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## **Development and Quality Evaluation of Finger Millet**

### **Noodles**

#### **Dissertation-1 Report**

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## CERTIFICATE

This is to certify that Yathagiri Venkata Naga Supraja (Registration No. 11707690) has personally completed M.Sc. Pre-dissertation entitled, “Development and Quality Evaluation of Finger Millet Noodles” under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfilment of the conditions for the evaluation leading to the award of Master of Food Technology.

Date:

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## **DECLARATION**

I hereby declare that the work presented in the Thesis entitled “Development and Quality Evaluation of Finger Millet Noodles” is my own and original. The work will be carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, under the guidance of Dr.NavanidhiChhikara Assistant Professor of School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

Date:

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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## **CHAPTER - 1 INTRODUCTION**

NOODLES which are form of extruded product is extremely popular in India even as Continental and Italian countries. Noodles are primary food of traditional Chinese cuisine, instigated from the China as early as 5000 BC (Fu, 2008; Hou and Kruk, 1998). Products are made from milled durum wheat (*Triticum durum*) which is mixed with water and then pressure is applied through metal die and produce extruded product. Instant Noodles is prepared by extrusion machine that is basically made of a stainless steel make strips, either rolled and Cut or Extruded. The properties of instant noodles like the taste, nutrition for health, convenience to carry, safety, longer shelf-life which helps to preserve and reasonable price have made them popular.

Instant noodles are made from wheat flour, starch, water, salt (an alkaline salt mixture of sodium carbonate, potassium carbonate, and sodium phosphate), and other ingredients that can improve the texture and flavour of noodles, they are partially cooked by steaming and further boiled in water and dehydrated by a deep-frying process (Kim, 1996a). Manufacturing of noodles involves process steps like sheeting and cutting of dough and low water absorption is done compared to other bakery products. (Corke and Bhattacharya, 1999; Miskelly, 1993).

Noodle was reported that 20-50% of wheat consumption in total Asia was used for the production of noodles (Hou, 2010). Noodles can be classified into fresh noodles, dried noodles, steamed noodles, boiled noodles, frozen boiled noodles, and instant noodles based on the various processing methods (Fu, 2008). Compared with the other sectors, instant noodles are more widely consumed and enjoyed in the global market due to low cost, ease of cooking, convenient storage, and unique texture and flavor.

Products can be produced as Hard & Brittle pieces, formed into different shapes by extruding, cutting and drying tough dough made from semolina mixed with water.

Consumption of noodles in India had been low, however, it has increased rapidly in the past five years that is by more than 5 times reported by WINA (2011), with the current consumption of 2,940 million packets. Asian countries considered wheat flour noodles as an important part of the diet (Hou, 2011; Jeong et al., 2017).

Gluten, present in the wheat flour produces the dough sheeting capability and act as a major factor that affects cooking properties of noodles, gluten-free cereal flours cannot be utilized to make instant noodles by the sheeting method.

### **Finger millet:**

Finger millet *Eleusinecoracana L*, is known as African millet, and Ragi in India. It is an important staple food in parts of eastern and central Africa and India. It is extensively grown in Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Bihar, Gujarat, and Maharashtra. It even grows in the hilly regions of Uttar Pradesh and Himachal Pradesh, with a total area of 2.5 million hectares and 2.2 million tonnes of production (aut,2017). Finger millet stands sixth in production of cereals after wheat, rice, maize, sorghum and bajra in India.

Nutritional composition of millets is higher as compared to other cereals like rice, wheat. It is a good substitute to wheat and rice. Finger millet contains proteins(5-8%), carbohydrates(65-75%), dietary fibre(15-20%), minerals(2.5-3.5%), and other extractives(1-2%). It has the highest calcium content (344 mg/100 g) as compared to all cereals.

Finger millet is especially valuable as it contains the amino acid methionine, which is lacking in the diets of hundreds of millions of the poor who live on starchy staples such as cassava, plantain, polished rice, or maize meal. Finger millet can be prepared into cakes, puddings or porridge which will help people to consume in a reasonable price. The grain is used as fermented drink or beer in Nepal and in many parts of Africa. The left-over meal(straw) from finger millet is used as animal fodder. It is also used for festivals as a flavoured drink.

The main constituents of the millet kernel are seed coat, embryo and endosperm. Among several varieties of finger millets such as **yellow, white, tan, red, brown, or violet color, only the red-coloured are cultivated extensively throughout world.** The unique feature of finger millet that it has five layered seed coat as compared to other millets such as foxtail millet, pearl millet, kodo millet and proso millet.

It is an essential cereal because of its excellent preservative properties and nutritive values of the grain which is higher than that of rice and equal to wheat (Van Wyk and Gericke, 2000).

## **CHAPTER - 2 OBJECTIVES**

To study the physical, chemical and nutritional composition of finger millet

To develop finger millet noodles as food products.

To study the shelf-life of the acceptable product.

### **CHAPTER – 3 PROBLEM BACKGROUND**

Noodles are the extruded products which are consumed by most of the population around the world. The innovation of noodles is done day by day throughout the world. Instead of using wheat flour in the noodles, we can use “Ragi flour” which has a good amount of calcium content among all cereals. It also contains other components like proteins, carbohydrates, fibres, fat, energy. Finger millet is a very good source of micronutrient, like minerals i.e. calcium, phosphorus, potassium, sodium, magnesium, iron and zinc. Even vitamins are also present but in very little amounts, thiamine, riboflavin, and nicotinic acid. The antinutritional factors present in the ragi are polyphenols that contain phytic acid and tannins in large amount and flavonoids in the small amount. The micronutrient present in the ragi can alleviate the micronutrient malnutrition in the developing countries. There are health benefits like imparting antimicrobial, anti-diabetic, antimutagenic properties.

## CHAPTER - 4 REVIEW OF LITERATURE

### **Nutritional value of finger millet:**

#### **Properties:**

Finger millet (*Eleusinecoracana L*) is known as ragi. Finger millet is a well known source of nutrients, mainly mineral contentlike calcium, iron, phosphorus, zinc, potassium, and other and dietary fibre. Finger millet is a good source of the various phenolic compounds which may have health benefits. The main polyphenols are phenolic acids and tannins while flavonoids are present in small quantities. Polyphenols have been known to impart antimicrobial, anti-diabetic, antimutagenic properties. Along with this functional property (gelatinization) which is also present in finger millet.

**Nutrient composition of Finger millet:** (Millet Network of India(<http://milletindia.org>)100g)

Proteins	7.7g
Fat	1.5g
Ash	2.6g
Crude fibre	3.6g
Carbohydrates	72.6g
Energy	336Kcal
Calcium	350mg
Neutral lipids mainly triglycerides	70 – 72mg
Total lipids	1.85 – 2.10mg
Glycolipids	10 – 12mg
Phospholipids	5 – 6mg



**Nutrient composition of cereals: (Source: Saldivar (2003))**

<b>Composition</b>	<b>Wheat</b>	<b>Rice</b>	<b>Maize</b>	<b>Finger Millet</b>
Protein (%)	14.4	7.5	12.1	7.3
Fat (%)	2.3	2.4	4.6	1.3
Crude Fibre (%)	2.9	10.2	2.3	3.6
Ash (%)	1.9	4.7	1.8	3.0
Starch (%)	64.0	77.2	62.3	59.0
Total Dietary Fibre (%)	12.1	3.7	12.8	19.1
Total phenol (mg/100g)	20.5	2.51	2.91	102

**Mineral and vitamin composition of cereal grains: (Source: Saldivar (2003))**

<b>Composition</b>	<b>Wheat</b>	<b>Rice</b>	<b>Maize</b>	<b>Finger millet</b>
Ca (%)	0.04	0.02	0.03	0.33
P (%)	0.35	0.12	0.29	0.24
K (%)	0.36	0.10	0.37	0.43
Na (%)	0.04	0.00	0.03	0.02
Mg (%)	0.14	0.03	0.14	0.11
Fe (%)	40.1	19.0	30.0	46.0
Mn (%)	40.0	12.0	5.0	7.5
Zn (%)	30.9	10.0	20.0	15.0
Thiamin (mg/100gm)	0.57	0.07	0.38	0.48
Riboflavin (mg/100gm)	0.12	0.03	0.14	0.12
Nicotinic Acid (mg/100gm)	7.40	1.60	2.80	0.30

**Amino acid profile of Wheat, Rice, Maize and Finger millet:**

<b>Composition</b>	<b>Wheat</b>	<b>Rice</b>	<b>Maize</b>	<b>Finger millet (g/100gm)</b>
Isoleucine	3.0	4.5	3.6	4.3
Leucine	6.3	8.1	12.4	10.8
Lysine	2.3	3.9	2.7	2.2
Methionine	1.2	1.7	1.9	2.9
Phenylalanine	4.6	5.2	4.8	6.0
Threonine	2.4	3.7	3.9	4.3
Valine	3.6	6.7	4.9	6.3
Histadine	2.0	2.5	2.9	2.3
Tryptophan	2.4	1.3	0.5	---

**Nutrient composition of finger millet:****Carbohydrates:**

Finger millet is a rich source of carbohydrates and contains free sugars (1.04%), starch (65.5%) and non-starchy polysaccharides. (Malleshi, Desikachar, & Tharanthan, 1986) and dietary fiber (11.5%). (Gopalan et al., 2009). The composition of carbohydrate varies according to the variety of finger millet percentages of starch(59.5 – 61.2%) ,pentosans(6.2 – 7.2%), cellulose(1.4 – 1.8%), and lignin(0.04 – 0.6%). (Wankhede, Shehnaj, and Raghavendra Rao, 1979a). The dietary fiber (11.5%) content of finger millet is much higher than the fibre content of brown rice, polished rice and all other millets such as foxtail, Kodo, and barnyard millet. The dietary fiber content of finger millet is compared to that of pearl millet and wheat and it is approximately same. The carbohydrate content of finger millet is comparable to that of wheat but lower than that of polished rice. Finger millet starch contains amylase and amylopectin. The amylose content of finger millet starch is lower than 16% (Wankhede, Shehnaj, and Raghavendra Rao, 1979b). Compared to the other millets such as sorghum (24.0%), pearl millet (21.0%), proso millet (28.2%), foxtail millet (17.5%) and Kodo millet (24.0%). (FAO, 1995).

Finger millet starch had the highest setback viscosity during cooling (from 930 to 5000°C) which is suggestive of its tendency to retrograde. (Wankhede et al., 1979b).

### **Proteins:**

Finger millet exists various difference in protein content. Prolamins are the major fractions of protein in finger millet. (Virupaksha, Ramachandra, &Nagaraju, 1975). Finger millet contains lysine and tryptophan in huge amount but wheat and rice is deficient in lysine and tryptophan (ICMR, 2010). Albumin and globulin fractions contains few fundamental amino acids, while prolamin parts contains a higher portion of glutamic acid, proline, valine, isoleucine, leucine, and phenylalanine but low lysine, arginine, and glycine. The albumin and globulin contain total protein content of the seed is about 8 – 15%. The amount of prolamin content in seed is about range of 35 – 50% that is of total protein and in the endosperm, flour is 29 – 41%. The chemical score of finger millet protein is 52% compared to other millets like sorghum 37% and pearl millet 6.3%. (FAO, 1995). Finger millet contains a higher level of sulphur-containing amino acids, like methionine and cystine, compared to milled rice. The tannin content present in the grain affects the protein digestibility of finger millet. (Ramachandra, Virupaksha, &Shadaksharaswamy, 1977). The consumption of finger millet with pulse-based diet was enough to maintain a positive nitrogen (10.4%N), calcium (3.0%Ca) and phosphorus (8.7%P) to balance the health of human adults. (Subrahmanyam, Narayana Rao, Rama Rao, and Swaminathan, 1955). The supplementing finger millet diet with lysine, improves the nutritional status, apparently protein digestibility and nitrogen storage in children. (Doraiswamy, Singh, and Daniel, 1969).

Finger Millet contains many number of essential amino acids which are required by human body like Valine, Methionine, Isoleucine, Threonine, and Tryptophan. Valine is an essential amino acid important for the repair of tissues, muscle coordination, and metabolism and also help to maintain the balance of nitrogen in the body.

Isoleucine ensures the blood formation, keeping a check on blood sugar levels. It also helps to heal and repair muscle tissues, bones and skin.

Tryptophan acts as a natural relaxant and fight against anxiety, depression. They reduce surplus appetite and help control weight gain; the growth hormones are also released.

Methionine is a sulphur-based amino acid and is important for various different activities in the human body. It promotes the growth of healthy skin and hair. The Sulphur which we get

from Methionine helps to produce a component called lecithin which helps in reduce the inner fat present in the liver and help to protect kidney functioning.

**Finger millet nutrition value (Aminoacid):**

Amino acid content	Amount (mg/g of protein)
Leucine	594
Valine	413
Phenylalanine	325
Isoleucine	275
Threonine	263
Methionine	194
Tryptophan	191
Lysine	181
Cystine	163

**Fat:**

Finger millet consists 1.85-2.10% of total lipids in seven breeding varieties of finger millet. (Mahadevappa and Raina, 1978). Finger millet lipids consist of similar lipids(70-72%) mainly triglycerides and small amount of sterols, glycol-lipids (10-12%) and phospholipids(5-6%). The total lipid content containing un-saturated polysaccharides in range like oleic acid(46-62%), palmitic acid(20-35%), linoleic acid(8-27%), and very little amount of linolenic acid. Finger millet contains fat content that is compared low to pearl millet, and foxtail millet. The lesser fat content could be one of the parts contributed for better preservative properties of finger millet which is differentiated to other millets.

The unrefined fat content of finger millet is in a range of 1.3 to 1.8% and it is reported by (Bhatt et al., 2003; Singh et al., 2003; Malleshi and Desikachar, 1986; Lupien, 1990) but crude fat has estimated approximately and reported as 2.1% (Antony et al., 1996). The varieties of finger millet in brown and white contains fat content ranged from 1.2 to 1.4%. (Seetharam, 2001). (Sridhar and Lakshmi Narayana 1994) Total lipid content in ragi to be

5.2% as it contains free lipids (2.2%); bound lipids (2.4%); and structural lipids (0.6%). Finger millet is less in fat content, but it contains more amount in polyunsaturated fatty acids. (Antony et al., 1996). The major fatty acid present in finger millet are oleic acid then it is followed by palmitic acid and last is linoleic acid. It also has a small amount of linolenic acid that would complete un-saturated fatty acids. The fatty acid present in finger millet shown saturated fatty acids are 25.6% while unsaturated fatty acids are 74.4% of total fatty acids content which is present. (Sridhar and Lakshmi Narayana, 1994).

### **Micro-nutrients:**

Finger millet is very good source of micro nutrients and high in calcium (344 mg) than compared to all other cereals and millets and contains phosphorous(234mg), iron(3.9mg) (Gopalan et al, 2009) and many other trace elements, vitamins, and potassium(408mg) content of finger millet is also high than compared to other cereals and millets. "Hamsa" variety of finger millet was reported to contain much higher levels of calcium (660mg). (Umopathy&Kulsum, 1976). The finger millet containing phytic acid content was lower than the levels present in common millet and foxtail millet and these values were ranging of 0.45-0.49g for different varieties of finger millet and in milligram the range of finger millet is 29-30mg (Ravindran, 1991) Total calcium content of finger millet is present in the husk which contains 49%. (Swaminathan, Subrahmanyam; 1959). The germination and fermentation of finger millet decreased the phytate content by 60% and improves bio-availability of minerals (Sripriya, Antony and Chandra, 1997). The process step decortication of finger millet decreases the total mineral contents but increased the bio-accessibility of calcium, iron, and zinc. Popping process of finger millet decreases the bio-accessibility of calcium but increases the bio-availability of iron and zinc. Malting of finger millet increased the bio-availability of calcium, iron, and zinc. (Rateesh, Usha, Malleshi, 2012).

Germination and fermentation processing can improve the bioavailability of finger millet which has high source of calcium and iron in finger millet, it should be considered as a good supplement for children and improving both health and haemoglobin for adolescents.

### **Nutritional Inhibitors:**

The presence of phytates, phenols, tannins and enzyme inhibitors are the utilized maximum for the nutrient of the millet. Tannins gets attached to both exogenous and endogenous proteins which include enzymes of the digestive track that affects the protein utilization. (Asquith and Butler, 1986). Finger millet has more amounts of tannins ranging from 0.04 to 3.74% of

catechin equal. (Rao, 1994; Antony and Chandra, 1998; Antony and Chandra, 1999). They also got to know that iron 50% present in the meal of human might be attached to tannins. Soaking of grains, roasting grains, boiling them, germination required, and fermentation are processes which have found to reduce tannin content. (Rao and Prabhavathi, 1982). Malting decreased in brown finger millet by 54% which is tannin content. (Rao, 1994). Strong binding capacity is done by phytic acid. According to various studies the range of phytate content in finger millet is 0.679 to 0.693g/mg. (Antony and Chandra, 1999). Phytin phosphorus content of finger millet is reduced by the malting of the grain. After germination and fermentation the phytic acid reduced upto 49.2 and 66.5%. The content of phytic acid can be decreased by using different methods in finger millet by 84.7%. Contribution to health benefits for their partial retention of phytates is helpful to contribute to human health such as antidiabetic, antioxidant and anticancer effects which are recently recognized. (Graf et al., 1987; Thompson, 1993).

**Mineral and vitamins of finger millet: (mg/100g) (USDA Database)**

Ca	334
P	283
Fe	3.9
Mg	137
Na	11
K	408
Cu	0.47
Mn	5.49
Zn	2.3
Thiamine	0.42
Riboflavin	0.19
Niacin	1.1

**Essential amino acid of finger millet: (g/100g) (USDA Database)**

Arginine	0.300
Histidine	0.130
Lysine	0.220
Tryptophan	0.100
Phenylalanine	0.310
Tyrosine	0.220
Methionine	0.210
Cystine	0.140
Threonine	0.240
Leucine	0.690
Isoleucine	0.400

Valine	0.480
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**HEALTH BENEFITS  
OF FINGER  
MILLET**

ANTI CANCER  
PROPERTY

ANTI-DIABETICS  
(TYPE 2 DIABETES  
MELITUS)

WOUND  
HEALING  
PROPERTY

ANTI  
OXDIANT  
PROPERTY

ANTI  
MICROBIAL  
PROPERTY

ANTI PROTIEIN  
GLYCATION  
PROPERTY

ALDOSE REDUCTASE  
ENZYME  
INHIBITORY

ANTI  
ULCREATIVE  
PROPERTY



## Health benefits and diseases:

Properties	Functional Role	Reference
Diabetes	<ul style="list-style-type: none"> <li>➤ finger millet feeding controls blood glucose level improves antioxidant status and hastens the dermal wound healing process in diabetic rats.</li> <li>➤ Finger millet based diet response to lower glycaemic effect due to the presence of antinutritional factors which reduce starch digestibility and absorption.</li> <li>➤ Finger millet seed coat phenolics acts as inhibitors decreasing the post prandial hyperglycaemia by blocking the action of enzymes needed for hydrolysis of complex carbohydrates.</li> </ul>	<p>(Hedge et al., 2005) (Rajasekaran et al., 2004) (Kumari, et al., 2002)</p>
Cardiovascular diseases	<ul style="list-style-type: none"> <li>➤ Investigated that finger millet may prevent cardiovascular disease by reducing plasma triglycerides in hyperlipidaemic rats.</li> <li>➤ Finger millet has lower concentration of serum triglycerides.</li> </ul>	<p>Lee, et al., (2010)</p>
Celiac diseases	<ul style="list-style-type: none"> <li>➤ Celiac disease is an immune mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals.</li> <li>➤ Finger millet is gluten free therefore excellent option for people suffering from celiac diseases and gluten sensitive patients often irritated by the gluten content of wheat and other more common cereal grains.</li> </ul>	<p>(Saleh et al., 2013)</p>

Cancer	<ul style="list-style-type: none"> <li>➤ Phenolics of millets may be effective in the prevention of cancer initiation and progression in vitro.</li> <li>➤ Phytate present in millets are associated with reduction in cancer risk.</li> </ul>	Chandrasekara, et al., (2011c) Coulibaly, et al., (2011)
Anti-inflammatory activity	<ul style="list-style-type: none"> <li>➤ Good antioxidant effects of finger millet on the dermal wound healing process in diabetes induced rats with oxidative stress mediated modulation of inflammation</li> </ul>	Rajasekaran et al., (2004)
Aging	<ul style="list-style-type: none"> <li>➤ It has been found that for inhibit glycation and cross linking of collagen to usefulness in the protection against aging.</li> </ul>	(Hegde, et al., 2002)
Cataract genesis	<ul style="list-style-type: none"> <li>➤ Finger millet seed coat phenolics such as gallic, vanilic, syringic, ferulic, quercetin, trans-cinnamic, p-coumaric, protocatechuic and p-hydroxybenzoic were identified for inhibiting cataract of the eye lens to inhibit reversibly aldose reductase <i>S. Niger</i>.</li> </ul>	Chethan, et al., (2008)
Antimicrobial activity	<ul style="list-style-type: none"> <li>➤ Protein extracts of millets were highly effective to inhibit the growth of pathogenic fungi such as <i>Rhizoctoniasolani</i>, <i>Macrophominaphaseolina</i>, and <i>Fusarium oxysporum</i>.</li> <li>➤ Millets polyphenols content showed antibacterial and antifungal activity.</li> </ul>	(Radhajejalakshmi, et al., 2003) Xu, et al., (2011)
Antibacterial activity	<ul style="list-style-type: none"> <li>➤ The phenolic content and flavonoids of finger millet inhibit oxidation of microbial membranes and microbial enzymes leading to inhibitory activities</li> </ul>	Banerjee, et al., (2012)

<p>of proliferation of bacterial cells such as <i>E. coli</i>, <i>B. Cereus</i>, <i>Listeria monocytogenes</i>, <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, <i>Serratia marcescens</i>, <i>Proteus mirabilis</i>, <i>Pseudomonas aeruginosa</i>, <i>Klebsiella pneumonia</i> and <i>Yersinia enterocolitica</i>.</p>	
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### **Polyphenols and dietary fibre:**

The grains of the Finger millet are having dark brown testa which are high source of polyphenols compared to other cereals like Rice, Wheat and Rice. The phenolic compounds are not located in the grain but are stored mainly in the outer aleurone layers, pericarp and testa of the fruit which together makes up the bran portion of the grain. The bran exist as free, soluble conjugates and insoluble-bound forms that are present in phenolic compounds. The major bounded phenolic compounds are Ferulic Acid (64%-96%) and p-Coumaric Acid (50%-99%) which are present in the Finger millets. The various types of Finger millets also have proanthocyanins (Condensed Tannins), those are larger molecular weight polyphenols which consists of polymerized flavan-3-O1 and/or flavan-3, 4-diol units. (Dykes and Rooney2006). Three classes of phenolic compounds are found in the finger millets those include, Hydroxybenzoic Acid derivatives, Hydroxycinnamic Acid derivatives, and Flavonoidshave the basic composition of C6-C1, C6-C3 and C6-C3-C6. These derived compounds are identified as p-Hydroxybenzoic Acid, Syringe Acid, Proanthocyanins, Ferulic Acid, Gallic Acid, Vanillin Acid, Trans-Cinnamic Acid – Coumaric Acid, Caffeic Acid, Synaptic Acid, Quercitannic, Protocatechuic Acid. The tannin content present in the outer layers of the finger millet acts as a physical barrier to majority of the fungi and also provides the grain with the resistance to the fungal attack. The phenolic compounds are highly concentrated in the seed coat than in that of the whole flour, hence these polyphenols present in the seed coat show show high antifungal and antibacterial activity as required to the flour extracts.

The Proanthocyanins are biologically active these less the nutritional value and bio-availability of the minerals and proteins present in sufficient quantities in the grains. (Chavan2001). The studies on the finger millet reports that, the local Finger millet had the

highest content ( $311.28 \pm 3.0$ ) followed by finger (Ravi), foxtail, little, pearl, and prose millets. These values for millets were higher than those for barley (Chandra Shekar & Shahidi. 2010). The tannins and total phenolic contents highly varies in the genotypes of the Finger millet grains.

The grains which are light in color have much lower values of the phenolic contents on comparison with grains which have dark brick red pigmented types. The red pigmented grains seed coat are known to be having high amount of tannins content and these compounds are said to be located in the tissues of the grain of the Finger millet.(Siwela et al 2007). It was observed that the brown coloured varieties of the grain had higher (1.2– 2.3%) proportions of the polyphenols than the white varieties (0.3– 0.5%) of the grains.varieties (Ramachandra, 1997). It was reported that there were approx. differences (0.19 3.37%) in the total phenolic contents in among 85 Indian varieties of finger millet (Shankara1991).

The studies have said that the good storage abilities of the Finger millet and its processed foods are due to the presence of the polyphenolic content which are higher than those of the barley (Chandrasekhara & Shahidi2010).

The free radicals are created due to the oxidation of cell components and microbial membranes with forms irreversible complexation with nucleophilic amino acids leads to inactivation of enzymes which contribute to the major biochemical benefits of polyphenols to the antifungal activity.

Tannins and Flavonoids present in millet outer seed coat are having multifunctional properties as they act as singlet oxygen quenchersreducing agents (free radical terminators), and metal chelators. Finger millet is a good source of antioxidants and these antioxidants have high radical-scavenging activity with higher than that of wheat, rice, and other millets. The brown or red variety of finger millet has higher activity (94%) using the DPPH method than white variety (4%), which had less activity.

Carbohydrates in finger millet are slow digesting and assimilating carbohydrates on comparison with present in other cereals. The daily consumption of finger millet having high polyphenols and dietary fibre contents are known to decrease the risk of diabetes mellitus and gastrointestinal tract disorders, and these are due to high polyphenolic and dietary fibre content. Finger millet are rich and good source of dietary fibre than any other portions of cereals.

Health benefits of finger millet are postpone in nutrient absorption, high fecal bulk, reducing of blood lipids, does not occur of colon cancer, the way to digestion. (Tharanathan& S. Mahadevamma.2003). It also contains a functional fibre parts known as RS, this escapes the enzymatic digestion, imparts useful effects by reducingmany intestinal disorders (Annison et al1994) (Gee1992 et al). Colonic bacteria escapes digestion and requires fermentable bacteria. It is also useful for such production of desirable metabolites, which contain short-chain fatty acids in the colon, mainly butyrate, which seems to stabilize colonic cell proliferation as a reduced mechanism for colon cancer. Its therapeutic effects, resistant starch (RS) provides better appearance, texture, and mouthfeel than required fibres (Martinez-Flores1999).

### Phenolic compounds identification in finger millet:

Class	Basic skeleton	Compounds	Reference
Phenolic acids Hydroxybenzoic acid derivatives	C6-C1	Gallic acid protocatechuic acid, p-hydroxybenzoic acid, vanilic acid, syringic acid.	McDonough et al. (1986), Rao and Murali Krishna (2002), Chethan and Malleshi (2007a),
Hydroxycinnamic acid derivatives	C6-C3	Ferulic acid, trans- cinnamic acid, p- coumaric acid, caffeic acid, sinapic acid.	Chethan et al. (2008a, b), Shobana et al. (2009)
Flavonoids	C6-C3-C6	Quercetin, proanthocyanidins (condensed tannins)	Chethan et al. (2008a, b), Dykes and Rooney (2006), Chandrasekara and Shahidi (2010)

### Total polyphenols content in few brown and white finger millet varieties:

Number of varieties	Polyphenols (%)	Tannins (%)	Reference

Brown			
26	0.08 – 2.44	0.12 – 3.47	Ramachandra et al. (1977)
1	-	0.36	Rao and Prabhavati (1982)
3	0.05 – 0.59	0.17 – 0.32	McDonough et al. (1986)
12	-	0.35 – 2.39	Rao and Deosthale (1988)
1	0.1	-	Sripriya et al. (1977)
5	1.3 – 2.3	-	Chethan and Malleshi (2007a)
18	0.34 – 1.84	0.02 – 2.08	Siwela et al. (2010)
White			
6	0.06 – 0.09	0.04 – 0.06	Ramachandra et al. (1977)
1	0.003	-	Sripriya et al. (1977)
2	0.3 – 0.5	-	Chethan and Malleshi (2007a)
4	ND – 0.09	ND	Siwela et al. (2010)
Hilly region			
3	-	0.34	Wadikar et al. (2006)
Base region			
7	-	0.53	Wadikar et al. (2006)

\*ND – not detected

### **Tannins:**

Finger millet is a good source of micronutrients like calcium, iron, phosphorus, zinc, and potassium. Due to the presence of anti-nutrients in the grain such as tannins and phytates, these micronutrients are less bioavailable. Eleusinecoracana popularly known as finger millet contain high amounts of tannins ranging from 0.04 to 3.47% (Ramachandra et al, 1997). The tannin content of ragi from 0.04 to 3.47 percent, most of the values fall around 0.6%.

Weight gains and impair feed conversion efficiency are reduced by tannins. Tannins reduce apparent digestibility of protein and energy. Tannins are known to have a direct effect on metabolism. Chickens fed high tannins sorghums develop leg abnormalities (Elkins et al, 1978). Tannins impart a bitter taste to the grains making them unpalatable and also interfere with protein digestibility. (Barry and Blaney, 1987) found that plasma growth hormone levels increased with increased intake of condensed tannins by sheep. Tannins react not only with dietary protein but also with enzymes of the gut wall and proteins in the saliva.

The total amount of phenol levels and tannin levels of finger millet varieties indicated wide variations in phenolic contents. White-grain varieties had lower phenolic content than the brown-grain varieties. In vitro protein, digestibility values of low tannin samples were higher than those of the high tannin samples. Dehulling had the effect of removing most of the phenolics from finger millet grain with a concomitant increase in vitro protein digestibility. Addition of tannic acid to low tannin or dehulled finger millet samples decreased the in vitro protein digestibility. Tannins were found to be associated mostly with the glutelin fraction of finger millet protein. Tannin content was estimated in hilly region varieties was found to be less compared to base region varieties. These noticeable differences between polyphenols content in white and brown varieties could be due to the presence of the red pigments, such as anthocyanins, which are generally polymerized phenolics present in brown cultivars.

It has been found that tannins reduce feed intake, impair nutrient digestibility and nitrogen retention thus causing growth depression of poultry. (Chang SI, Fuller HL, 1964) (Mohammedian GM et al, 1986).

### **Cyanide:**

Cyanide readily and reversibly binds to a number of proteins and enzymes in the body particularly those with a metallic component. It has a specific affinity for iron in its trivalent (ferric) state and is capable of binding to all enzymes and proteins containing iron, including hemoglobin, myoglobin, catalase and the cytochromes system (Ahmed et al, 1996; Uvere et al, 2000). It's most significant interaction is its binding to the ferric iron of the mitochondrial cytochrome oxidase system. Cyanide binds to the cytochrome amino acids complex, thus inhibiting oxidative phosphorylation and paralyzing cellular respiration. This results in anaerobic metabolism, increased lactic acid production, reduced ATP stores and anoxic cell death. The organ systems that are most sensitive to cyanide toxicity are those with the highest oxygen utilization that cannot tolerate hypoxic stress, for instance, the central nervous system and the myocardium (Muzanila, 1993).

The average fatal dose of cyanide for an adult human is between 50 and 60mg per 100g of dry matter (Panasiuk and Bills, 1984). (EFSA (2004) and Salami (1994)) quote values of 0.5 – 3.5 mg/kg body weight as a lethal dose. (The World Health Organisation (2003) guidelines specify a figure of 0.05 mg/kg body weight/day.

The frequency of ingestion and the small body weight of infants may still result in health risk. Any procedure that reduces the cyanide content will thus be highly recommended in the preparation of complementary foods for infants.

**Phytate:**

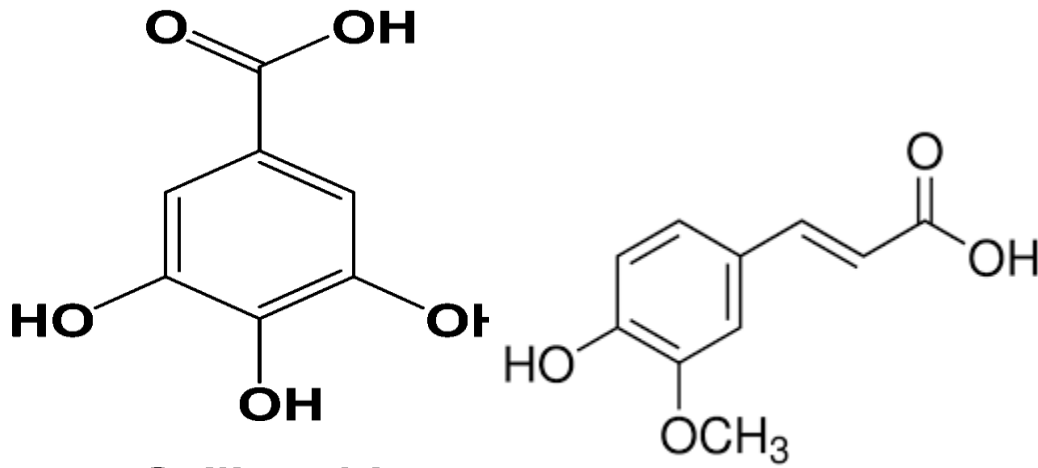
Phytate is a naturally occurring phosphorus compound which significantly influences the functional and nutritional properties of foods. It is the main phosphorus store in mature seeds. Phytate interferes with mineral absorption especially calcium and zinc. The high quantities of this anti-nutrient in the seed reduce their food value hence causing low utilization unless processed prior to use. (Udayasekhara Rao and Deosthale, (1998); Mukuru, (1992).

Phytate is an effective chelator of the positively charged cation. When consumed in feeds it will bind the nutritionally important mineral cations that it encounters in the intestinal tract, such as calcium, iron, and zinc and to proteins as well, forming complexes, the net result is reduced protein and mineral bioavailability.

Total Pentosans	3.31%
Cellulose	3.03%
Pectins	1.76 %
Total Non starch Polysaccharides	9.4 %
Tannins	0.04 – 3.47 %
Low amount of tannins (White grain varieties)	0.05%
High amount of tannins (African varieties)	3.42 – 3.47 %
Dark brown and brown varieties	0.61%
Phytates	0.48%
Polyphenols	0.61%

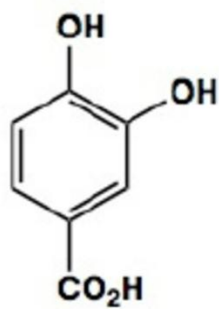


Structure of major phenolic compounds present in finger millet:

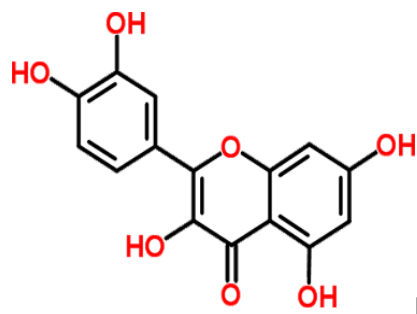


**Gallic acid**

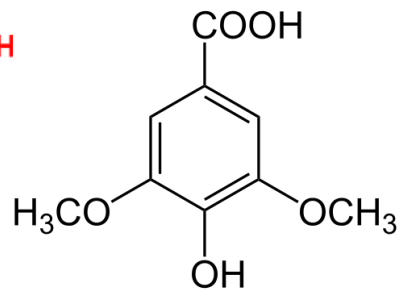
Ferulic acid



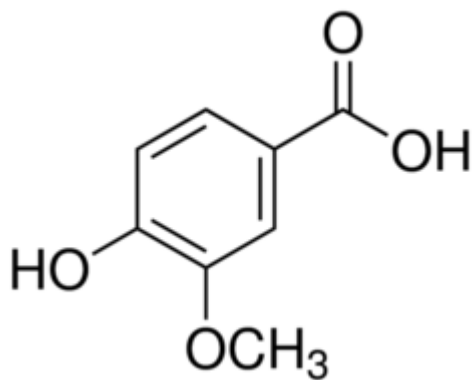
Protocatechuic acid



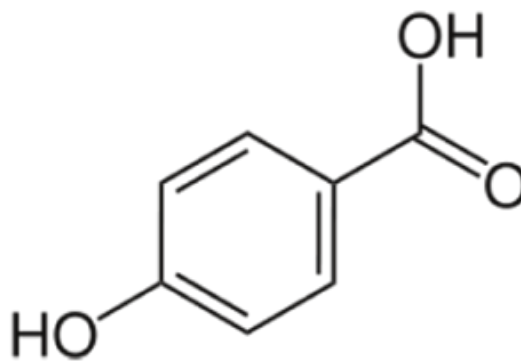
**Quercetin**  
C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>



Syringic acid



hydrobenzoic acid



p-  
Vanillic acid

## CHAPTER- 5 MATERIAL AND METHODS

### **Physical analysis of grain**

#### **Bulk Density:**

Bulk density of a powder is the ratio of the mass of an entrapped powder sample and its volume including the contribution of the interparticulate void volume. Hence the bulk density depends on both the density of powder particles and spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/ml) although the international unit is kilogram per cubic meter ( $1 \text{ g/ml} = 1000 \text{ kg/m}^3$ ) because the measurements are made using cylinders.

It's may also have expressed in grams per cubic centimeter.

#### **True density:**

Determination take 10 grains randomly from the lot. The exact volume of these grains is found by liquid displacement method. For this take a 100 ml capacity measuring cylinder and fill it with toluene to a predetermined level. Drop, randomly selected 10 grains in the cylinder and note the change in volume accurately. This gives the volume of 10 grains. Now weigh these 10 grains in an analytical balance (or) digital balance.

### **1000 kernel weight:**

One hundred grains of any cereal/pulse are collected manually or grains are spread on a counting plate with 100 dents equal to the size of the grains. Grains are carefully spread over the counting plate so that all the dents are filled. Extra grains are removed from the plate; grains are collected by turning plate upside down. The weight of these grains is noted by weighing on an analytical balance or digital balance. Repeat the experiment at least ten times and report the average value.

### **Chemical Analysis of grain**

**To estimate the protein content in the given sample:**

#### **A. Equipment**

- Test tubes
- Graduated cylinder
- Weight Balance
- UV spectrophotometer

#### **B. Reagents:-**

**A.** 2%  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH

**B.** 1% NaK Tartrate in  $\text{H}_2\text{O}$

**C.** 0.5%  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  in  $\text{H}_2\text{O}$

**D. Reagent I:** 48 ml of A, 1 ml of B, 1 ml C

**E. Reagent II-** 1 part Folin-Phenol [2 N]: 1 part water

**BSA Standard** - 1 mg/ml

#### **Procedure: -**

- 0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.

- The test tube with 1 ml distilled water serves as blank.
- Add 4.5 ml of Reagent I and incubate for 10 minutes.
- After incubation add 0.5 ml of reagent II and incubate for 30 minutes
- Measure the absorbance at 660 nm and plot the standard graph.
- Estimate the amount of protein present in the given sample from the Standard graph.

**2. AIM:** - To Estimate the amount of carbohydrates in the grain sample.

**Reagents:-**

1. Glucose stock standard: 100 mg of glucose was dissolved in 100 ml of water in a standard flask.
2. Working standard: 10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose.
3. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use
4. 2.5 N HCl.

**Procedure**

1. Weigh 100mg of the sample into a boiling tube, hydrolyze by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cool to room temperature.
2. Neutralize it with solid sodium carbonate until the effervescence cease. make up the volume to 100 ml and centrifuge.
3. Collect the supernatant and take 0.2 to 1.0 ml for analysis.
4. Prepare the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank make up the volume to 1.0 ml in all the tubes with distilled water, then add 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath.
5. Cool rapidly and read the green to dark green color at 630 nm.

**Estimation of Amino Acids (Ninhydrin method):**

**Reagents:-**

i. Dissolve 50mg leucine in 50ml of water in a volumetric flask. Take 10ml of this stock standard and dilute to 100ml in another volumetric flask for working standard solution. A series of volume from 0.1-1 ml of this standard solution gives a concentration range 10 µg-100µg. Proceed as that of the sample and read the color.

ii. Ninhydrin: Dissolve 0.8 stannous chlorides in 500 ml of 0.2 M citrate buffer (pH 5.0). Add this solution to 20g of Ninhydrin in 500ml of methyl cellosolve (2 methoxyethanol)

iii. 0.2M Citrate buffer pH 0.5

iv. Diluent solvent: Mix equal volumes of water and n-propanol and use.

### **Procedure**

1. To 0.1 ml of extract, add 1ml of Ninhydrin solution
2. Make up the volume to 2ml with distilled water
3. Heat the tube in a boiling water bath for 20min.
4. Add 5ml of the diluents and mix the contents.
5. After 15min read the intensity of the purple color against a reagent blank in a colorimeter at 570 nm. The color is stable for 1h.
6. Prepare the reagent blank as above by taking 0.1ml of 80% ethanol instead of the extract.

### **To estimate the fat content by soxhlet method:**

#### **REQUIREMENTS:**

- Weighing balance
- Soxhlet apparatus
- Drying oven
- Thimble
- Heating mantle
- Glass rod
- Desiccator with silica gel
- Petroleum ether (Boiling temperature 60°-80°c)
- Cotton plugs

#### **PROCEDURE:**

1. First of all, rinse all the glass apparatus by petroleum ether and dry it in the oven at 102°C and after removing it keep in the desiccator.
2. Weigh 5 gram of grounded and dried sample and place it in the thimble.
3. Place the thimble in the soxhlet extractor.
4. Take a 150ml round bottom flask and clean it and fill the flask with 90 ml petroleum ether.
5. Place the whole setting on a heating mantle and allow the petroleum ether to boil.
6. Continue the extraction process for several hours, almost 6 hours.
7. Remove the condensing unit from extraction unit and allow the sample to cool down. Finally, it removes all the lipid.
8. Collect almost all the solvent after distillation.
9. Place the sample in the oven and after removing it place in the desiccator.
10. Take the weight of the sample.
11. As a result, we get a defat sample.

**To determine moisture content in grain sample:**

**Requirements:** - Hot air oven (thermo statistically controlled)

Weighing balance

Desiccator (with active desiccant)

Weighing pots

**Procedure:-**

1. Weigh accurately 5g of material in a dish previously dried and weighed.
2. Place the dish along with lid in an electric oven maintained at 105°C.
3. Cool the dish to room temperature in a desiccator and weigh with lid on.
4. Repeat the process until three consecutive readings are same.
5. Note down the weight.

**Calculations:-**

Weight of the weighing dish with lid =  $W_1 = \dots\dots\dots$ g.

Weight of the dish with lid and material =  $W_2 = \dots\dots\dots$ g.

Weight of the dish with lid and dried material =  $W_3 = \dots\dots\dots$ g.

Weight of the material = (weight of the sample – weight of the dish) = (W<sub>2</sub>-W<sub>1</sub>)  
=.....g.

Quantity of the moisture in the material = (weight of the material before drying – weight of the material after drying) = (W<sub>2</sub>-W<sub>3</sub>) =.....g.

**Percent moisture in the material** = quantity of the moisture in the material \* 100



Weight of the material

### **Determination of Ash Content:**

Method:

Take a clean crucible which was dried in the oven, then cool it and weigh it (W<sub>1</sub>). then take 5g of sample in the crucible and weigh it again (W<sub>2</sub> ). the sample will be churned and placed into muffle furnace for proper ashing at 550°C and left for 5-6 hours. The crucible containing the remaining ash is removed and weighed (W<sub>3</sub> ).

Caluclations:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Materials:

Crucible, muffle furnace

### **Estimation of Tannins by Folin- Denis method.**

#### **Reagents**

**1. Folin-Denis reagent:** Dissolve 100g of sodium tungstate and 20 g phosphomolybdic acid in 750ml distilled water in suitable flask and add 50ml phosphoric acid. Reflux with mixture for 2 hours and make up to one liter with distilled water, protect the reagent from exposure to light.

**2. Sodium carbonate solution:** Dissolve 350g sodium carbonate in one litre of water at 70°C-80°C. Filter through glass wool after allowing it to stand overnight.

#### **3. Tannic acid solution:**

Stock standard: Dissolve 100mg tannic acid in 100ml of distilled water.

Working standard: Dissolve 5ml of stock solution in 100ml with distilled water (concentration 50 $\mu$ g/ml)

**Procedure:**

1. Extraction of Tannin: Weigh 0.5g of the powdered sample and transfer to 250ml conical flask. Add 75ml of water. Heat the flask gently and boil for 30mins. Centrifuge at 2000rpm for 20mins and collect the supernatant in 100ml volumetric flask and make up the volume.
2. Transfer 1ml of the sample extract to 100ml volumetric flask containing 75ml water.
3. Add 5ml of Folin-Denis reagent, 10ml of sodium carbonate solution and dilute to 100ml with water.
4. Shake well. Read the absorbance at 700nm after 30mins.
5. Prepare a standard graph using 0-100 $\mu$ g tannic acid.

**Estimation of total antioxidant Activity:**

**Reagents required**

**1. Standard solution:-**

50mg of Ascorbic acid is dissolved in 50ml standard flask using distilled water. (conc., 1mg/ml)

**2. Extract solution:-**

50mg of methanolic dried extract is dissolved in 50ml standard flask using distilled water. (conc., 1mg/ml).

**3. Phosphomolybdenum Reagent:-**

0.6M H<sub>2</sub>S<sub>4</sub>.

28mM sodium phosphate.

4mM ammonium molybdate.

**Procedure**

1. Prepare (50-250 $\mu$ g) concentration of standard & extract solution, from that take 0.3ml of each sample respectively.
2. To all the tubes add 3.0ml of Phosphomolybdenum reagent.
3. 0.3ml of water and 3.0 ml of reagent alone serves as blank.
4. All the tubes incubate at 97 $^{\circ}$ C for 90minutes.



5. Cooled and the absorbance was measured at 695nm using an UV/Vis spectrophotometrically against the blank. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

#### **Determination of polyphenols:**

Method:

To an aliquot of the extract, 15ml of 20% sodium carbonate was added, mixed well, and after 15min 5ml of Folin-ciocalteus reagent was added and the reaction mixture incubated for 30min at room temperature. The contents were diluted to 100 ml distilled water and the absorbance was measured at 760 nm using UV Spectrophotometer.

#### **Determination of phytate:**

Method:

We have to have take a particular weight of the sample and ground and it has to be soaked in to 100ml of 2% HCl for 5hours and filter. Take 25ml of filtrated sample in to a conical flask, add 50ml of 0.3% potassium thiocyanate solution. The mixture was titrated with a standard solution of  $\text{FeCl}_3$  until a brown-yellow colour persisted for 5 mintues. The concentration of the  $\text{FeCl}_3$  was 1.04% w/v calculations; mole ratio of Fe to phytate = 1:1.

#### **Determination of total phenols:**

Method:

Take 2gm finely grounded sample was extracted with 5-10ml of 80% alcohol in a pestle mortar and the homogenate was boiled in water bath for 5-10 minutes, centrifuged and supernatant was collected and volume made up to 200ml in the same flask (T), then 1.0 ml Folin-ciocalteu's reagent and 0.8ml sodium carbonate (7.5%) were added into 'T' test tube. The absorbance of sample was measured at 760nm after incubating at 30°C for 1.5hr.

Calculation:

Results were expressed as milligram of gallic acid equivalent (GAE) per gram of fresh weight. Standard curve was drawn by plotting the absorbance against concentration of gallic acid.



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