

# Phytochemical Screening and Standardization of *Kaempferia galanga* L. leaves

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**Abstract:** Standardization of drugs means confirmation of its identity and determination of its quality and purity. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. The most common plant secondary metabolites occur in the following groups; alkaloids, anthraquinones, coumarins, essential oils, flavonoids, steroids and terpenoids.

**Keywords:** Phytochemicals, bioactive constituents, standardization, evaluation

## I. INTRODUCTION

The subject of phytochemistry, or plant chemistry, has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. Methods are needed for separation, purification and identification of the many different constituents present in plants [1]. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. The crude drugs, its standardized extracts or the phytopharmaceuticals needs to be studied extensively for its quality, purity, potency, safety and efficacy [2].

Evaluation of crude drugs involves the determination of authenticity, quality, purity, potency, safety, efficacy, reliability and reproducibility [3]. On the basis of the types of studies carried out for various evaluation of the quality of drugs. The ash of any organic material that is composed of their nonvolatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material [4]. This value varies with fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs [5]. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. The moisture requirements for the active growth of some of the common molds and bacteria that may be found in or on drugs are relatively low [6]. Therefore, the drying process should reduce the moisture content of the drug below this critical or threshold level [7].

## II. PLANT PROFILE

Common names: Aromatic ginger, Resurrection lily, Sand ginger, Cekur, Kencur.

Vernacular names: Malayalam: Kachhulam, katjulam; Tamil: Kacholum, Pulankilanku; Hindi: Chandramula, Sidhoul [8]

*Kaempferia galanga* L. is a rhizomatous medicinal plant belongs to the family, Zingiberaceae **Figure 1**. It is native to India and distributed in China, Bangladesh, Myanmar, Sri Lanka, Japan, Thailand, Indonesia, Malaysia, Vietnam, Java and Sudan, Nigeria and South Africa [9]. The edible fresh leaves of *Kaempferia galanga* L. represent a potential food value. The constituents present is moisture, protein, starch, soluble sugar, lipid, dietary fibre, ash. It exhibits the highest level of Potassium, Calcium, Magnesium, Iron and Copper. Its rhizome contains volatile oil, several alkaloids, starch, protein, amino acids, minerals and fatty matter [10]. The major chemical constituents of the essential oil of *Kaempferia galanga* L. are ethyl-trans p-methoxy-cinnamate, pentadecane, 1, 8-cineole, g-carene and borneol. In addition, it contains camphene, kaempferol, kaempferide, cinnamaldehyde, p-methoxy cinnamic acid and ethyl cinnamate [11]. *Kaempferia galanga* L. has traditionally been used as an expectorant, stimulant, diuretic, carminative and anti-pyretic. In addition, *Kaempferia galanga* L. is used to treat diabetes, hypertension, cough, asthma, joint fractures, rheumatism, urticarial, vertigo and intestinal injuries [12]. Leaves and flowers of *Kaempferia galanga* L. contain flavanoids. The leaves possess antioxidant, antinociceptive and anti-inflammatory activities that help in treatment of mouth ulcers and headaches [13]. Leaf infusions can be used as a beneficial drink for pregnant women. The ashes of leaves are rubbed on swollen breasts after child birth while fresh leaves are chewed for relieving coughs [11].

## III. MATERIALS AND METHODS

The leaves of *Kaempferia galanga* L. were collected from Punalur, Kollam District, Kerala on 18.06.2022 and authenticated by Botanist Dr. Jacob Thomas M.Sc., B. Ed., Ph. D, FISCA, Asst. Professor and Research Guide, Mar Thoma College, Tiruvalla on 20.06.2022 and the herbarium deposited in the department with the Accession number MTCHT 2056.

### Extraction method

Maceration is the extraction method used for the extraction of plant material. The coarsely powdered leaves material **Figure 2** is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for seven days. The content is stirred periodically and is placed inside bottle it should be shaken time to time to ensure

complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath [14].

### **Phytochemical screening**

The phytochemical screening method is performed with the aqueous and alcoholic extract of the plant leaf extract.

#### **Test for alkaloids**

**Dragendorff's test:** 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange red precipitate formed indicates the presence of alkaloids.

**Wagner's test:** 1mL of extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. Appearance of reddish-brown precipitate signifies the existence of alkaloids.

**Mayer's test:** 1mL of extract was taken and placed into a test tube. Then 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids.

**Hager's test:** 1mL of solution of an extract was taken and placed into a test tube. Then 1mL of saturated ferric solution (Hager's reagent) was added and shaken. Formation of yellow-coloured precipitate indicates the existence of alkaloids.

#### **Test for glycosides**

**Bontrager's test (modified):** One gram of the crude extract was first weighed, placed into a test tube, and dissolved in 5mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution was added. The mixture was shaken and placed over water bath. Then the mixture was allowed to boil for 10min, cooled, and filtered. Afterward, the mixture was then extracted again with benzene. Finally, equal volume of ammonia solution was added to benzene layer. Appearance of pink colour indicates the presence of anthraquinone glycosides.

**Legals test:** 1mL of an extract was taken, and then an equal volume of sodium nitroprusside was added followed by few quantities of sodium hydroxide solution and shaken. Formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside.

**Keller-Killiani test:** 2mL of the extract was taken and diluted with equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride were added. Again, the whole mixture was placed into a test tube containing 2mL of sulphuric acid. Emergence of reddish-brown layer that turns bluish green implies the presence of digitoxose.

#### **Test for steroids and triterpenoids**

**Liebermann Burchard's test:** This method is utilized for an alcoholic extract. Extract need to dry out first through evaporation, then extracted again with chloroform. Add few drops of acetic anhydrites followed by sulphuric acid from the side of the test tube. Formation of violet to blue-coloured ring at the junction of the two liquids indicated the presence of steroids.

**Salkowski's test:** 1mL solution of the extract was taken and 2mL of chloroform was added, shaken, and filtered. Few drops of concentrated sulphuric acid were added to filtrate, shaken, and allowed to stand. Development of golden-yellow precipitate indicates the presence of triterpenes.

#### **Test for tannins**

**Ferric chloride solution test:** To 1ml of the extract, ferric chloride solution was added. Formation of a dark blue or greenish black colour shows the presence of tannins.

**Gelatine's test:** 1mL of extract was taken and placed in a test tube. Then 1% gelatine solution containing sodium chloride added and shaken. Appearance of white precipitate indicates the presence of tannins.

**Lead acetate test:** A few ml of 10% lead solution was added to the test solution. The formation of a voluminous white precipitate indicates the presence of tannins.

#### **Test for flavonoids**

**Shinoda's test:** 1mL of extract was taken and placed into a test tube. Then, few drops of concentrated hydrochloric acid were added followed by 0.5mg of magnesium turnings and shaken. Emergence of pink coloration indicates the presence of flavonoids.

**Lead acetate test:** To detect the presence of flavonoids, 1mL of extract was taken and placed into a test tube. Then few drops of lead acetate added and shaken. Formation of yellow precipitate signifies the presence of flavonoids.

**Alkaline reagent test:** 1mL of extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. Emergence of intense yellow colour that turns to colourless after adding dilute acid implies the existence of flavonoids.

#### **Test for protein**

**Biuret test:** Some quantity of an extract was taken and 4% sodium hydroxide solution of the drug was produced. This is followed by the addition of 1% copper sulphite. Appearance of violet colour implies the existence of peptide linkage.

**Ninhydrin test:** 1mL of an extract was taken and placed into a test tube. Then 0.25% of Ninhydrin reagent was added and shaken. The mixture was then boiled for few minutes. Formation of blue colour signifies the presence of protein.

**Xanthoproteic test:** 1mL of the extract was taken and placed it into a test tube. Then few drops of nitric acid were added and shaken. Emergence of yellow-colour indicates presence of protein.

#### **Test for carbohydrate**

**Molish's test:** To 2-3 ml of test solution, added few drops of Molish's reagent solution and was shaken. Concentrated sulphuric acid was added from the sides of the test tube. Violet ring was formed at the junction of two liquids.

**Fehling's test:** To 1 ml of the test solution, equal quantities of Fehling's solution A and B was added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

**Iodine test:** To 3 ml of test solution few drops of iodine solution is added. Blue colour appeared which was disappeared on boiling and reappeared on cooling.

Benedict's test: To 5 ml of Benedict's solution, add 1 ml of the test solution and shake each tube. Place the tube in a boiling water bath and heat for 3 minutes. Remove the tube from the heat and allow them to cool. Formation of green-red or yellow precipitate has been observed.

Test for fats and oil

Solubility test: Oils are soluble in ether, benzene and chloroform, but insoluble in ethanol and water.

Filter paper test: Filter paper gets permanently stained with oils.

Test for saponins

Foam test: The extracts were vigorously shaken with water. Persistent foam formation indicates presence of saponins.

Lieberman Burchard's test: To drug extracts few drops of glacial acetic acid and 2 drops of concentrated sulfuric acid were added.

Colour changes from rose red, violet, blue to green reveals the presence of steroidal saponins [15].

### **Standardization methods**

#### **Determination of Total Ash Value**

Weigh and ignite flat, thin, porcelain or a tarred silica crucible. Weigh about 2g of the powdered drug into the dish or crucible. Support the dish on a pipe-dry triangle placed on a ring tripod stand. Heat with a burner, using a flame about 2cm high and supporting the dish about 7cm above the flame, heat till vapour almost cease to be evolved, then lower the dish and heat more strongly until all the carbon is burnt off. Cool in a desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug

#### **Determination of Acid-insoluble Ash Value**

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug, further by using 25ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into a 100ml beaker. Place a wire gauze over a Bunsen burner and boil for 5 min. Filter through an ash less filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter paper and residue together into the crucible; heat gently until vapour cease to be evolved and then more strongly until carbon has been removed. Cool in a desiccator. Weigh the residue and calculate acid insoluble ash of the crude drug with reference to the air-dried sample of the crude drug.

#### **Determination of Water-Soluble Ash Value**

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug, further by using 25ml of water, wash the ash from the dish used for total ash into a 100ml beaker. Place a wire gauze over a Bunsen burner and boil for 5 min. Filter through an ash less filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter paper and residue together into the crucible; heat gently until vapour cease to be evolved and then more strongly until carbon has been removed. Cool in a desiccator. Weigh the residue and calculate water soluble ash of the crude drug with reference to the air-dried sample of the crude drug.

#### **Determination of Alcohol-soluble Extractive Value**

Weigh about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a 100ml graduated flask to the delivery mark with a solvent 90% alcohol, wash out the weighing bottle and pour the washings together. Cork the flask and set aside for 24 hours shaking frequently (maceration). Filter into a 50ml cylinder, when sufficient filter was collected, transfer 25ml of the filtrate to a weighed, thin porcelain dish as used for the ash value determination. Evaporate to dryness on a water bath and complete the drying in an oven at 100°C. Cool in a desiccator and weigh. Calculate the percentage w/w of extractive with reference to the air-dried drug.

#### **Determination of Water-Soluble Extractive Value**

Weigh about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a 100ml graduated flask to the delivery mark with, chloroform water wash out the weighing bottle and pour the washings together. Cork the flask and set aside for 24 hours shaking frequently (maceration). Filter into a 50ml cylinder, when sufficient filter was collected, transfer 25ml of the filtrate to a weighed, and thin porcelain dish as used for the ash value determination. Evaporate to dryness on a water bath and complete the drying in an oven at 100°C. Cool in a desiccator and weigh. Calculate the percentage w/w of extractive with reference to the air-dried drug.

#### **Determination of Moisture (Loss on drying)**

Weigh about 1.5g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100°C or 105°C. Cool in a desiccator and watch. The loss in weight is usually recorded as moisture [15].

## **IV.RESULTS**

### **Morphological characters**

Colour : Dark green; Odour : Aromatic characteristics; Taste : Pungent; Size : 8-15 cm wide and length varies; Shape: Round ovate.

## Figures and Tables

Figure 1 *Kaempferia galanga* L.

Figure 2 Dried powdered leaf drug

Table 1 Phytochemical screening

Sl. no.	Chemical tests	Aqueous extract of <i>Kaempferia galanga</i> L.leaves	Ethanollic extract of <i>Kaempferia galanga</i> L. leaves
1	Alkaloids	-	+
2	Glycosides	-	+
3	Steroids and triterpenoids	+	-
4	Tannins	+	+
5	Flavonoids	+	+
6	Protein	-	+
7	Carbohydrate	-	+
8	Fats and oils	-	+
9	Saponins	+	-
10	Volatile oils	+	+

(+) Present, (-) Absent

Table 2 Physiochemical analysis

Sl No:	Parameters	Percentage(% w/w)
1	Total ash	17.5
2	Acid insoluble ash	2.5
3	Water soluble ash	6.5
4	Alcohol soluble extractive	25.6
5	Water soluble extractive	20
6	Moisture content	5.5

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