Estimation of Biochemical and Amino acid Compositions of Rhizome of In vitro Regenerated *Zingiber zerumbet* (L.) Sm. — Medicinally important Wild Ginger

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ABSTRACT *A wild ginger Zingiber zerumbet (L.) Sm. is also known as bitter ginger, pinecone lilly belonging to the family Zingiberaceae. It is an important herbal medicinal plant used all over the world to treat a wide array of ailments. The aim of the present study was to estimate the biochemical and amino acid compositions of methanol extract of the rhizome from the in vitro regenerated Z. zerumbet. The percentage of biochemical such as the total protein, total carbohydrates and total lipid contents were estimated quantitatively at 22.54 ± 0.24, 34.31 ± 0.17 and 20.20 ± 0.17 respectively. Twenty different amino acids found with varied quantities in the methanol extract of the rhizome which is expressed in µg/g dry wt. Among different amino acids, aspartic acid was estimated to be higher in amount (695.31± 0.161), whereas, glutamine was estimated to be lesser in amount (20.29 ± 0.179). The findings of the biochemical and amino acid compositions of the rhizome from the in vitro regenerated plant clearly show that the values are more or less similar to the rhizome of ex vitro grown plant. Hence, it can be concluded that the in vitro regeneration technique can be used for mass cultivation and also to propagate disease free Z. zerumbet regardless of seasons.*

Keywords: *Zingiber zerumbet, bio chemicals, amino acid, methanol extract, rhizome.*

INTRODUCTION

Medicinal plants are the local heritage with global importance. Humans are endowed with a rich wealth of medicinal plants. It also plays an important role in the lives of rural people, particularly in remote parts of developing countries with few health care facilities. Plants have been used in the traditional health care system from time immemorial, particularly among tribal communities (Moshrafuddin Ahmed, 2010). They are the richest bio-resource of drugs of traditional system of medicines, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008).

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. Apart from India, these systems of medicines are prevalent in Korea, China, Singapore, West Asia and many other countries. Medicinal plants are a significant source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. Isolated active constituents are used for applied research. Over the last few decades, phytochemistry has been making rapid progress and herbal products are becoming popular. Besides the demands made by these as raw material, the demand for medicinal plants made by the modern pharmaceutical industries has also increased day by day. Thus, medicinal plants constitute a group of industrially important crops which brings appreciable income to the country by way of export (Bhattacharjee, 2008).

Zingiber zerumbet (L.) Sm. well known as *lempuyang*, is a wild ginger belonging to the Zingiberaceae family. Zingiberaceae is one of the largest monocotyledonous families of the plant kingdom. It provide many important useful products for food, spices, medicines, dyes, perfume and aesthetics to man. *Z. zerumbet* is a well known medicinal plant, employed to cure various diseases (Joy et al., 1998). The raw ginger is useful in anorexia, vitiated conditions of vata and kapha, dyspepsia, pharyngopathy and inflammations. The dry ginger is useful in dropsy, otalgia, cephalagia, asthma, cough, colic, diarrhea, flatulence, anorexia, vitiated conditions of vata and kapha, dyspepsia, cardiopathy, pharyngopathy, cholera, nausea, vomiting, elephantiasis and inflammations. It is also used in several domestic preparations Warrier et al., 1994. In the Unani system, ginger is used rather extensively in such preparation as "Hub-gul-pista" for clearing the

respiratory system, "Sufuf Shirin" for dysentery, "Majun Izaraqi" as a tonic and "Qurs Podina" and "Murraba-adrak" as Carminatives (Thakur et al., 1989).

However, our knowledge of medicinal plants has mostly been inherited traditionally. Usage of medicinal plants for cure of various ailments is not confined to the doctors only but extends to several households as well. Spreading and preserving this knowledge on medicinal plants and their uses has become an important and vital need for human existence. There is a growing tendency all over the world to shift from synthetic to natural based products including medicinal plants. There is a need for conservation of all useful plant species, and also cultivation, maintenance and assessment of germplasm for further uses.

Plant tissue culture is employed to meet all these requirements but at the same it is necessary to analyze the chemical constituents of the *in vitro* regenerated plants to that of wild plants to validate the usage of *in vitro* regenerated plants for herbal remedies and in pharmaceutical industries. Hence, the aim of the present study was to estimate the biochemical and amino acid compositions of methanol extract of the rhizome from the *in vitro* regenerated *Z. zerumbet*.

MATERIALS AND METHODS

Plant material

In vitro regenerated plants of *Z. zerumbet* (L.) Sm. was obtained on Murashige and Skoog (MS) medium supplemented with 8.88µM/L of BAP, 1.10µM/L of NAA and 10.86µM/L of AdS. The *in vitro* regenerated plantlets were hardened and established in the field and maintained in the garden of Department of Botany, Pachaiyappa's College, Chennai, Tamilnadu. The mature rhizomes were collected from 12 months old plant for this study; 500 grams of fresh rhizome were collected separately and were washed thoroughly with tap water to remove the adhered soil particles on the surface of rhizome. These rhizomes were cut in to small pieces, dried in oven (50ºC) for about 48 hours and were then coarsely powdered.

Preparation of extract

The coarsely powdered sample was extracted in 1:10 ratio at room temperature with 99% methanol. The extract was filtered with Whatman No.1 filter paper and was concentrated by distillation and desiccated. Ultimately 10%w/w of semi solid residues was recovered and the extract was subjected for the estimation of the biochemical and amino acid studies.

Estimation of total protein

The total protein was evaluated using the Folin-Ciocalteu Phenol method of Lowry *et al*., (1951).

Estimation of total carbohydrates

The total carbohydrate was evaluated following the Phenol-sulphuric acid method of Dubois *et al*., (1956).

Estimation of total lipids

The extraction of lipid was determined by the chloroform-methanol mixture by following Folch *et al*., method (1957).

Amino acid analysis

Amino acids were determined by high-performance liquid chromatography (HPLC) according to the method described by Rajendra (1987). 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, acetonitrite 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 ± 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).

RESULTS AND DISCUSSION

Biochemical compositions

The methanol extract of rhizome from *in vitro* regenerated *Z. zerumbet* was subjected to a quantitative estimation of total protein, total carbohydrate, total lipids and amino acids. The results demonstrated a significant biochemical composition and the values are all expressed in percentage (Table -1).

Proteins are one of the most important components of living things. The presence of higher protein level in the plant points towards their possible increase food value. Dietary protein may play an important role in the prevention of metabolic dysfunctions Thomsen et al., (1991). The total protein content in the methanol extract of rhizome was 22.54 ± 0.24%. Sharma Pradeep Kumar *et al.,* (2013) reported the total protein content of *Z. officinale and Curcuma longa* as 31.5% and 10.5%. According this report the percentage of total protein content of *Z. Zerumbet* is higher than the *Z. officinale* and lower than the *C. longa.* Ajayi *et al.,* (2013) reported that, the total protein content of white and yellow type of *Z. officinale* was 12.05 and 11.65% respectively which is lower than the report of present study.

Carbohydrate is plays vital role in metabolism by supplying the energy needed for respiration (Bligh and Dyer, 1959). The soluble carbohydrates analyzed by Hashimah *et al.,* (1998) ranged from 2.14g to 5.96g in different Zingiberaceae species. The percentage of total carbohydrate content of methanol extract of *in vitro* regenerated *Z. zerumbet* was found to be 20.43 ± 0.28%. This value is contrary to the report of Ajayi *et al.,* (2013) in white and yellow type of *Z. officinale*. Indrayan *et al.,* (2009) reported the carbohydrate content with different quantities from some species of Zingiberace such as *Alpinia galangal* (78.9%), *A. officinarum* (76.9%), *A. zerumbet* (76.0%), *A. calcarata* (75.0%) and *Kaempferia galangal* (76.0%) and in rhizome of *Z. cassumunar* (387.33 ± 6.69 µg/mL) by Majaw *et al.,* (2009).

Table 1: Biochemical compositions of rhizome from *in vitro* regenerated *Z.zerumbet**

S. No	Bio Chemicals	Percentage (%) of dry Weight
1	Total Protein	22.54 ± 0.24[a]
2	Total Carbohydrate	34.31 ± 0.17[b]
3	Total Lipids	20.20 ± 0.17[c]
F- Value		130.888
P- Value		0.00

**Values are expressed as Mean ± SE, n=3. Means in each column with different superscripts Letters are significantly different at p<0.05.*

The total lipid composition was the least among the biochemical composition in the present study. Lipids have vital role in oxidation processes than other biological compounds by providing more energy (Jain, 2004). The total lipid content of rhizome from *in vitro* regenerated plant was found to be 20.20 ± 0.17%. Ajayi *et al.,* (2013) reported the total fat content of white and yellow type of *Z. officinale* which were 17.11 and 9.89% respectively. Indrayan *et al.,* (2009) reported the crude fat content in some Zingiberaceae species, namely *A. officinarum* (2.26%), *A.* galanga (1.14%), *A. zerumbet* (2.01%) *A. calcarata* (1.68%) and *Kaempferia galaga* (1.70%) and these quantities are comparatively less than that of in rhizome of *in vitro* regenerated *Z. zerumbet* .

Amino acids estimation

The methanol extract of rhizome of *in vitro* regenerated plant consists of 20 different amino acids with various quantities (µg/g Dry weight). The protein hydrolysate found to contain 20 amino acids and out of which amino acids 11 were Essential Amino Acids (EAA) and 9 were Non Essential Amino Acids (N-EAA) were present (Table 2 and 3).

Amino acids are building blocks of protein and more than 300 amino acids have been described, but only 20 amino acids take part in protein synthesis. All twenty amino acids did not appear simultaneously in nature. Instead some of them appear early, while others were added into the genetic code later. It is necessary to amino acids take them in the diet because it cannot be synthesized by animals and humans (Akram et al., 2011). High composition of total essential amino acid was found with 62.79%, whereas the non-essential amino acid was found to be 37.21% in the rhizome of *in vitro* regenerated plant. The amount of aspartic acid (454.47 ± 0.145) and tyrosine (50.4033 ± 0.054) were the maximum and minimum among essential amino acids respectively, whereas, aspartic acid (695.31 ± 0.161) and glutamine (20.29 ± 0.179) were the maximum and minimum amount among non- essential amino acids (Table 3).

Table 2: Amino acids compositions of rhizome from *in vitro* regenerated *Z. zerumbet**

S.No	Amino acids	(µg/g Dry weight)
1	Aspartic acid	695.31±0.161[t]
2	Glutamic acid	259.22±0.141[n]
3	Aspragine	73.15±0.175[e]
4	Serine	127.67±0.042[a]
5	Glutamine	20.29±0.179[b]
6	Glycine	80.1267±0.137[g]

7	Threonine	78.6333±0.088[f]
8	Arginine	213.27±0.142[k]
9	Alanine	189.40±0.100[h]
10	Cysteine	211.29±0.076[j]
11	Tyrosine	50.4033±0.054[c]
12	Histidine	414.50±0.115[q]
13	Valine	57.5000±0.115[d]
14	Methionine	254.53±0.145[m]
15	Iso leucine	193.37±0.088[i]
16	Phenylanine	413.63±0.088[p]
17	Leucine	214.37±0.120[l]
18	Lysine	454.47±0.145[r]
19	Proline	515.23±0.093[s]
20	Tryptophan	314.43±0.120[o]
	Total Amino acid	4830.7933
F- Value		231.4
P- Value		0.00

*Values are expressed as Mean ± SE, n=3. Means in each column with different superscripts Letters are significantly different at p<0.05.

Table 3: Percentage of essential and non-essential amino acids

Amino acids	%
Essential amino acids	
Threonine	1.63
Cystine	4.37
Tyrosine	1.043
Histidine	8.58
Valine	1.19
Methionine	5.27
Isoleucine	4.003
Phenyl alanine	8.562
Leucine	4.44
Lysine	9.41
Tryptophan	6.51
Non-essential amino acids	
Aspartic acid	14.393
Glutamic acid	5.366
Asparagine	1.514
Serine	2.643
Glutamine	0.42
Glycine	1.66
Arginine	4.414
Alanine	3.92
Proline	10.67
Total EAA (%)	55
Total non-EAA (%)	45
Total amino acids (%)	100
EAA/ Non-EAA	1.22
EAA/ Total Amino acid	0.55

The aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis. glutamic acid and glycine participate in the synthesis of glutathione increasing the antioxidant capacity of the plant. Valine maintains the balance of branched chain amino acids, whereas alanine is involved in hepatic autophagy, gluconeogenesis and transamination. Leucine regulates the protein turnover and gene expression (Akram *et al*., 2011; Wu, 2009). Glycine, lysine, threonine and glutamate help to maintain intestinal integrity and health (Rhoads and Wu, 2009; Wang et al., 2009). The amino acids, aspartic acid, glutamic acid, serine, glycine, alanine and leucine are naturally involved in osmolyte synthesis, cell

metabolism, ammonia detoxification, antioxidant activity and alkaloid synthesis, suggesting that the therapeutic properties (Moran-Palacio *et al*., 2014).

CONCLUSION

The quantitative estimation of biochemical and amino acid compositions of the rhizome of *in vitro* regenerated *Z. zerembut* was achieved. Their biochemical compositions and amino acids estimation depict that they are more or less similar to that of in rhizome of *ex vitro* grown plant. Variation in biochemical quantity and conflicting results to other reports may be related to the changes in temperature and light intensity, geographical origin and seasonal periods. It can be concluded that tissue culture technique can be used to mass cultivation of *Z. zerembut* since it has high demand as herbal food and medicinal adjuncts and also as raw material in pharmaceutical industries.

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