

Estimation of Biochemical and Amino acid Compositions of *Zingiber zerumbet* (L.) Sm — Wild Ginger

¹Gandhi, K, ^{2*}Saravanan, S

Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai-600 030, Tamil Nadu, India.

*Corresponding author's Email: sstc1965@yahoo.co.in

Abstract: *Zingiber zerumbet* (L.) Sm. a wild ginger used as herbal medicines all over the world for a wide array of ailments. The present study aimed to determine the biochemical and amino acid compositions in the methanol extract of the rhizome. The percentage of total protein, total carbohydrates and total lipid contents were 16.27 ± 0.13 , 20.43 ± 0.28 and 19.99 ± 0.13 respectively. The methanol extract of the rhizome was found to contain 20 different amino acids with varied quantities which are expressed in $\mu\text{g/g}$ dry wt. Among amino acid compositions, Tryptophan (612.30 ± 0.115) was present in higher amount, whereas, serine (0.4467 ± 0.31) was present in lesser amounts. The study concluded that the rhizome of the *Zingiber zerumbet* was found to be a good source of nutraceutical agent.

Keywords: *Zingiber zerumbet*, wild ginger, Biochemicals, Amino acid, Nutraceutical.

I. INTRODUCTION

Medicinal plants are generally known as “Chemical Goldmines” as they contain natural chemicals, which are acceptable to human and animal systems. Green plants synthesize and preserve a variety of bio chemical products, many of which are extractable and are used as chemical feed stocks or as raw material for various scientific investigations and industrial utilization. Many secondary metabolites of plants are commercially used as pharmaceutical compounds. Human beings depend on plants for their various needs since the beginning of civilization [1]. In India, the usages of medicinal plants to cure specific ailments have been in vogue from ancient times. The Indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. These systems of medicine cater to the needs of nearly 70% of our population residing in the villages. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal in nature. About 5000 species are extensively used in traditional systems of medicine but the research work on these species is still insufficient [2].

Zingiber zerumbet (L.) Sm. is a monocot herbaceous and rhizomatous perennial belonging to the Zingiberaceae family. It is a wild ginger with high medicinal properties and a well known medicinal plant, employed to cure various diseases. No wonder ginger is called “MahaOushadhi” in Ayurveda, the ancient science of life. Ginger is an important “dravya” in very many Ayurvedic preparations. The rhizomes of *Z. zerumbet* are used like the officinal ginger (*Z. officinale*). It is employed as a hot remedy for coughs, asthma, worm's leprosy and other skin diseases. It is one of the reputed drugs of Ayurvedists and is employed in indigenous systems of medicine for very long period. Traditionally, the *Z. zerumbet* is used in the treatment of swelling, sores and loss of appetite, the juice of the boiled rhizomes is also used as a medicine for worm infestation in children [3]. Decoction prepared from the rhizome is used to treat jaundice [4]. The rhizome of *Z. zerumbet* is used to relieve stomachache and macerated in alcohol as tonic or stimulant in China and in Southeast Asia and it is used for the treatment of fever, constipation and to relieve pain [5]. Although, it is used traditionally for long period of time but the data on the quantitative biochemical and amino acid analysis in this highly medicinal plant is less. Hence, the aim of the present study is to analysis the biochemical and amino acid compositions of the rhizome of *Z. zerumbet*.

II. MATERIAL AND METHODS

Mother plant source

The seed rhizomes *Zingiber zerumbet* (L.) Sm. was collected from Spices Board, Ministry of Commerce & Industry Government of India, Cochin, Kerala, India. The seed rhizomes were cultivated in the garden and the well grown one year old rhizomes were used for this study.

Sample preparation

The rhizome of *Z. zerumbet* was shade dried, dehydrated and pulverized using electric blender. The powder sample was stored in a container in the dark and experimental study was performed in triplicates from the pulverized rhizome powder.

Estimation of total protein

The total protein was evaluated using the Folin-Ciocalteu Phenol method of Lowry *et al.*, [6].

Estimation of total carbohydrates

The total carbohydrate was evaluated following the Phenol-sulphuric acid method of Dubois *et al.*, [7].

Estimation of total lipids

The extraction of lipid was determined by the chloroform-methanol mixture by following Folch *et al.*, method [8].

Amino acid analysis

Amino acids were determined by high-performance liquid chromatography (HPLC) according to the method described by Rajendra [9]. Hydrolysis tube containing a rhizome powder of 75 mg was added with 2 ml of 6.0 N HCL. The solution was incubated in an oven at 110°C for 18 h and dried in vacuo using rotavapor. Equal volume (20µL) of the OPA reagent and amino acid sample was added in a vial together for derivatization for 2 min. After this, 50µL of 1M Borate buffer with pH of 9.0 was added and mixed well. Filtered and derivatized amino acid sample (20µL) was injected into a HPLC containing a C18 reverse phase, ion exchange chromatography (Shimatzu-High Performance Liquid Chromatography LC 6A) and were analyzed using sodium acetate buffer with tetrahydrofuran (THF), triethylamine (TEA) and sodium acetate with methanol, acetonitrile as mobile phase A and B respectively. A variety of amino acid standards were injected simultaneously. By comparing the sample retention time (Rt) with that of the standard amino acids run at identical conditions, the amino acids present in the sample were identified and quantified.

Statistical analysis

The data were analyzed statistically using the SPSS 16.0 software (SPSS Inc., Chicago, USA) and the mean values are expressed as mean \pm SE of three experiments. The significance of differences among means was carried out at $p < 0.05$ probability level using Duncan's Multiple Range Test (DMRT).

III. RESULTS AND DISCUSSION

The rhizome is widely used in a variety of food preparation because of its nutritional composition and flavouring compounds. The *Z. zerumbet* rhizomes are rich source of carbohydrates, proteins and lipids. The rhizome extract was subjected to a quantitative estimation of total protein, total carbohydrate, total lipids and amino acids, the results were summarized.

Biochemical compositions

The rhizome extract of *Z. zerumbet* demonstrated a significant biochemical composition and the results are all expressed in percentage (Table -1). Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport, storage; mechanical sustentation, growth and cellular differentiation control [10]. According to earlier reports of various researchers, the total protein content varied in different plant species. The total protein content of rhizome was $16.27 \pm 0.13\%$. The total protein composition was the least among the biochemical composition in the present study. Zanariah *et al.*, [11] reported that the total protein content of some Zingiberaceae species, 0.48g/100g of protein in the rhizome of *Z. zerumbet*, 0.7g/100g of protein in *Z. officinale*, 0.9g/100g of protein in rhizome of *Curcuma domestica*, 0.8g/100g of protein in *Alpinia galanga* and 0.4g/100g in *Curcuma mangga*. Similarly, the total protein content 31.5% in *Z. officinale* and 10.5% in *C. longa* was reported [12].

Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes [13]. The soluble carbohydrates analysed by Hashimah *et al.*, [14] ranged from 2.14g to 5.96g in different Zingiberaceae species. In the present study, the total carbohydrate content of dried rhizome was found to be $20.43 \pm 0.28\%$. Similarly, total carbohydrate content of various species of Zingiberaceae was reported viz., 76.9% in *Alpinia officinarum*, 78.9% in *A. galanga*, 76.0% in *A. zerumbet*, 75.0% in *A. calcarata* and 76.0% in *Kaempferia galanga* [15]. In *Zingiber cassumunar* rhizome, the total carbohydrate content was $387.33 \pm 6.69 \mu\text{g/mL}$ [16]. The total carbohydrate content of rhizome of *Z. zerumbet* was 8.90g/100g, in *Z. officinale* 8.10 g/100g as reported by Zanariah *et al.*, [11].

The lipids are important constituents of the diet because of their high energy value, and are a heterogeneous group of compounds related to fatty acids and include fats, oils, waxes and other related substances. Lipids are rich in -C=O- bonds, providing much more energy in oxidation processes than other biological compounds [17]. In the present study, the total lipid content of dried rhizome was found to be $19.99 \pm 0.13\%$. Similarly, the total lipid content of various species was also reported by different researchers. Zanariah *et al.*, [11] reported that the total fat content of rhizome of *Z. zerumbet* was 0.7g/100g, in *Z. officinale* 0.9g/100g, in *Curcuma domestica* 0.5g/100g, in *Alpinia galanga* 0.7g/100g and in *Curcuma mangga* 1.2g/100g. Variation in quantity of the lipid content in the present study may be related to the changes in temperature and light intensity, geographical origin and seasonal periods [18].

TABLE 1: Biochemical analysis of rhizome of *Zingiber zerumbet**

S. No	Bio Chemicals	Percentage (%) of dry Weight
1	Total Protein	16.27 ± 0.13^a
2	Total Carbohydrate	20.43 ± 0.28^b
3	Total Lipids	19.99 ± 0.13^c
F- Value		130.888
P- Value		0.00

*Values are expressed as Mean \pm SE, n=3. Means in each column with different superscripts

Letters are significantly different at $p < 0.05$.

Amino acids estimation

The dried rhizome powder of *Z. zerumbet* consists of 20 different amino acids with various quantities in percentage of dry weight. Totally 20 amino acids have been identified in the protein hydrolysate of rhizome of *Z. zerumbet*. Out of 20 amino acids which 11 Essential Amino Acids (EAA) and 9 Non Essential Amino Acids (N-EAA) were present (Table 2 and 3). The amino acid analysis of rhizome, showed a relatively high composition of total essential amino acid with 62.79%, whereas the percentage of non-essential amino acid was 37.21%. The maximum and minimum percentage of essential amino acids were Tryptophan 16.45% and Tyrosine 1.17% respectively, whereas, non-essential amino acid Aspartic acid showed a higher amount of 12.255% and Glutamine showed minimum amount 0.37%. The amino acid composition is one of the common criteria for nutritive quality of protein in food and the amino acids are the basic structures of proteins and each type of protein depends on the arrangement of the amino acids. Increasing amino acids content could be related to the degradation of proteins under elevated CO_2 conditions and hydrolysis to free amino acids [19]. Till date, there are no contemporary data available on the amino acid composition of the proteins of rhizome of *Z. zerumbet*. Generally, amino acid profile is an indication of the nutrition value of the protein component [20]. The main amino acids in most of the species studied are aspartic acid and glutamic acid. However, bonglai and lempoyang have rather high phenylalanine content of over 19g/100g protein. Turmeric, ginger and greater galangal rhizomes which are commonly used in cooking, are rich in lysine, an essential amino acid deficient in most cereals especially wheat [11]. Jeevani Osadee Wijekoon *et al.*, [21] reported that, essential and non-essential amino acids to constitute 47.1% and 52.9%, respectively of the total protein in *Etlingera elatior*. Some of the amino acids derived from different food sources have proven their potential as antioxidants, antimicrobial, anti-inflammatory and immune stimulating agents [22]; [23]. Hence, the presence of these in rhizome of *Z. zerumbet* provides a more nutraceutical purpose.

Amino acids are precursors for the synthesis of secondary metabolites such as alkaloids, which provide chemical defense for plants that confer beneficial physiological effects in consumers. Alkaloids provide protection to plants from a variety of herbivores and some of them possess significant pharmacological activity such as analgesic, antibacterial and antibiotic [24].

TABLE 2: Amino acids content of rhizome of *Zingiber zerumbet**

S.No	Amino acids	($\mu\text{g/g}$ Dry weight)
1	Aspartic acid	456.26 \pm 0.224 ^f
2	Glutamic acid	191.53 \pm 0.145 ^l
3	Asparagine	45.64 \pm 0.145 ^d
4	Serine	0.4467 \pm 0.31 ^a
5	Glutamine	13.6167 \pm 0.222 ^b
6	Glycine	67.8467 \pm 0.112 ^e
7	Threonine	91.1467 \pm 0.181 ^g
8	Arginine	104.63 \pm 0.120 ⁱ
9	Alanine	95.5800 \pm 0.061 ^h
10	Cysteine	109.47 \pm 0.087 ^j
11	Tyrosine	43.4100 \pm 0.106 ^c
12	Histidine	198.53 \pm 0.145 ^m
13	Valine	78.4667 \pm 0.088 ^f
14	Methionine	184.90 \pm 0.173 ^k
15	Iso leucine	217.60 \pm 0.115 ^o
16	Phenylalanine	209.27 \pm 0.120 ⁿ
17	Leucine	198.27 \pm 0.120 ^m
18	Lysine	394.47 \pm 0.202 ^p
19	Proline	409.53 \pm 0.120 ^q
20	Tryptophan	612.30 \pm 0.115 ^s
	Total Amino acid	3722.9135
F- Value		1.353E6
P- Value		0.00

*Values are expressed as Mean \pm SEM, n=3; Means in each column with different superscripts letters are significantly different at p<0.05.

TABLE 3: Percentage of amino acids of rhizome of *Zingiber zerumbet*

Amino acids	%
Essential amino acids	
Threonine	2.44
Cystine	2.94
Tyrosine	1.17
Histidine	5.332
Valine	2.107
Methionine	4.966
Isoleucine	5.844
Phenyl alanine	5.621
Leucine	5.32
Lysine	10.6
Tryptophan	16.45
Non-essential amino acids	
Aspartic acid	12.255
Glutamic acid	5.14
Asparagine	1.231
Serine	0.014
Glutamine	0.37

Glycine	1.822
Arginine	2.8
Alanine	2.57
Proline	11
Total EAA (%)	62.79
Total non-EAA (%)	37.21
Total amino acids (%)	100
EAA/ Non-EAA	1.68
EAA/ Total Amino acid	0.628

IV. CONCLUSION

The present study has delivered some basic information on the biochemical compositions and amino acid contents of the *Zingiber zerembut*. Their biochemical compositions and amino acids analysis depict them as rich source of carbohydrates, proteins and lipids and also amino acids hence they are used mainly as food and medicinal adjuncts. It can be concluded that *Zingiber zerembut* is a good source of amino acids and hence, apart from its medicinal properties, it can also be used as a nutraceutical food.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Science and Technology, Government of India, New Delhi for awarding INSPIRE fellowship (Grant no: DST/INSPIRE/2010/(178) to pursue this study.

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