ABSTRACT
Cassia auriculata, Centella asiatica and Zingiber officinale are belongs to the family Leguminosae, Apiaceae and Zingiberaceae respectively. These herbs possess abundant remedial benefits and are used traditionally to treat numerous illnesses. The standardization of these crude drugs is essential to gain information on their identity and quality. WHO recommended parameters were used to standardize the plant materials. Dried raw powder of Cassia auriculata leaf, Centella asiatica leaf and Zingiber officinale rhizome were subjected to macroscopic and microscopic evaluation followed by physicochemical evaluations such as loss on drying, ash values and extractive values. Further, the plant materials were extracted with hydroalcoholic solvent (Alcohol: Water ratio is 70:30) by cold maceration process and subjected to qualitative phytochemical investigation. The identity and purity of the plant material had been proven by its organoleptic characteristics. Detailed structural characteristics were obtained from powder microscopy. Physico-chemical parameters displayed the quality of raw powder. Qualitative phytochemical analysis showed the presence of various phytoconstituents. The pharmacognostical standardization has provided with a referential information on the identity, quality and purity of the crude drugs.

Keywords: Cassia auriculata, Centella asiatica, Microscopy, Physicochemical, Standardization, Zingiber officinale.

INTRODUCTION
Herbal medicines have gained greater importance in the modern world, mainly due to their safety and efficacy as compared to the synthetic drugs [1]. Plants are believed to have mystical and supernatural healing powers. They are thought to be the base for many medicines [2]. Its popularity and demand are rising day by day. Due to their accessibility and affordability, WHO encourages and recommends the use of herbal medications in the treatment of various ailments. This led to the inception of distinct active research to establish efficient standardized extracts and to isolate new lead phytoconstituents. However, inadequate quality control and standardization has hampered the acceptability of herbal remedies in the developed countries. Herbal medications are susceptible to contamination and degradation which might cause variation in their active constituents resulting in reduced therapeutic efficacy [3].

It is vitally essential to standardize herbal drugs to reduce the amount of adulteration and misinterpretation. Therefore, in the present study, an effort has been made to standardize the leaves of Cassia auriculata, Centella asiatica and dried rhizome of Zingiber officinale by assessing both qualitative and quantitative parameters. This might be crucial in developing a referential standard for a specific herb.

Cassia auriculata L (CAr) often known as “Tanner’s cassia” (Caesalpiniaceae) is a medicinal plant, which grows abundantly all over India [4]. CA is a branching shrub with smooth, brown bark and branchlets that are densely pubescent. Its leaves have alternate, stipulate and slender in nature. It has bright yellow colored irregular, bisexual flowers blooming throughout the year. Fruits are short legume, oblong, thin and pale brown colored [5]. Traditionally, the leaves of CA are used for the treatment of diabetes, liver diseases, jaundice, ulcers and leprosy [6]. Additionally, it possesses antiviral, antipyretic and antispasmodic property [7].

Centella asiatica (CAs) commonly called Indian pennywort (Umbelliferae) is widely distributed in the areas of India, Asia and the Middle East. It is a perennial herb that can reach a height of 30 cm and has fan-shaped leaves [8]. It is widely used in CNS disorders like schizophrenia, epilepsy and cognitive dysfunction. It is also used in renal stones, leprosy, skin diseases, asthma and anorexia. In addition to that, it can also be used in the management of diarrhoea, jaundice, cholera, measles, leukorrhoea, hematemesis, urethritis, hepatitis, toothache, smallpox, syphilis, rheumatism and varices. It has antipyretic, analgesic and anti-inflammatory property [9].
Ginger is a common spice in the Indian kitchen. It is an underground rhizome of the plant Zingiber officinale Roscoe belonging to the family Zingiberaceae. Ginger rhizomes have pungent, thick lobed, pale yellowish leaves that are narrowly oblanceolate and alternate distichously. Z. officinale has long been used to treat a variety of illnesses, including nausea, vomiting, cough, asthma, palpitations, inflammation, pain, loss of appetite, dyspepsia, constipation and indigestion in Ayurveda, Siddha, Chinese, Arabian, and African medical systems.

**MATERIAL AND METHOD**

**Procurement of Chemicals and reagents**

All the chemicals and reagents used in this study have been procured from Sisco Research Laboratories Pvt. Ltd., Taloja, Maharashtra, India and Yarrow Chem Products, Mumbai, Maharashtra, India.

**Plant material**

In the present study, leaves of *Cassia auriculata*, leaves of *Centella asiatica* and rhizomes of *Zingiber officinale* were collected from Nagamangala Taluk, Mandya District, Karnataka. Plant parts were authenticated in the herbarium centre, Foundation for Revitalization of Local Health Traditions (FRLHT), 74/2, Jarak Bande Kaval, Post Attur, Via Yalahanka, Bangalore, Karnataka, India (FRLHT Acc. No. of plants is 5551, 5552 and 5553 for *Centella asiatica*, *Cassia auriculata*, and *Zingiber officinale* respectively. The plant materials were cleaned thoroughly to make them free from contamination. Parts were dried under shade for more than a week and subjected to a coarse powder. Powders were stored in an air-tight container and kept in a clean hygienic place for further use.

**Macroscopic and Microscopic Evaluation**

Macroscopic evaluation gives extensive information on the identity and purity of the material. Various organoleptic characteristics like physical appearance, taste and odour of the powdered crude drug had been evaluated and compared with reference samples.

Powder microscopy gives detailed structural characteristics of the drugs. Microscopic examination has been done by mounting a pinch of the powdered sample on a microscopic slide followed by the addition of a drop of phloroglucinol and concentrated hydrochloric acid. The characteristics of raw powder were observed using Inverted Biological microscope (FM-BM-B200, Fison Instruments Ltd, Glasgow G2 4JR, UK).

**Physico-chemical Evaluation**

The crude plant materials were subjected to physico-chemical evaluation such as loss on drying, total ash, acid insoluble ash, water soluble ash, water soluble extractive, alcohol soluble extractive and ether soluble extractive value. The amount of water and volatile substances in the crude drug is determined by the loss on drying. The excess water in the crude drug will foster microbial growth that leads to deterioration. The presence of water content in the crude drug provides information about the quality and shelf life of the drugs. The total ash method is used to measure the total quantity of material that remains after ignition. Acid-insoluble ash is the residue leftover after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This determines the amount of silica present. Water-soluble ash is the difference between the weight of total ash and the residue following the treatment of the total ash with water. The extractive value calculates the extent of active ingredients that can be extracted using suitable solvents from a crude extract. The extraction of a crude drug with a specific solvent gives a solution containing various phytoconstituents that provides preliminary indications on sample quality.

**Preparation of Extract**

Extraction is the primary step associated with phytochemical investigation. Ethanol is considered to be the universal solvent for extraction. In the present study hydro-alcohol (Alcohol: Water ratio is 70:30) was used as an extracting solvent. The dried powder of *Cassia auriculata* leaves, *Centella asiatica* leaves and *Zingiber officinale* rhizome were extracted by cold maceration method.

**Preliminary Phytochemical Investigation**

Qualitative phytochemical analysis of *Cassia auriculata* leaf extract, *Centella Asiatica* leaf extract, *Zingiber officinale* rhizome extract were carried out using standard procedures.

**RESULTS AND DISCUSSION**

**Macroscopic and Microscopic evaluation**

The macroscopic characteristics of crude plants powder is depicted in Table 1 and microscopic features are given in Table 2 and Figure 1 to 3 respectively.

| Name of the plant & part | Nature | Colour | Odour | Taste |
|--------------------------|--------|--------|-------|-------|
| *Cassia auriculata* leaves | Coarse powder | Green | Pungent | Bitter |
| *Centella asiatica* leaves | Coarse powder | Yellowish green | Faintly aromatic | Slightly bitter |
| *Zingiber officinale* rhizome | Coarse powder | Light brown or Buff | Pleasant, Aromatic | Pungent |

| Plant name | Observation |
|------------|-------------|
| *Cassia auriculata* (Dried Leaf powder) | Presence of unicellular trichome, Epidermal cells with paracytic stomata, Simple prismatic crystals, Polygonal epidermal cells. |
| *Centella asiatica* (Dried Leaf powder) | Presence of uniserite unicellular glandular and covering trichome, Polygonal epidermal cells, Epidermal cells with stomata, Simple prismatic crystals, Epidermal cells with calcium oxalate crystals, Pitted vessels. |
| *Zingiber officinale* (Dried rhizome powder) | Presence of Scalariform xylem vessels, Oleoresins, Parenchyma cells, Simple starch grains, cork, Group of fibres. |
**Figure 1:** Microscopy of Cassia auriculata dried leaf powder. Where A: Unicellular Trichome, B: Epidermal cells with paracytic stomata, C: Simple prismatic crystals, D: Polygonal epidermal cells (Magnification: 10X, Staining agent: Phloroglucinol-HCl).

**Figure 2:** Microscopy of Centella asiatica dried leaf powder. Where A1: Unicellular glandular trichome, A2: Unicellular covering trichome, B: Polygonal epidermal cells, C: Epidermal cells with stomata, D: Simple prismatic crystals, E: Epidermal cells with calcium oxalate crystals, F: Pitted vessels (Magnification: 10X, Staining agent: Phloroglucinol-HCl)
Physico-chemical Evaluation

The results of loss on drying are shown in Table 3. The total ash, acid insoluble ash, water soluble ash values are given in Table 4, and the results of water-soluble extractives, alcohol soluble extractives and ether soluble extractives are depicted in Table 5.

Table 3: Loss on drying

| Plant name     | LOD (% w/w) |
|----------------|-------------|
| Cassia auriculata | 8.8%        |
| Centella asiatica      | 7.6%        |
| Zingiber officinale     | 10.8%       |

Table 4: Ash content

| Plant name             | Total ash (% w/w) | Acid insoluble ash (% w/w) | Water soluble ash (% w/w) |
|------------------------|-------------------|-----------------------------|---------------------------|
| Cassia auriculata      | 7.66%             | 2%                          | 2.33%                     |
| Centella asiatica      | 9%                | 3.66%                       | 2%                        |
| Zingiber officinale     | 5.66%             | 1.3%                        | 1%                        |

Table 5: Extractive value

| Plant name             | Water soluble extractive (%) | Alcohol soluble extractive (%) | Ether soluble extractive (%) |
|------------------------|-----------------------------|--------------------------------|-----------------------------|
| Cassia auriculata      | 27%                         | 26%                            | 6%                          |
| Centella asiatica      | 24%                         | 23%                            | 6%                          |
| Zingiber officinale     | 19%                         | 16%                            | 5%                          |

Phytochemical Analysis

The chemical tests for various Phytoconstituents in the extract were carried out and the results are displayed in Table 6.

Table 6: Phytochemical Analysis

| Phytoconstituents | Cassia auriculata | Centella asiatica | Zingiber officinale |
|-------------------|-------------------|-------------------|---------------------|
| Carbohydrates     | +                 | +                 | +                   |
| Proteins          | +                 | +                 | +                   |
| Alkaloids         | +                 | +                 | +                   |
| Glycosides        | +                 | +                 | +                   |
| Phenolic compounds| +                 | +                 | +                   |
| Flavonoids        | +                 | +                 | +                   |
| Tannins           | +                 | +                 | +                   |
| Sterols           | +                 | +                 | +                   |
| Saponins          | +                 | +                 | +                   |
| Terpenoids        | +                 | +                 | +                   |

CONCLUSION

Pharmacognostical standardizations an integral part of establishing the correct identity and quality of a crude drug. This can be achieved by evaluating its macroscopy, microscopy and physico–chemical parameters. The present study provides various qualitative and quantitative standards of Cassia auriculata leaves, Centella asiatica leaves and Zingiber officinale rhizome. This pharmacognostical standardization will provide referential information on the identity, quality and purity of the crude drugs and will prevent their adulteration with a drug of the same or other genus having low potency.

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Conflict of interest

Declared None
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