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Nutritional Profile and Antioxidant Activity of *Momordica tuberosa*

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ABSTRACT

Chemical, fatty acid, amino acid composition and antioxidant activity of dehydrated *Momordica tuberosa* Roxb. (Adavi Kakara) was investigated. The dehydrated *M. tuberosa* possessed 21.76% protein and 33.96% fibre. The total lipid of seed was rich in oleic (22.05%), linoleic (25.09%) and linolenic (6.17%) acids. Major amino acids of the seeds are glutamic acid, arginine, leucine, aspartic acid and alanine. The ratio of essential to nonessential amino acids was 0.57 and in which, essential amino acids contributed to an extent of 36.36 g/100 g seed proteins. Inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical at 50% was higher with pulp (6 mg) than whole fruit (14 mg) and seeds (20 mg). The ABTS radical inhibition assay was found to be high in whole fruit (98.4%) and followed by pulp (50.76%) and seed (16.14%) at 2 mg. Marginal differences in ferric ion reducing power were observed in all the samples at 10 mg level.

Keywords: *Momordica tuberosa*, Chemical Composition, Fatty Acid, Amino Acid, Antioxidant Activity

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INTRODUCTION

Adavi kakara (*Momordica tuberosa* Roxb.) is an underutilized vegetable and popularly consumed in southern Indian states of Andhra Pradesh, Maharastra, Tamilnadu, and Karnataka. It is available during August to November every year[1] Generally, *M. tuberosa* is used for the preparation of a traditional curry. Parvathi and Kumar [2] have reported its nutritional composition and standardized the recipes for the preparation of different products such as poriyal, fry, pulikulambu, pickle and vadagam using fresh *M. tuberosa* . They reported that the vegetable is rich source of fiber (6.42%), potassium (500 mg/100 g) and vitamin C (290 mg/100 g). The vegetable is used in the treatment of gout, rheumatism, acute cases of the spleen and liver diseases. Aqueous vegetable extract is used for the treatment of diabeties, malaria, sores and wounds. The seed and pulp extracts have shown antihelimintic activity[3]. Antioxidant activity and hepatoprotective activity of tuber extract of *M. tuberosa* in rats was also reported[4]. It is used as abortifaciant and antiovulatory agent[5]. Fruits were also reported to possess hypoglycaemic activity[6]. The total lipid extraction and fatty acid profile of vegetable was also reported in the literature[7]. Rutin a flavonoid was found to an extent of 0.27% in methanolic extract of *M. tuberosa*[8]. The present study was taken up to prepare dehydrated whole vegetable, pulp and seeds of *M. tuberosa* and evaluate its chemical, fatty acid, amino acid composition and antioxidant activity.

MATERIALS AND METHOD

Materials

Freshly harvested *M. tuberosa* vegetable (4 kg) were collected in 3 batches from different vendors on different days from Rhythu Bazar at Kurnool, Andhra Pradesh, India. The material was stored under refrigerated conditions (4-7 °C) and transported to the laboratory in a insulated box and the analysis was carried with in 24 h after collection. Reagents and solvents used in the study were of analytical and laboratory grade respectively and procured from Sd Fine-Chem Ltd. (Mumbai, India). Chemicals used in antioxidant assays were purchased from Sigma Aldrich, Philadelphia, USA.

Preparation of dehydrated *M. tuberosa*

Whole *M. tuberosa* was washed, stalks removed manually and air dried. Individual parts like pulp, seeds were separately dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55 ± 2 °C for 8 h. The dried materials were ground using a high speed mixer (M/s. Sumeet, Nasik, India), passed through BS 72 (220 μ) mesh. Powder were packed in metallized polyester polyethylene (MPE) laminate pouches.

Physico-chemical composition

Physico-chemical composition such as moisture, ash, fat, protein and fiber of *M. tuberosa* vegetable was carried out using standard methods[9,10]. The percent carbohydrate content was calculated by difference method as follows.

% Carbohydrate = [100 –% (moisture + total ash + protein + fiber + fat)].

Estimation of total polyphenols

Quantification of total polyphenol content (TPC) in dehydrated parts of whole fruit, pulp and seeds of *M. tuberosa* was conducted using a method reported by Sadasivam and Manickam[11].

Determination of total lipid and fatty acid composition by GC and GC-MS

Dehydrated seed (100 g) was made into powder and extracted with a mixture of solvents (chloroform-methanol, 2:1, v/v) at room temperature (RT). The fatty acid methyl esters (FAME) of total lipid were prepared by using mixture of sulphuric acid in methanol (2%, v/v) and were analyzed by Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC-MS) as per the method reported earlier[12].

Estimation of amino acid composition

Dehydrated *M. tuberosa* seeds amino acid profile was determined using an automatic amino acid analyzer. Amino acid profile was determined by high performance liquid chromatography (Shimadzu Model LC 10A, Japan)[13]. Cysteine and methionine contents were determined according to the method reported by Moore[14].

Determination of antioxidant activity

DPPH radical scavenging activity, ABTS assay and ferric ion reducing power was used to determine the antioxidant activity of dehydrated whole *M. tuberosa*, pulp and seeds in the range 0.4-20 mg and compared with that of Trolox at 5-30 µg/ml[15-17].

Statistical analysis

Chemical composition, amino acid composition and antioxidant activity were carried out in triplicate and mean values with standard deviation (SD) were computed by using MS excel, 2007. The seed fatty acid composition was analysed in duplicate.

RESULTS AND DISCUSSION

Chemical composition of dehydrated M. tuberosa

Fresh *M. tuberosa* yielded 21.40% of dehydrated powder. The physico-chemical composition of

dehydrated *M. tuberosa* whole fruit was presented in Table 1. The dehydrated *M. tuberosa* possessed good quantities of protein 21.69% and fibre 33.69% The results are comparable with reported values by Parvathi and Kumar[2].

Table 1:Physico-chemical and polyphenol content of *M. tuberosa*

Parameter, %	Value
Moisture	10.26 ± 0.65
Total ash	8.43 ± 1.13
Crude fat	3.80 ± 0.83
Crude protein	21.76 ± 0.87
Crude fiber	33.96 ± 1.40
Carbohydrates by difference	21.79 ± 1.70
Total polyphenol content, mg/100g	450.00 ± 0.43

a Values are average of triplicate analysis with ± SD

Total polyphenols

In the present study, an amount of 0.45 g/100 g total polyphenols were observed in dehydrated *M. tuberosa*. Higher amount of 0.98 g/100 g in fruit pulp and 0.49 g/100 g total polyphenols in seeds were observed. Earlier, naturally occurring phenols such as flavonoids to an extent of 0.47% (w/w) were reported in *M. tuberosa*[8]. The total phenolic content of the methanolic fraction of *Momordica cymbalaria* was found to be 132 mg/g on dry matter[18]. Polyphenols such as rutin, kaempferol, quercetin, etc., are some important plant flavonoids known for their anti-inflammatory, anti-allergic, antithrombitic, hepatoprotective, antispasmodic and anticancer properties reported in the literature[19]. Natural antioxidants are found in various parts of plants such as leaves, fruits, seeds, roots and bark[20,21]. Antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already being exploited commercially either as antioxidant additives or as nutritional supplements[22].

Fatty acid composition of total lipid

The dehydrated *M. tuberosa* seed yielded 13.37% total lipid by extraction with a mixture of chloroform and methanol. The results of GC and GC-MS analysis of *M. tuberosa* seeds total lipid are presented in Table 2. The SFA and PUFA together constituted an amount of ~76.67% and monounsaturated accounted for 23.33% of total lipid. PUFA contributed to an extent of 38.3% of the total lipid. The fatty acid composition of dehydrated *M. tuberosa* seed was characterized by substantial amounts of palmitic (17.7%), linoleic (22.1%), linolenic (6.2%) and oleic (22.5%). The total lipid was composed of high concentration of saturated fatty acids (38.37%) and the major quantities of oleic, linoleic and linolenic acids which together accounted for 53.31%. Similar observations on the polyunsaturated fatty acid content of 36.7% comprising linoleic acid (28.83%) and eicosadienoic acid (4.98%) from the total lipid of *Sterculia urens* seed were reported[23]. Oleic,

linoleic and linolenic acids together accounted for 74.2% of total lipid and oleic acid contributed to an extent of 20% in the total lipid of *Aegle marmelos* seeds[24]. Jatropha seed oil possessed palmitic acid (14.2%) and linoleic acid (32.8%)[25]. The presence of 17.9% palmitic acid in the Brazilian nuts, 23.9% oleic acid in pine nuts and 17.8% in walnuts and 26.1% linoleic acid in pistachio nuts[26]. These fatty acids were present in *M. tuberosa* seed lipid in identical quantities in the present study. Higher saturated (38.37%) and polyunsaturated (38.3%) and lower monounsaturated (23.33%) fatty acids were noticed in *M. tuberose* seeds lipid. High intake of diets enriched with MUFA protect against atherosclerosis, lower serum cholesterol levels by decreasing oxidative stress and promoting antioxidant defense[27]. The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) was found to be 0.99 and the ratio of polyunsaturated to monounsaturated fatty acids (PUFA/MUFA) was 1.64 in the total lipid. High PUFAs/SFA and PUFAs/MUFAs ratio increases the level of very low density lipoprotein in plasma but reduces the effect of dietary cholesterol in elevating the triglycerides level in liver. PUFA + MUFA/SFA ratio was found to be 1.60. The effects PUFA + MUFA/SFA on plasma and liver lipid concentrations in rats were reported earlier[28]. It was suggested that the requirement for keeping low plasma and liver lipid concentration are low MUFA/SFA ratio and high PUFA/MUFA ratio, which is in good agreement with *M. tuberosa* seed lipids in the present work (0.61 and 1.64). The ratio of PUFA/SFA is generally used to evaluate the nutritional value of lipid. The fixed oil present in *M. tuberosa* vegetable was reported to contain palmitic acid, oleic acid, stearic acid, α-eleostearic acid and γ-linolenic acid[7]. Apart from these fatty acids other fatty acids such as 24:1 (0.4%), 22:0 (0.6%), 20:1 (0.9%) and 20:5 (6.4%) were also observed in the total lipid.

Table 2: Fatty acid composition of *M. tuberosa* seed total lipid[a]

Fatty acid	Retentiontime(min.)	Composition (Wt.%)
Lauric acid (12:0)	5.46	0.29
Myristic acid (14:0)	6.60	0.34
Palmitic acid (16:0)	9.35	17.66
Stearic acid (18:0)	12.29	20.08
Saturated		38.37
Oleic acid (18:1)	12.56	22.05
Docosa monoenoic acid (20:1)	15.99	0.87
Nervonic acid (24:1)	22.41	0.41
Monounsaturated		23.33
Linoleic acid (18:2)	13.12	25.09
Linolenic acid (18:3)	13.78	6.17
Docosapentaenoic acid (20:5)	17.04	6.42
Arachidic acid (22:0)	18.11	0.62
Polyunsaturated	-	38.3

[a]Values are mean of duplicate analyses

Amino acid composition

Amino acid composition of the dehydrated *M. tuberosa* seed is presented as g/100 g seed protein in Table 3. The protein content was found to an extent of 21.2% in seed. Higher amounts of glutamic acid (17.82 g/100 g), arginine, leucine, aspartic acid and alanine were found in *M. tuberosa* seed. In our study, *M. tuberosa* seed was observed to contain essential amino acids leucine (9.28 g) and lysine (5.21 g) followed by valine and phenylalanine and the results are comparable to soy protein isolate (SPI) and casein where leucine (7.0, 8.4 g) and lysine (5.39, 7.1 g) respectively were reported by Tang *et al.*[29].

Table 3: Amino acid composition of *M. tuberosa*[a]

Amino acid	Value, g/100g protein	FAO/WHO Requirements (g/day/70 kg adult)
Alanine	7.39 ± 0.04	
Arginine	11.31 ± 0.20	
Aspartic acid[b]	8.93 ± 0.04	
Cysteine	2.20 ± 0.07	
Glutamic acid[c]	17.82 ± 0.06	
Glycine	5.46 ± 0.04	
Histidine	2.65 ± 0.04	
Isoleucine	3.82 ± 0.07	1.40
Leucine	9.28 ± 0.09	2.73
Lysine	5.21 ± 0.05	2.10
Methionine	1.53 ± 0.04	1.05
Phenylalanine	4.55 ± 0.06	1.75
Proline	4.60 ± 0.06	
Serine	4.80 ± 0.02	
Threonine	3.85 ± 0.11	1.05
Tyrosine	1.13 ± 0.01	
Valine	5.47 ± 0.01	1.82
Total	100	

[a]Values are mean of triplicate analyses
[b]Values include aspartic acid and asparagine
[c]Glutamic acid included glutamine and glutamic acid

The ratio of essential to non essential amino acids in *M. tuberosa* seed was found to be 0.57. It was reported that blending of soy, sesame and peanut flours in 1.1:1.7:0.7 ratios resulted in a product with well balanced essential amino acid profile[30]. It was found that 100 g the resultant protein mix provides leucine (9.28 g) and isoleucine (3.82g). The proportion of the essential amino acids available in the protein were much higher when compared to the requirement as per FAO/WHO[31]. The recommended values of essential amino acids (mg/kg body weight per day) for adult humans are isoleucine (20), leucine (39), lysine (30), methionine and cysteine (15), phenylalanine and tyrosine (25), threonine (15), tryptophan (4) and valine (26). These investigations indicated *M.*

tuberosa seed can be explored further to prepare the protein concentrates, isolates and hydrolysates.

Antioxidant activity of ethanolic extracts

Data on DPPH radical scavenging activity, ABTS assay and ferric ion reducing power of whole, pulp and seed of dehydrated *M. tuberosa* was presented in Table 4. In all the methods the activity of extracts was dose dependent. The activity was more pronounced in the method of ABTS and DPPH assays as seen from the data. IC_{50} value of ABTS radical scavenging activity of whole fruit extract was found to be 1.5 mg/ml when compared to pulp (2 mg) and seed (9 mg) whereas, the standard trolox showed 56.88% inhibition at 5 µg/ml. It was observed that the inhibition was 51.97% with 14 mg/ml of extract, which increased to 69.55% with 20 mg/ml. In ferric ion reducing power, the increase in optical density was much higher for pulp (0.509) followed by seed (0.420) and whole fruit (0.292) at 8 mg/ml. The activity was much lower when compared to standard antioxidant Trolox. Trolox at varying concentrations of 5-30 µg/ml showed an activity in the range of 37.12 to 84.62% inhibition by DPPH assay, 56.88 to 99.14% inhibition by ABTS assay and optical density values of 0.047 to 0.152 by ferric ion reducing power. The variation in total polyphenol content on changes in% inhibition in dehydrated *M. tuberosa* parts might be one of the responsible factors, apart from the other chemical constituents possessing antioxidant activity. The DPPH activity of ethanolic extract and aqueous extract of stevia (20 - 200 µg//ml) increased from 36.93 - 68.76% and 40 - 72.37% in a dose dependent manner and the total phenolic contents were measured as 6.15 and 5.67%[32,33]. The higher total phenol (131 µg) content in stevia leaf extract showed greater antioxidant activity than stevia callus extract (44 µg/ml)[34].The higher DPPH radical scavenging activity (77.7%) was reported when 250 µg/ml methanolic extract of stevia leaf was used[35]. Ferric ion reducing power of aerial parts extract was found to be higher compared to fruits and root extract from *Momordica cymbalaria*. A similar trend was observed in the cases of ABTS radical scavenging activity and total antioxidant activity. IC_{50} value of ABTS radical scavenging activity of fruits extract was found to 13 µg//ml[18]. The antioxidant activity exhibited by annatto seed extract by DPPH assay and ferric ion reducing power may be due to the presence of phenolics[36]. It was reported that the % inhibition activity of standard ascorbic acid and annatto seed extract at concentration range of 0.5- 2.5 µg/ml showed a moderate antiradical activity against DPPH radical with inhibition of about 5.5% to 48.9% and 2.9% to 41.5% respectively. Phytochemicals of plant origin have been found to possess antioxidant or free radical scavenging activity which find application pharmaceutical formulations in oxidative stress associated disorders[37]. Proteins and bioactive components from soybean, canola, maize and capalin

were also found to be responsible for antioxidant activity. All individual amino acids have been shown to have antioxidant activity in some systems, which probably reflect the antioxidant nature of the amino group. The use of a protein or a peptide for the enhancement of the antioxidative activity in functional foods might be a more practical approach than the use of individual amino acids[38]. The polyphenols and proteins of mushroom, tomato and orange were found to responsible for antioxidant activity as reported in the literature[39-41]. Antioxidant activity of the pulp fractions of *M. tuberosa* was found to be higher than the seeds or the whole vegetable, which correlates with the polyphenol contents of the different vegetable parts

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Table-4 Antioxidant activity of whole, pulp and seeds of dehydrated *M. tuberosa*[a]

DPPH, % Inhibition				ABTS, % Inhibition				FRS, Optical density 700nm			
Concentration (mg/ml)	Whole fruit	Pulp	Seed	Concentration (mg/ml)	Whole fruit	Pulp	Seed	Concentration (mg/ml)	Whole fruit	Pulp	Seed
2	14.73	22.96	7.60	0.4	9.77	18.45	-	2	-	-	0.135
4	18.03	42.37	9.05	0.8	18.95	31.52	-	4	0.188	0.342	0.241
6	27.06	53.85	12.41	1.2	25.70	39.42	-	5	-		0.299
8	28.31	66.65	15.78	1.6	57.42	48.91	-	8	0.292	0.509	0.420
10	33.39	80.95	20.58	2.0	98.40	50.76	16.14	10	-	-	0.501
12	35.31	-	25.46	4	-	-	29.51	12	0.444	0.712	-
14	51.97	-	28.91	6	-	-	35.75	14	-	-	-
16	60.71	-	29.61	8	-	-	46.22	16	0.555	0.801	-
18	63.86	-	41.40	10	-	-	54.73	18	-		-
20	69.55	-	50.07		-	-	-	20	0.656	0.925	-

[a] Values are average of triplicate analysis with ± SD (0.001-1.5)
DPPH: 2,2-diphenyl-1-picrylhydrazyl 1,1-diphenyl-2-picrylhydrazyl radical
ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid
FRP: Ferric reducing power

CONCLUSION

The chemical, fatty acid, amino acid composition and antioxidant activity investigations of dehydrated *M. tuberosa* revealed that it is rich in protein and fiber. The seed total lipid was rich in oleic, linoleic and linolenic and low saturated fatty acids. The superior quality protein of *M. tuberosa* seed is due to the presence of essential amino acids. All parts of *M. tuberosa* have shown good antioxidant activity among which highest antioxidant activity was found in whole fruit followed by pulp and seeds. The study reveals that the consumption of *M. tuberosa* would exert several beneficial effects by virtue of their nutritional profile in terms of chemical composition, fatty acid, amino acid, and antioxidant activity.

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