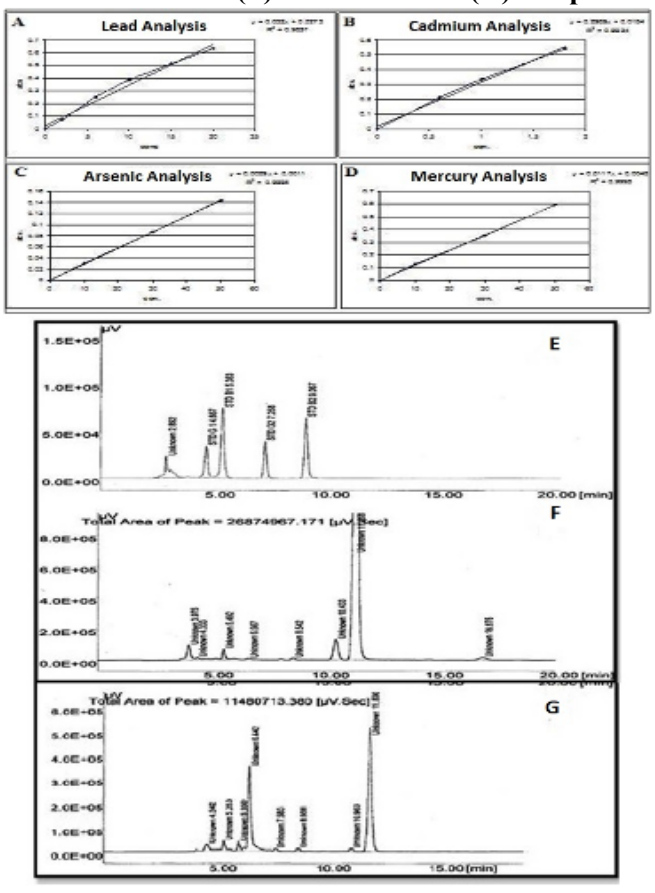


Table 1: The results of physicochemical evaluation of both drug samples (n=3)

S. No	Parameters		Cassia	Amomum
			(mean ± SD*)	
1	**Foreign matter**		1.37 ± 0.31	0.83 ± 0.071
2	**LOD**		6.21 ± 0.29	7.73 ± 0.17
3	**Moisture content**		5.76 ± 0.31	6.03 ± 0.91
4	**Ash values** (% w/w)	Total Ash	3.34 ± 0.22	3.16 ± 0.18
		Acid insoluble Ash	1.28 ± 0.12	1.62 ± 0.14
		Water soluble Ash	2.18 ± 0.18	0.68 ± 0.07
5	**pH**	10 % solution	6.42 ± 0.14	5.12 ± 0.19
		1 % solution	5.27 ± 0.18	5.07 ± 0.24
6	**Successive extraction** (% w/w)	Petroleum ether	2.04 ± 0.27	1.82 ± 0.15
		Chloroform	1.26 ± 0.25	3.12 ± 0.36
		Acetone	2.22 ± 0.14	0.68 ± 0.16
		Methanol	15.18 ± 0.39	3.84 ± 0.16
		Water	14.04 ± 0.68	13.83 ± 0.75
7	**Soluble extractives** (% w/w)	Ether soluble extractives	6.73 ± 0.38	2.87 ± 0.21
		Water soluble extractives	13.88 ± 0.76	17.92 ± 1.53
		Alcohol soluble extractives	11.82 ± 0.92	7.05 ± 0.64

Table: 2-HTLC fingerprint data of different extracts of both drugs with individual solvent system, detection UV wavelength and detected R_f Values of respected phyto-constituents.

S. No.	Plant Name	Type of Extract	Solvent system	Detection	No of spots and R_f values
4	Cassia (*Cinnamomum cassia*)	Petroleum ether extract	Chloroform: hexane: (1: 3, v/v)	At 450 nm*	**(8)** 0.13, 0.22, 0.32, 0.37, 0.42, 0.49, 0.64, 0.96
		Methanol extract	Ethyl acetate: hexane (1: 4, v/v)	At 366 nm	**(10)** 0.1, 0.17, 0.21, 0.31, 0.41, 0.53, 0.6, 0.79, 0.87, 0.94
6	Amomum (*Amomum subulatum*)	Petroleum ether extract	Methanol: ethyl acetate: Toluene (0.5: 5: 5, v/v/v)	At 530 nm*	**(9)** 0.09, 0.26, 0.34, 0.39, 0.48, 0.69, 0.74, 0.81, 0.89
		Methanol extract	Formic acid: ethyl acetate: Toluene (1: 4: 5, v/v/v)	At 560 nm*	**(11)** 0.17, 0.30, 0.36, 0.38, 0.47, 0.57, 0.59, 0.70, 0.74, 0.78, 0.87

*After spraying with anisaldehyde sulphuric acid

Table: 3-Results of heavy metals analysis of both the samples along with limit set by the World Health Organization

Sample	Results (in ppm)			
	Lead	Cadmium	Mercury	Arsenic
Amomum seed	0.09859	0.00718	0.48	0.503
Cassia leaves	0.12378	0.00486	0.24	0.283
Limit (safe up to)	10	0.3	0.50	3.0

Discussion

The present work has been based on the Unani and Ayurvedic systems of medicine, which offers a wide range of formulations that are effective in curing a number of chronic diseases. But these formulations are not accepted globally because of inadequate quality control. In the current study, two medications were chosen from a list of 63 plants that a Persian physician named Ibn-E-Sina reported as having both preventive and therapeutic effects on cardiac disorders. These plants are a component of some unani formulas that have been used to treat and protect against heart problems. The chosen herbs are also utilized as ingredients in Khamira, a semisolid remedy that is well-known for its tonic properties as well as its cardioprotective properties. The medications were standardised in accordance with WHO recommendations with particular attention to microscopy, chemo-profiling by thin layer chromatography, and safety evaluation. The chosen medications were procured, and a pharmacognostical assessment was accomplished in which macroscopic and microscopic characteristics were examined. Foreign materials, drying loss, moisture content, ash values, pH values, extractive values by subsequent extraction, and soluble extractives were all considered in the physicochemical evaluation. By creating various solvent systems with thin layer chromatography, different extracts of each medication were chemo-profiled. A precise scientific definition of the raw material serves as the foundation for the quality standards for herbal medications. Physical constants should be studied to standardize the medicine, and adulterations must be verified for identification and quality. The estimation of contaminants is crucial factors for ensuring the safety in terms of heavy metals, aflatoxins, and pesticides.

One should be noticed that when using herbs as a form of treatment for various illnesses, they may also have hazardous side effects due to the presence of heavy metal contaminants. Controlling the amount of impurities in pharmaceutical raw materials is crucial for these reasons. Atomic absorption spectrometry is frequently advised among the several techniques reported in the literature for the trace measurement of the aforementioned metals in plant material (17–19). Lead, mercury, and arsenic were present in some of the most widely used Ayurvedic herbal medicines. Theses toxic metals were tested in both medication powders and found to be within the limits, studies on the

presence of these toxic metals in botanical preparation beyond established norms are also available (14).

Aflatoxins are mycotoxins made by some Aspergillus fungi, which can be found on different food products and therapeutic herbs. Aflatoxin toxins fall into one of four categories: B1, B2, G1, or G2. The occurence of extra double bond in aflatoxins B1 & G1 causes the production of harmful epoxies during hepatic metabolism, making them more hazardous than aflatoxins B2 and G2. These have the potential to severely harm the human body's key organs' structural and functional integrity. According to research, aflatoxins B1 many a time found to cause of the human hepatocellular carcinoma (20, 27). When medicinal food or herbs are harvested or stored, certain environmental factors (moisture and temperature) accelerate the development of Aspergillus and aflatoxins harmful infections. Both the herbal drugs were assessed to find the aflatoxin content using the HPLC method. Aflatoxin was not found in any of the drug samples, according to the chromatogram of combined standard aflatoxins and samples.

Many nations have fixed their highest acceptable residual levels of pesticides in herbal drugs as a result of research showing that pesticides can cause a number of health risks in humans, including cancer (21), neurological diseases (22–23), and male infertility (28). In order to identify pesticides in medicinal plants, various analytical techniques are shown in the literature (24). Gas chromatography is an appropriate method for this (25-26, 29-31); therefore, pesticide detection was achieved through GC-MS. Followed by comparing the chromatograms of the standard pesticides viz. 12 organophosphorus insecticides, 19 organochlorine and pyrethroid insecticides and both drug samples, it has been concluded that there were no pesticides in the medications.

Conclusion

Thus, the information provided is very helpful in describing the microscopic characteristics of cassia leaf and amomum seed in detail. The chemo-profiling of various extracts using thin layer chromatography is exceptional in terms of separating chemical compounds with various polarities. The World Health Organization's strategy for ensuring the safety of plant-based medicines is strongly emphasized by the heavy metal analysis, aflatoxins evaluation, and pesticides assessment.

Declaration of Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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