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### Analysis of Primary and Secondary Metabolite Profile of *Costus speciosus* (Koen Ex.Retz.) Sm. Rhizome

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#### ABSTRACT

*Costus speciosus* (Keon. Ex Retz.) Sm. has been used as a medicinal plant by various traditional healers and its rhizome has been consumed as a vegetable especially in Assam and also in some other parts of India. The primary and secondary metabolites play a vital role in the growth and maintenance of health of plants. The study of their role in human diet is a prime area of scientific research and various plants have been studied for their contribution to human nutrition. The present study showed that the rhizome of *C. speciosus* has an energy value of  $159.63 \pm 4.12$  kcal/100 g with high protein content ( $24.05 \pm 0.67\%$ ) which contributes to approximately 60% of the total energy value. The rhizome is also a source of secondary metabolites like phenolics, flavonoids, tannins, saponins, steroids and glycosides which are reported for various biological effects including antimicrobial and antioxidant activities. The findings of the study suggest that the rhizome of *C. speciosus* has the potential for use in diet for nutritive and health benefits.

**Key words:** *Costus speciosus*, Primary metabolites, Secondary metabolites

#### INTRODUCTION

Plants have great significance due to their nutritive value and are a major source of not only nutrition but also medicines. Plants have served mankind throughout the history of human civilization [1]. 30 to 40% of the currently available drugs are based on the medicinal & curative properties of various plants and are in use as herbal supplements, botanicals and nutraceuticals [2,3]. Wild edible plants, many of which are potentially valuable as alternative food for human beings, can play an important role to strike a balance between population explosion and limited agricultural productivity, especially in developing countries [4]. Most of the developing countries depend on starch-based food products as primary source of energy and proteins. This fact partly accounts for the prevailing protein deficiency in such countries as recognized by Food and Agricultural Organization [5]. In India, Malaysia and Thailand, about 150 wild plants species have been identified which have the potential of use as emergency foods [6].

Medicinal plants are important not only for their biologically active secondary metabolites but also for their primary metabolites like carbohydrates, proteins and lipids. These primary metabolites are centrally important for the growth and development of a plant and are carriers of chemical energy to the subsequent trophic levels of the food chain. The carbohydrates, proteins and fats are referred to as the proximate principles and form the major portion of diet of herbivores and omnivores [7]. On the other hand, secondary metabolites are produced as by-products of metabolic pathways and though not essential for the survival of the plant, are important in their defence system. A variety of secondary metabolites has been isolated from plants and includes alkaloids, tannins, polyphenols, quinines, flavonoids, coumarins, terpenoids and saponins. These major groups represent classes of structurally and chemically diverse groups of compounds that exert strong physiological effects in humans. Their therapeutic properties have been utilized since long and research is still in progress to explore their applications as medicines [8, 9].

Considering the extensive research on medicinal plants, the present study was conducted to evaluate the availability of primary and secondary metabolites in a traditionally used plant of Assam, India. For the purpose of study, *Costus speciosus* (Keon. Ex Retz.) Sm. rhizome was selected since different part of the plant has been consumed as a vegetable by the natives and by people in other parts of the country. [10, 11]. The rhizome is used in the traditional system to treat bronchitis, fevers, dyspepsia, inflammations, anaemia, rheumatism, lumbago and hiccough [12]. The natives of North-east India use the rhizome in urinary troubles, fever, headaches and also to dissolve kidney stones [13, 14].

## MATERIALS AND METHODS

The plant samples were collected from Dibrugarh University campus (27°29' N 95°00'E- 27.48°N 95°E) and identified botanically at the Department of Life Sciences, Dibrugarh University, Assam, India and the voucher specimen (DULSC 464) was deposited in the department. The rhizomes were washed, cleaned and sliced before shade drying. The dried material was ground into fine powder in an electric blender.

### Quantitative estimation of Primary metabolites:

#### Determination of moisture content

The moisture content in the plant samples was determined by following standard procedures outlined by AOAC, 1990 [15]. 5g of fresh, clean plant material was taken in a flat-bottom dish and kept overnight in an air oven at 100–110°C and weighed. The loss in weight was calculated and reported as moisture content in mg per g of fresh plant material.

#### Determination of ash

The ash remaining following ignition of medicinal plant materials was determined by three different methods as outlined by WHO, 1998 [16]. These measured total ash, acid-insoluble ash and water-soluble ash.

#### Determination of total carbohydrate content

Total carbohydrate content was determined by the method of Sadasivam & Manickam, 1996 [17]. Standard curve of glucose was prepared using different concentrations to calculate the amount of carbohydrate in the samples.

#### Determination of total protein content

Total protein was estimated by Folin–Ciocalteu method as modified by Lowry et al. 1951 [18].

A standard curve was prepared using different concentrations of bovine serum albumin (BSA) to calculate the amount of protein in the samples.

#### Determination of total lipids

Total lipids were determined by the method as outlined by Aberoumand, 2010 [19]. Two grams of powdered air-dried plant material was taken in a porous thimble. The thimble was placed in the extraction chamber of a Soxhlet apparatus containing petroleum ether (b.p. 40–60°C) and was extracted for 8 hours. The extract was taken in a pre-weighed beaker. The beaker was heated in the oven at 100°C to evaporate the solvent, then cooled in a desiccator, and weighed.

#### Determination of crude fibre

Crude fibre content was determined by the method of Aberoumand, 2010 [19]. It was estimated by acid-base digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> (prepared by diluting 7.2 ml of 94% conc. acid of specific gravity 1.835 g/ml per 1000 ml distilled water) and 1.25% NaOH (12.5 g per 1000 ml distilled water) solutions.

#### Determination of energy value

The energy value of each plant sample was determined from the percentage of total lipids, total carbohydrates and total proteins using the formula as given below [20].

$$\text{kilocalories (kcal)/100 g} = 9 \{ \text{Total lipids (\%)} \} + 4 \{ (\text{Total carbohydrates (\%)} + \text{Total proteins (\%)}) \}$$

### Qualitative analysis of secondary metabolites:

#### Preparation of extracts:

The dried plant material was extracted successively by cold maceration in petroleum ether, ethyl acetate and ethanol in the ratio 1:10 w/v for 48 hours with intermittent shaking. The extracts were dried in IKA RV 10 rotatory vacuum evaporator. The residues thus obtained were stored aseptically at 5°C for further use. The aqueous extract was prepared by soaking dried plant material in sterile distilled water in the ratio 1:10 w/v (containing 1% chloroform)

for 48 hours with intermittent shaking. The extract was filtered through a double layer muslin cloth followed by centrifugation at 3500 rpm for 20 minutes. The supernatant was filtered through Whatman No. 1 filter paper, and then by 0.2  $\mu$ m membrane filter. The extract was dried and preserved aseptically at 5°C for further use.

Qualitative analysis of secondary metabolites was performed for alkaloids, saponins, flavonoids, phenols and tannins, sterols, cardiac glycosides and anthraquinone glycosides by following the standard methods [21, 22].

**Alkaloids:** (Extract + 1% HCl, filtered) 1ml of filtrate+ few drops of Dragendorff's reagents/Mayer's reagents/ Hager's reagents/ Wagner's reagent, Orange brown precipitate/ Cream colored precipitate/ Yellow precipitate/ Red brown precipitate respectively indicated the presence of alkaloids.

#### **Tannins and Phenolics:**

- Small quantity of the extract dissolved in distilled water + 10% Lead acetate solution, white precipitate indicated the presence of tannins.
- Small quantity of the extract dissolved in distilled water + few ml of 1% gelatin + 10% sodium chloride, white precipitate indicated the presence of tannins.

#### **Flavonoids:**

- Plant residue + 10% NaOH, yellow coloration indicated the presence of flavonoids.
- Extract + con.  $\text{H}_2\text{SO}_4$ , formation of yellow or orange color indicated the presence of flavonoids.

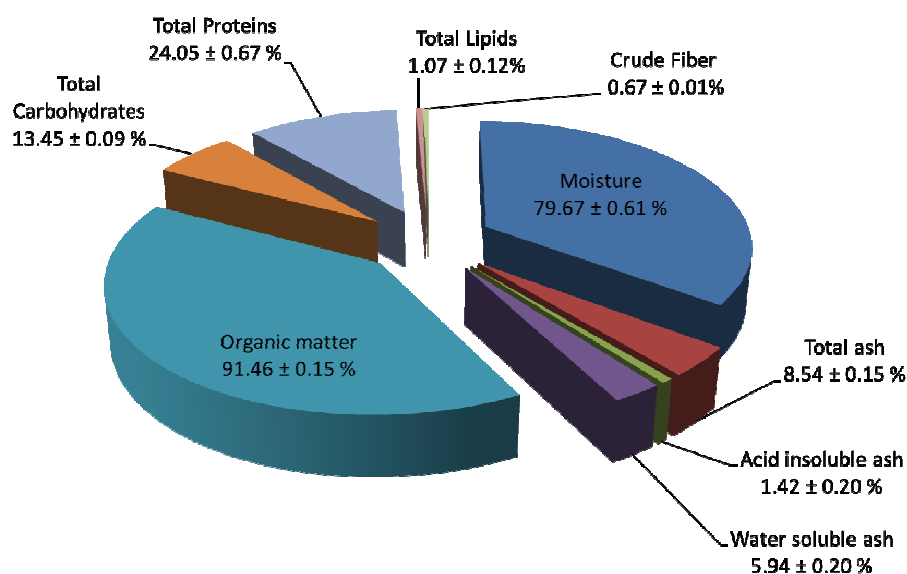
#### **Saponin:**

- Foam Test: small quantity of the residue was diluted with distilled water to 20 ml and shaken vigorously; formation of one cm layer of foam which was stable for 10 minutes indicated the presence of saponin.
- Alcoholic extract + Sodium bicarbonate + shaken well, honey comb like frothing confirmed the presence of saponin.

**Cardiac glycosides:** Kellar-Killani test: 2 ml of extract + 1 ml glacial acetic acid + one drop 5%  $\text{FeCl}_3$  + Conc.  $\text{H}_2\text{SO}_4$ , reddish brown color appears at junction of the two liquid layers and upper appears bluish green, indicates the presence of cardiac glycosides.

**Anthraquinone glycosides:** 5 ml of extract + 5 ml 5%  $\text{FeCl}_3$  + 5 ml dilHCl + Heat, cool + chloroform, shaken well, separated the organic layer and added dilute ammonia solution. Pinkish color indicated the presence of anthraquinone glycosides.

**Figure 1: Primary metabolites in *C. speciosus* rhizome**



#### **Steroids:**

- Liebermann-Burchard's test: The extract was dissolved in chloroform and 1-2 mL of acetic anhydride was added along with few drops of con.sulphuric acid. Formation of red colour which turn into blue and finally into green colour indicated the presence of steroid.

b) Liebermann's test: The extract was dissolved in 3 mL of acetic anhydride and heated. On cooling few drops of con. sulphuric acid was added. Formation of blue colour indicated the presence of steroid.

c) Salkowski test: The extract was mixed with 2 ml of chloroform and equal volume of con. sulphuric acid and shaken well. Formation of red colour in chloroform layer and greenish yellow fluorescence in acid layer indicated the presence of steroid.

### Statistical Analysis

Statistical analysis was performed using SigmaStat 3.5 and the results were expressed as the mean of the three replicates  $\pm$  standard deviation of the replicates.

## RESULT AND DISCUSSION

Table 1: Physical characteristics of *C. speciosus* rhizome extracts

Extract	Color	Texture
Petroleum ether	Creamish- white	Soapy
Ethyl acetate	Light brown	Soapy
Ethanol	Brown	Soapy
Aqueous	Dark brown	Powder

Table 2: Secondary metabolite analysis of *C. speciosus* rhizome

Phytochemicals tested	Name of the test	Observations			
		Petroleum ether extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids	a Dragendroff's Reagent	-	-	-	-
	b Mayer's Reagent	-	-	-	-
	c Hager's Reagent	-	-	-	-
	d Wagner's reagent	-	-	-	-
Phenolics & Tannins	a Ferric Chloride Test	-	-	+	+
	b Lead Acetate Test	-	-	+	+
	c Gelatin Test	+	-	+	+
Flavonoids	a Shinoda	-	-	-	+
	b Sodium Hydroxide Test	+	+	+	+
	c Lead Acetate Test	-	-	+	+
	d FeCl <sub>3</sub> - Amyl alcohol- NaOH Test	+	+	+	+
	e Test for Flavones	+	+	+	+
	f Test for Coumarins	+	-	+	-
Saponins	a Foam test	-	-	+	+
	b Sodium bicarbonate test	-	-	+	+
Steroids	a Liebermann-burchard's test	+	+	-	-
	b Liebermann's test	+	+	-	-
	c Salkowski test	+	+	-	-
Glycosides	a Borntrager's test for Anthraquinone glycosides	-	-	-	+
	b Kellar – Kiliani test for Cardiac glycosides	+	+	+	-
+ = Present		-	= Absent		

The primary metabolite profile of *C. speciosus* is shown in Figure 1. The ash content, which is a measure of mineral content, was obtained as  $8.54 \pm 0.15\%$ . The acid insoluble ash which represents the "non-physiological" ash is obtained

from extraneous matter (e.g. dust, sand and soil) adhering to the plant surface. It was found to be  $1.42 \pm 0.20\%$  while the water soluble ash was recorded as  $5.94 \pm 0.20\%$ . The moisture content was found to be  $79.67 \pm 0.61\%$ . Moisture content is known to affect the processing, preservation and storage of food and herbal products [23]. High moisture content renders plant products susceptible to microbial attack and thus leads to spoilage and lowered shelf life [24]. The crude fibre content was found to be  $0.67 \pm 0.01\%$ . Crude fibre represents the non-digestible carbohydrates and lignins. The content of crude fibre is known to enhance digestibility but high levels lead to intestinal irritation, lowered digestibility and decreased nutrient absorption [25]. The content of crude fibre in *C. speciosus* rhizome is less than the rhizomes of *Alpinia zerumbet*, *A. officinarum*, *A. galanga*, *A. calcarata* and *Kaempferia galanga* [26] and may be considered appropriate for consumption [24]. Lipids are a rich source of energy and aid in the transport of fat soluble vitamins, insulate and protect internal tissues and contribute to important cell processes [27, 28]. The total lipid content in *C. speciosus* rhizome was found to be  $1.07 \pm 0.12\%$ . The total protein content was obtained as  $24.05 \pm 0.67\%$  which was 3-5 times higher than that of *Alpinia zerumbet*, *A. officinarum*, *A. galanga*, *A. calcarata* and *Kaempferia galanga* [26]. According to Pearson (1976), a plant-based food that provides more than 12% of its calorific value from protein is considered as a good source of proteins [29]. In the case of *C. speciosus* rhizomes, total proteins contribute to approximately 60 % of the total calorific value, thus making it a rich source of proteins. The plant is a moderate source of carbohydrates ( $13.45 \pm 0.09\%$ ) when compared with the Recommended Dietary Allowance (RDA) values for children, adults, pregnant and lactating mothers [30]. The nutritive value was calculated to be  $159.63 \pm 4.12$  kilocalories (kcal) per 100 grams which was lower than that for the other rhizomes as reported by Indrayan et al, 2009 [26].

The physical properties of the extracts prepared using petroleum ether, ethyl acetate, ethanol and water are shown in Table 1. The four extracts of *C. speciosus* rhizome showed the presence of most of the secondary metabolites viz. phenolics, tannins, flavonoids, saponins, steroids and glycosides (Table 2). Alkaloids were absent in all the four extracts. Phenolic compounds are reported to exert a wide spectrum of biological effects such as- antioxidant and free radical scavenging activity and antimicrobial activity [31, 32]. Similarly, tannins are well known for their antioxidant and antimicrobial properties as well as for skin regeneration, anti-inflammatory and diuretic properties [33, 32]. Flavonoids are widely recognised for exerting antioxidant, antimicrobial, anti-carcinogenic and antitumor properties [33, 32]. Many pharmacological activities such as antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer activities have been reported for saponins [34]. Steroids have been reported to exert analgesic properties [35] while cardiac and anthraquinone glycosides are reported to have antibacterial and antifungal activity [36, 37]. The presence of these secondary metabolites in *C. speciosus* rhizome suggests that *C. speciosus* may be a potent source of pharmacologically active metabolites and further research in this regard may yield novel compounds.

## CONCLUSION

The present results show that *C. speciosus* rhizome is a rich source of primary metabolites and has the potentiality of use as a non-conventional food to supplement the nutritional needs of the under-nourished population. Due to the ignorance of the wild plants as food products, there is a high demand of commercial crops, the production of which is seldom enough to meet the nutritional requirement of the population. Hence, it becomes essential and urgent to create community awareness so that the people may accept the wild food plants to be as useful as the cultivated ones. Moreover, the presence of different types of secondary metabolites in the studied plant makes it suitable for health benefits and for use in herbal medicine to combat different diseases.

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