Characterization of nutrients, amino acids, polyphenols and antioxidant activity of Ridge gourd (*Luffa acutangula*) peel

M. P. Swetha¹ · S. P. Muthukumar¹

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Abstract Ridge gourd (*Luffa acutangula*) is consumed as a vegetable after peeling off the skin which is a domestic waste. *Luffa acutangula* peel (LAP) was observed to be a good source of fiber (20.6 %) and minerals (7.7 %). Amino acid analysis revealed presence of the highest content of Carnosine followed by aspartic acid and aminoadipic acid. Antioxidant activity of different extracts showed that ethyl acetate extract was more potent when compared to other solvent extractions. It exhibited a significant amount of phenolic acids like p-coumaric acid (68.64 mg/100 g of dry weight) followed by gallic acid (34.98 mg/100 g of dry weight), protocatechuic acid (30.52 mg/100 g of dry weight) in free form and ferulic acid (13.04 mg/100 g of dry weight) in bound form.

Keywords *Luffa acutangula* peel · Antioxidant activity · Free phenolic · Bound phenolic nutritional composition · Amino acids

Introduction

The family *Cucurbitaceae* comprises members that were cultivated throughout the world as a source of food, fiber and indigenous medicines. *Luffa acutangula* (L.) Roxb commonly known as Ridge gourd belonging to family *Cucurbitaceae* is an annual herb found in parts of India,

especially along the coastal lines. It is dark green in colour and has a tapering end to it. The pulp of this vegetable is white in color and consumed after peeling off the skin (Anon 2014a, b).

Luffa acutangula fruit include carbohydrates, carotene, fat, protein, phytin, amino acids, alanine, arginine, cystine, glutamic acid, glycine, hydroxyproline, leucine, serine, tryptophan and pipecolic acid. Its leaves and flowers contain flavonoids (Schilling and Heiser 1981) and herb contains saponins and acutosides (Nagao et al. 1991). The seeds contain a fixed oil which consists of glycerides of palmitic, stearic and myristic acids (Jaysingrao and Sunil 2012).

Luffa acutangula has high nutritive value and is often called a nutrition powerhouse because of its rich and varied nutrient content. It has vitamin C, riboflavin, niacin, and essential amino acids (Anon 2014a, b). The charantin and peptide which are present in this vegetable have insulin regulatory properties and thus helps in lowering blood sugar levels as well as urine sugar levels. Its high fiber content helps with healthy digestion and proper functioning of the excretory system. It helps in cooling down the body to a large extent, is a natural detoxifier, thus helps in purifying the blood and it also helps in building immune system. It makes the skin glow. The whole plant is also used for the treatment of ulcers and sores (Arunachalam et al. 2012).

From the viewpoint of nutritional value, *Luffa acutangula* seeds are an excellent agricultural product and its kernel have been found potentially rich in protein and fat (39 and 44 %) which are higher than those contained in many plant seeds. The fatty acid profile of *Luffa acutangula* seeds indicates that the glycerides of oleic and linoleic acid constitute 68 % of the total kernel oil. The seeds are also found to be a good source of certain amino acids,

✉ S. P. Muthukumar
muthukumar@cftri.res.in

¹ CSIR – Central Food Technological Research Institute,
Mysore, Karnataka 570020, India

phosphorous, iron, and magnesium (Kamel Basil and Bernice 1982).

Growing interest in the replacement of synthetic food antioxidants by natural ones has fostered research on vegetable sources and screening of raw material to identify potential antioxidants; this applies in particular to plant materials that act as alternative antioxidant sources. From this point of view, the present study was aimed to evaluate the antioxidant potential of LAP.

Materials and methods

Materials

Raw *Luffa acutangula* vegetables were procured from a local market. Hexane, methanol, ethyl acetate, acetone, ethanol, trifluoroacetic acid, hydrochloric acid, sulphuric acid, sodium hydroxide and FC (Folin-Ciocalteu) reagent was procured from Rankem (Bangalore, India). All the reagents were of analytical grade. HPLC (High-pressure liquid chromatography) grade gallic acid, catechin, epicatechin, p-coumaric acid, quercetin, ferulic acid, vanillin, caffeic acid, protocatechuic acid, cinnamic acid and DPPH (2, 2-diphenyl-1-picryl-hydrazyl) were procured from Sigma-Aldrich Chemicals Pvt Ltd (Bangalore, India). HPLC column (10u C18) was procured from Waters India Pvt Ltd (Bangalore).

Sample preparation

Luffa acutangula vegetables were cleaned and peeled. Peels were dried in hot air oven at 40 °C for 12 h, powdered using a motor and pestle and stored in airtight container for further use.

Proximate analysis of LAP

Proximate composition of LAP like moisture, protein, fat, minerals, fiber and carbohydrate content were analyzed as per AOAC method (AOAC 2005).

Amino acid composition

The sample (7 mg, equivalent to 1 mg protein) was placed in a hydrolysis tube and dried. The lyophilized protein was hydrolysed with 200 µL of Hydrolysis Solution (6 N hydrochloric acid containing 0.1 to 1.0 % of phenol) per 500 µg. The sample tubes were frozen in dry ice- acetone bath, and flame sealed in a vacuum. Samples were hydrolyzed at 110 °C for 24 h in vacuum or inert atmosphere to prevent oxidation. To the hydrolyzed samples, coupling reagent (derivitization mixture of 10 µl phenyl

isothiocyanate, 70 µl methanol, 10 µl triethylamine and 10 µl filtered Milli-Q water) was added and kept in thermomixer for 1 h at 45 °C. Then 200 µl of 10 mM sodium acetate buffer was added, and 20 µl volume was injected into HPLC instrument (Agilent 1100 series). Amino acids were separated using Agilent TC-C18 analytical 4.6 × 250 mm 5 µ, a solvent system consisting of buffer A: 10 mM sodium acetate adjusted to pH 6.4 with 6 % acetic acid, buffer B: 10 mM sodium acetate and 60 % acetonitrile adjusted to pH 6.4 with 6 % acetic acid at the rate of 1 ml/min. The compounds eluted were monitored at 254 nm wavelength.

DPPH assay

The free radical scavenging activity was measured by using DPPH as described by Kalpna et al. 2011. About 3 ml reaction mixture consisting of DPPH in methanol (0.1 mM) and 150 µl different concentrations of the solvent extracts were incubated for 30 min in the dark, followed by which the absorbance was measured at 517 nm. Absolute methanol was used as blank, DPPH as control and ascorbic acid as a positive control. Percent of radical scavenging activity was calculated using the formula:

$$\text{Radical scavenging activity (Percentage)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

The IC₅₀ value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 %, was calculated from the results and used for comparison of the quality of antioxidant extracts. The radical scavenging activity of vitamin C was also measured in the same condition for comparison purpose.

Total polyphenol content

The total polyphenol content of the extracts was assessed using the FC assay (Adel et al. 2010). Briefly, standard-curve was derived using gallic acid at different concentrations ranging from 50 to 500 mg/L. For the analysis, different concentrations of sample extract, 100 µl of FC reagent (1:1 dilution) and 300 µl of 200 gL⁻¹ of sodium carbonate were added and incubated in shaking incubator for 30 min at 40 °C. The absorbance was then measured at 760 nm. The results were expressed in mg gallic acid equivalent/g dry weight (mg GAE/g of DW). All samples were tested in triplicate.

Free and bound phenolic extraction

Cleaned and dried LAP (~25 g) was extracted five times with 100 ml of 70 % methanol at 37 °C. After filtration,

the combined supernatants were evaporated under vacuum at 45 °C to a 20 ml volume. The aqueous suspension was adjusted to pH 2 by using 6 N HCL and centrifuged to separate a cloudy precipitate. The clear supernatant was extracted five times with ethyl acetate at a solvent to the water phase ratio of 1:1. The ethyl acetate extracts were evaporated to dryness under vacuum at 45 °C. The dry residues were re-dissolved in HPLC grade methanol. The residues from the methanol extractions were extracted with 100 ml of 4 N NaOH under nitrogen gas atmosphere. After acidification and centrifugation, the clear supernatants were obtained with ethyl acetate as described above (Krygier et al. 1982).

Identification and quantification of free and bound phenolic profile

The phenolic acids fractions of LAP were analyzed using HPLC. Individual phenolic acids were identified and quantified through reverse phase C18 column on HPLC using solvent A (10 % methanol and 1 µm trifluoroacetic acid) and solvent B (80 % methanol trifluoroacetic acid). The solvent gradient was 90 % solvent A and 10 % solvent B to 100 % solvent B for 60 min. Peaks eluted were monitored at 280 and 320 nm to detect various compounds. The retention times of these peaks were compared with standards of phenolic acids, and their identifications were confirmed by spiking with authentic standards. Identified peaks were quantified by calculating peak area with a known concentration of standard peak area.

Results and discussion

Proximate analysis

The proximate analysis (Table 1) indicated that LAP has an appreciable amount of nutrients. The total protein content in DMSF was 14.26 %, moisture content was 12.40 % and fat content 6.10 %. The carbohydrate content in DMSF was determined after acid hydrolysis and it was 38.94 %. The crude fibre content was 20.60 % and the ash content was 7.70 %. These results have shown that LAP was good source of fiber and minerals.

Amino acid composition

The amino acid profile of LAP is presented in Table 2. Carnosine was highest in LAP (23.20 µg/mg of protein). Carnosine is a dipeptide of the amino acids beta-alanine and histidine. It was found to inhibit diabetic nephropathy, retard cancer growth, and acts as antiglycating agent (Reddy et al. 2005). The amino acids profile revealed that

Table 1 Proximate composition of *Luffa acutangula* peel

Parameters	Percentage (DMB)
Moisture content (%)	12.40 ± 0.23
Carbohydrate content (%)	38.94 ± 0.49
Protein content (%)	14.26 ± 0.17
Fat content (%)	6.10 ± 1.41
Fiber content (%)	20.60 ± 0.16
Ash content (%)	7.70 ± 0.45

Values are means (n = 3)

DMB Dry matter base

Table 2 Amino acid composition of *Luffa acutangula* peel

Amino acids	µg/mg of protein
Alanine	12.12 ± 0.41
Amino adipic acid	21.20 ± 0.73
Anserine	3.93 ± 0.74
Arginine	1.68 ± 0.19
Aspartic acid	21.77 ± 0.78
Carnosine	23.20 ± 0.12
Cysteine	10.00 ± 0.08
Glutamic acid	5.14 ± 0.45
Histidine	2.34 ± 0.43
Hydroxyl lysine	8.00 ± 0.11
Isoleucine	15.61 ± 0.19
Leucine	5.40 ± 0.81
Lysine	6.95 ± 0.51
Methionine	2.51 ± 0.19
1-Methyl histidine	0.73 ± 0.13
Phosphoethanolamine	5.61 ± 0.56
Proline	15.42 ± 0.48
Taurine	0.49 ± 0.28
Tryptophan	3.23 ± 0.71
Tyrosine	14.01 ± 0.13

Values are mean (n = 3) ± SD

LAP contained five essential amino acids namely leucine (05.40 ± 0.81 µg/mg protein), isoleucine (15.61 ± 0.19 µg/mg protein), lysine (06.95 ± 0.51 µg/mg protein), methionine (02.51 ± 0.19 µg/mg protein) and histidine (02.34 ± 0.43 µg/mg protein). These amino acids are purported to play a role in the biological system such as regulating the blood sugar concentrations, growth and repair of muscles/tissues, hormone production, wound healing, energy production, development of haemoglobin. They also aid in the production of antibodies and enzymes and prevent arterial fat build up (Anon 2014a, b). Among nonessential amino acids LAP contained aspartic acid (21.77 ± 0.78 µg/mg protein), glutamic acid

(05.14 ± 0.45 µg/mg protein), alanine (12.12 ± 0.41 µg/mg protein), arginine (01.68 ± 0.19 µg/mg protein), proline (15.42 ± 0.48 µg/mg protein), tyrosine (14.01 ± 0.13 µg/mg protein), and cysteine (10.00 ± 0.08 µg/mg protein). Cysteine acts as an antioxidant and protects the body from radiation and pollution. It also aids in protein synthesis and prevents cellular changes (Nagano et al. 1999). Also, it deactivates free radicals and neutralizes toxins. LAP also contained amino acid like aminoadipic acid which was reported an intermediate in the aminoadipic acid pathway and organic acids like taurine and anserine (3.93 µg/mg of protein) both of which were dipeptide containing β-alanine and histidine (Anhwange et al. 2004). The results indicated that LAP was a good source of essential amino acids.

DPPH assay

Radical scavenging capacity is a widely accepted method for estimation of antioxidant activity in vitro. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reactions (Sreeramulu and Raghunath 2010). There are different types of radicals, such as cation ABTS⁺ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), radical DPPH⁺ and peroxy radical. The advantage of the radical DPPH⁺ scavenging capacity assay is that DPPH⁺ is a stable radical and the test is relatively easy to perform. This assay relies on the decreasing absorbance when DPPH⁺ radicals are quenched by antioxidants. The stable DPPH in alcoholic solution reduces to the yellow-coloured diphenyl-picrylhydrazine (non-radical form DPPH) in presence of antioxidant. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Mohan et al. 2012).

In the present study, the DPPH⁺ scavenging capacity of various ridge gourd peel extracts was analyzed. Ascorbic acid was used as positive control/reference antioxidant to compare our results. Extracts were able to quench the DPPH⁺ radical, but the capacity varied among different extracts. Among five different extracts, aqueous extract showed comparatively more scavenging activity (24.71 %) followed by ethanol (18.87), acetone (13.05), methanol (11.13) and ethyl acetate extracts (7.14) respectively (Table 3). Water extract was found to be a potent free radical scavenger with IC₅₀ value 0.05 µg/ml followed by ethanol (0.07 µg/ml) as shown in Table 3.

Total polyphenol content

The FC method provides a convenient, rapid and simple estimation of the content of total phenolics (der Jens et al. 2009). The total phenolic content of methanol, aqueous,

Table 3 Radical scavenging activity of different extracts of Ridge gourd peel

Extracts	Percentage of scavenging activity	IC ₅₀ value (µg/ml)
Acetone	13.05	0.11 ± 0.01
Ethanol	18.87	0.07 ± 0.01
Water	24.71	0.05 ± 0.01
Methanol	11.13	0.13 ± 0.05
Ethyl acetate	7.14	0.26 ± 0.09

Values are mean (n = 3) ± SD

acetone, ethanol and ethyl acetate extracts was found to be 00.43 ± 00.1, 00.26 ± 00.1, 0.063 ± 0.01, 0.043 ± 0.03, 0.041 ± 0.01 mg/100 g of LAP, respectively. Aqueous extract exhibited good antioxidant activity and high phenolic content compared to other solvent extracts as LAP is rich in vitamin C, a water-soluble compound might have contributed to the activity. This method is also used to estimate other oxidation substrates hence the FC assay performed with ascorbate found to be 94–96 % of the compound recovery after a spiking test, so there is potential for significant interference using these molecules (Singleton et al. 1999).

Identification and quantification of polyphenols

Polyphenols are known as a potential source of antioxidants (Katalinic et al. 2006; Wojdylo et al. 2007) because they protect human beings against both non-communicable and communicable diseases (Cai et al. 2004; Yao et al. 2004; Jiangrong and Jiang 2007). The number of hydroxyl groups on phenol ring is directly proportional to its antioxidant activity. All types of phenolic acids have shown potential antioxidant activities such as p-coumaric acid (Niwa et al. 2001; Tapia et al. 2004), ferulic acid and gallic acid (Karamac and Amarowicz 2004; Balasobashini et al. 2004). Though many reports are available for the presence of these phenolic acids in plants (Ozkan and Baydar 2006; Olszewska 2007), there is very limited literature about LAP.

Flavonoids are well-known as free radical scavengers by fast donation of hydrogen atoms to free radicals which mainly depends on the availability of phenolic hydrogen atom and substitution pattern of hydroxyl groups. Among flavonoids, Quercetin and catechin are potent free radical scavengers (Bouchet et al. 1998; Levites et al. 2001; Silva et al. 2002; Materska and Perucka 2005; Dukic et al. 2008; Jahan et al. 2013).

Liquid chromatography is a routinely used procedure for the separation of individual phenolic compounds from complex mixtures, based on their different affinities for a resin-packed column. Changing the pH and ionic strength

of the solution helps to allow the compounds of interest to elute in a sequential manner (Mandal and Dey 2008). RP-HPLC was used for the identification and quantification of important phenolic acids and flavonol from ridge gourd peel. The compounds were numbered according to their order of elution from the lowest to the highest RT (Retention Time) and compared with that of the standards to identify the unknown. In this study, a total of 8 phenolic compounds were determined by comparing RT (Chandrasekara and Shahidi 2011).

Five phenolic acids including gallic acid, p-coumaric acid, ferulic acid, vanillin, protocatechuic acid and two flavanols namely catechin and quercetin were determined and quantified by HPLC (Table 4). For quantitative validation by HPLC, Individual phenolic acids were collected and spiked with known concentration of standard. p-coumaric acid, gallic acid, and protocatechuic acid are the main phenolic acids present in LAP. In free form, p-coumaric acid (68.64 ± 1.46 mg/100 g dw) shows highest concentration followed by gallic acid (34.98 ± 1.89 mg/100 g dw),

protocatechuic acid (30.52 ± 1.25 mg/100 g dw), quercetin (28.84 ± 1.22 mg/100 g dw), catechin (23.36 ± 0.63 mg/100 g dw), ferulic acid (3.84 ± 1.25 mg/100 g dw) and cinnamic acid (1.48 ± 0.14 mg/100 g dw) (Fig. 1). In bound form, catechin (22.80 ± 1.28 mg/100 g dw) is found in high concentration followed by quercetin (21.72 ± 0.17 mg/100 g dw), vanillin (19.60 ± 0.65 mg/100 g dw), ferulic acid (13.04 ± 0.31 mg/100 g dw), p-coumaric acid (7.00 ± 0.07 mg/100 g dw), gallic acid (0.20 ± 0.01 mg/100 g dw) and cinnamic acid (0.22 ± 0.01 mg/100 g dw) (Fig. 2). LAP is an amalgamation of various phenolic acids and flavanols which collectively contribute to its strong antioxidant activity.

Conclusion

The results indicate that LAP contains a significant amount of fiber, essential and nonessential amino acids. The water and ethanol extract of LAP seeds possess high

Table 4 Free and bound phenolic profile identified through HPLC

Polyphenol	Free phenolic (mg/100 g of DW)	Bound phenolic (mg/100 g of DW)
Gallic acid	34.98 ± 1.89	0.20 ± 0.01
Protocatechuic acid	30.52 ± 1.25	–
Vanillin	–	19.60 ± 0.65
Catechin	23.36 ± 0.63	22.80 ± 1.28
p-coumaric acid	68.64 ± 1.46	7.00 ± 0.07
Ferulic acid	3.84 ± 0.43	13.04 ± 0.31
Cinnamic acid	1.48 ± 0.14	0.22 ± 0.01
Quercetin	28.84 ± 1.22	21.72 ± 0.17

Values are mean ($n = 3$) $\pm$ SD

Fig. 1 HPLC chromatograph of free phenolics of *Luffa acutangula* peel; 1 gallic acid, 2 protocatechuic acid, 3 catechin, 4 p-coumaric acid, 5 ferulic acid, 6 cinnamic acid, 7 quercetin

Fig. 2 HPLC chromatograph of bound phenolics of *Luffa acutangula* peel; 1 gallic acid, 2 vanillin, 3 catechin, 4 p-coumaric acid, 5 ferulic acid, 6 cinnamic acid, 7 quercetin

antioxidative properties compared to other solvent extracts. Presence of varied number of flavonols and phenolic acids which are known to possess therapeutic benefits indicates the potential of LAP to be used for the alleviation of many disorders. Moreover, HPLC with diode array detection has shown the presence of gallic acid, protocatechuic acid, catechin, p-coumaric acid, ferulic acid, cinnamic acid and quercetin both in free and bound form, whereas vanillin only in bound form. The biological activity of these compounds requires further investigation to understand their potential health benefits.

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References

- Anhwange BA, Ajibola VO, Oniye SJ (2004) Amino acid composition of the seeds of *Moringa oleifera* (Lam), *Detarium microcarpum* (Guill & Sperr) and *Bauhinia monandra* (Linn.). J Chem Class 1:9–13
- Anon (2014) Reference guide for Amino acids. <http://www.realtime.net/anr/aminoacd.html>. Accessed 20 Oct 2015
- Anon (2014) Learn and share India. Spiceflair publishing. <http://www.spiceflair.com/ridge-gourd/>. Accessed 5 Nov 2015
- AOAC international Methods (2005) Official methods of analysis of association of official analytical chemists, 18th edn. AOAC international Methods, Washington
- Arunachalam A, Selvakumar S, Jeganath S (2012) Toxicological studies on ethanol extract of luffa acutangula in albino wistar rats. Int J Curr Pharm Clin Res 2(1):29–33
- Balasobashini MS, Rukkumani R, Viswanalhan P, Venugopal M (2004) Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother Res 18:310–314
- Bouchet N, Barrier L, Favconneav B (1998) Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae). Phytother Res 12:564–582
- Cai LQ, Sun M, Croke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plant associated with anticancer. Life Sci 74:2157–2184. doi:10.1016/j.lfs.2003.09.047
- Chandrasekara A, Shahidi F (2011) Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS. J Funct Food 3(3):144–158. doi:10.1016/j.jff.2011.03.007
- der Jens MA, Rawel H, Lothar Kroh W (2009) Composition of phenolic compounds and glycoalkaloids r-solanine and r-chaconine during commercial potato processing. J Agric Food Chem 57:6292–6297
- Dukic NM, Simin N, Cvejic J, Jovin D, Orcic E, Bozin B (2008) Phenolic compounds in field Horsetail (*Equisetum arvense* L.) as natural antioxidant. Molecules 13:1455–1465. doi:10.3390/molecules13071455
- Jahan N, Khalil-ur-rahman AS, Asi RM (2013) Phenolic acid and flavonol contents of gemmo-modified and native extracts of some indigenous medicinal plants. Pak J Bot 45(5):1515–1519
- Jaysingrao JS, Sunil CN (2012) Fatty acid profile of fruits of *Luffa acutangula* var. amara c.b.clarke. Global J Res Med Plant Ind Med 1(8):323–327
- Jiangrong L, Jiang Y (2007) Litchi flavonoids: isolation, identification and biological activity. Molecules 12:745–758. doi:10.3390/12040745
- Kalpna R, Mital K, Sumitra C (2011) Full length research paper vegetable and fruit peels as a novel source of antioxidants. J Med Plan Res 5(1):63–71
- Kamel Basil S, Bernice B (1982) Nutritional and oil characteristics of the seeds of angled Luffa *Luffa acutangula*. Food Chem 9(4):277–282
- Karamac M, Amarowicz R (2004) Antioxidant activity of phenolic fractions of white bean (*Phaseolus vulgaris*). J Food Lipids 11:165–177. doi:10.1111/j.1745-4522.2004.tb00268.x
- Katalinic V, Milos M, Kulisic T, Jukic M (2006) Screening of 70 medicinal plant extract for antioxidant capacity and total polyphenols. Food Chem 94:550–557. doi:10.1016/j.foodchem.2004.12.004
- Krygier K, Sosulski F, Hogge L (1982) Free, esterified and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J Agric Food Chem 30:330–334
- Levites Y, Winreb O, Maor G, Yondim MB, Mande S (2001) Green tea polyphenol-epigallo, catechin-3-gallate prevent N-methyl-4-phenyl-1-2-3-6-tetra hydro pyridine induced dopaminergic

- neuron degeneration. *J Neurochem* 78:1073–1082. doi:[10.1046/j.1471-4159.2001.00490.x](https://doi.org/10.1046/j.1471-4159.2001.00490.x)
- Mandal SM, Dey S (2008) LC-MALDI-TOF MS-based rapid identification of phenolic acids. *J Biol Tech* 19:116–121
- Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit. *J Agri Food Chem* 53:1750–1756
- Mohan R, Birari R, Karmase A, Jagtap S, Bhutani KK (2012) Antioxidant activity of a new phenolic glycoside from *Lagenaria siceraria* stand. *Fruits Food Chem* 132(1):244–251. doi:[10.1016/j.foodchem.2011.10.063](https://doi.org/10.1016/j.foodchem.2011.10.063)
- Mohdaly AAA, Sarhan MA, Smetanska I, Mahmoud A (2010) Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *J Sci Food Agric* 90:218–226
- Nagano N, Ota M, Nishikawa K (1999) Strong hydrophobic nature of cysteine residues in proteins. *FEBS Lett* 458(1):69–71
- Nagao T, Tanaka R, Iwase Y, Hanazono H, Okabe H (1991) Studies on the constituents of *Luffa acutangula*. ROXB structures of acutosides A—G oleanane type triterpene, saponins isolated from herb. *Chem Pharm Bull* 39(3):599–606
- Niwa U, Doi Y, Kato Y, Osawa T (2001) Antioxidant properties of phenolic antioxidants isolated from corn steep liquor. *J Agric Food Chem* 49:177–182
- Olszewska M (2007) Quantitative HPLC analysis of flavonoids and cholorogenic acid in the leaves and inflorescences of *Prunus serotina* Ehrh. *ACTA Chroma* 19:253–269
- Ozkan G, Baydar NG (2006) Direct RP-HPLC determination of phenolic compounds in Turkish red wines. *Med Univ Fac Agric J* 19(2):229–234
- Reddy VP, Garrett MR, Perry G, Smith MA (2005) Carnosine: a versatile antioxidant and antiglycating agent. *Sci Aging Knowl Environ* 18:12. doi:[10.1126/sageke.2005.18.pe12](https://doi.org/10.1126/sageke.2005.18.pe12)
- Schilling EE, Heiser CB (1981) Flavonoids and the systematics of *Luffa acutangula*. *Biochem Syst Ecol* 9(4):263–265
- Silva MM, Santos MR, Caroco G, Rocha R, Justiuo G, Mira L (2002) Structure antioxidant activity relationships of flavonoids: a re-examination. *Free Radic Res* 36:1219–1227
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178
- Sreeramulu D, Raghunath M (2010) Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. *Food Res Int* 43(4):1017–1020. doi:[10.1016/j.foodres.2010.01.009](https://doi.org/10.1016/j.foodres.2010.01.009)
- Tapia A, Rodriguez J, Theoduloz C, Lopez S, Feresin GE, Schmeda-Hirschman G (2004) Free radical scavengers and antioxidants from *Baccharis grisebachii*. *J Ethnopharmacol* 95:155–161. doi:[10.1016/j.jep.2004.06.035](https://doi.org/10.1016/j.jep.2004.06.035)
- Wojdylo A, Oszmianski J, Gzemerys R (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 10:940–949. doi:[10.1016/j.foodchem.2007.04.038](https://doi.org/10.1016/j.foodchem.2007.04.038)
- Yao LH, Jiang YM, Shi J, Barberan T, Datta FA, Siganusong R, Chen S (2004) Flavonoids in food and their benefits. *Plan Food Hum Nutr* 59:113–122