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Original article

Antioxidant potential and amino acid analysis of underutilized tropical fruit *Limonia acidissima* L.

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ABSTRACT

Objective: *Limonia acidissima* L. an underutilized edible fruit was evaluated for its antioxidant activity, free radical scavenging ability, proximate and amino acid analysis using established *in vitro* assay models.
Methods: 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging assay, trolox equivalent antioxidant capacity (TEAC) assay, hydroxyl radical scavenging activity (HRSA), ferric reducing antioxidant power (FRAP) assay, nitric oxide radical (NO$^{\bullet}$) scavenging activity, total antioxidant activity (TAA) were carried out. The total phenolic (TP) and flavonoid contents (TF) of the extracts were determined and expressed as gallic acid and quercetin equivalents.
Results: The highest percentage of phenol and flavonoid contents were observed in methanol and the lowest content was found in chloroform extract. Also, methanol extract recorded higher activity in DPPH$^{\bullet}$, HRSA, FRAP and TAA whereas, ethyl acetate extract of the fruit was found to be active for ABTS$^{\bullet+}$ radical scavenging activity. Further, water extract of the fruit exhibited potentially high nitric oxide radical scavenging activity than other solvent extracts. Moreover, the phenolic and flavonoid contents of the fruit extract significantly correlated with antioxidant capacity. Amino acid analysis revealed that among, all essential amino acids, the concentrations of isoleucine, phenylalanine and tryptophan were found to be present in higher amounts.
Conclusion: Positive correlation was observed between polyphenolic contents and the antioxidant capacities. It is evident from the study that the fruit possess potent antioxidant activity with enormous health benefits and thus may be used in food and pharmaceutical applications.

1. Introduction

Limonia acidissima L. Swingle Syn. *Feronia elephantum Correa*, *Schinus limonia* L. (Rutaceae), is a tropical plant species in the Indian subcontinent. Indigenous to India and was locally known as elephant apple, monkey fruit, curd fruit and katha bel.[1,2] The fruits are woody, rough used in treatment of diarrheoa, dysentery,[3] wounds, cardiac debility, liver tonic hiccough, sore throat, gum disease and hepatitis.[1] The fruits are sour and sweet, has been used in Indian folk medicine, in Ayurveda for the treatment of blood impurities, leucorrhoea and in Yunani medicine as diuretic.[4] Fruit pulp showed anti inflammatory, antipyretic, analgesic activity[5] larvicidal and antimicrobial activity.[6] The fruits of *L. acidissima* contains flavonoids, phytosterols, glycosides, saponins, tannins, coumarins, triterpenoids, carbohydrates, vitamins, amino acids as its chemical constituents[7] and tyramine derivatives have also been isolated.[8] Besides, it is an effective herbal remedy for diabetes, it is experimentally proved for the blood glucose lowering potential.[9] Antioxidant activities and the traditional claims of the *L. acidissima* fruits possessing wound healing property were demonstrated by Ilango et al (2010).[10] Saima et al (2000)[11] isolated the pectic polysaccharide, FL-1a-I from the fruits of *Feronia limonia*, which exhibited *in vivo* antitumour activity of Ehrlich ascites carcinoma in the murine model. The unripe fruit contains 0.015% stigmasterol[12] and seeds contain oil high in saturated fatty acids and lowered the blood glucose levels in streptozotocin-induced diabetic male albino rats.[4] The fruit pulp has also been studied for antiulcer activity.[13] It is recognized that naturally occurring substances in higher plants have antioxidant activity. Reports have shown that presence of natural antioxidants from medicinal and aromatic plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis.[14]

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Plant phenols also exhibit significant antioxidant, antitumour, antiviral and antibiotic properties.[15] Safety and efficacy of the synthetic antioxidants used in the food industry are frequently questioned because such antioxidants are unstable and highly volatile[16] therefore, interest in finding naturally occurring antioxidants that have the potential to protect human beings from damage induced by oxidative stress has intensified.[17] Therefore, present study evaluates the antioxidant activities of the fruit pulp of *L. acidissima.*

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid (trolox), potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), 2,4,6-tripyridyl-S-triazine (TPTZ), ferrous ammonium sulphate, ferric chloride, ethylene diamine tetra acetic acid (EDTA) disodium salt, trichloroacetic acid (TCA), Potassium ferricyanide, Folin–Ciocalteu's reagent, aluminium chloride, ascorbic acid, ammonium acetate, glacial acetic acid, acetyl acetone, sodium nitroprusside, sulfanilic acid, naphthyl ethylene diamine dihydrochloride, sodium phosphate and ammonium molybdate were obtained from Himedia (Mumbai, India), Merck (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.2. Plant material and preparation of extracts

The well matured fruits of *L. acidissima* were collected from Vellingiri hills, Coimbatore (T.N.) India was taxonomically identified and authenticated at Botanical Survey of India (BSI), Coimbatore. The rind was cracked and fruit pulp was shade dried, powdered and extracts were prepared by using solvents of varying polarity. A portion (50 gm) of dried fruit material was extracted with 250 ml (1:5) of petroleum ether followed by chloroform, ethyl acetate, methanol and water in a Soxhlet apparatus (8 h for each solvent). Subsequently, the solvent extract was evaporated to dryness at 40 °C in a rotary evaporator (Buchi type, Flawil/Schweiz, Switzerland). The water extract was cooled and lyophilized (Alpha 1-2 LD model, CHRIST, German). The yield of each fruit extract constituents were calculated and stored in the dark at 4 °C prior to use.

2.3. Proximate composition

Chemical analysis to determine proximate composition of sample was carried out using standard procedures of AOAC (1990).[18] The Kjeldahl method was used for total nitrogen determination using a Kjeltec System. Protein was calculated from total nitrogen using a factor of 6.25. The Soxhlet method was used for total fat determination. Total fat was obtained from 6 h hexane extraction. Crude fibre was obtained after samples digestion with boiling diluted acid and alkali. Moisture was determined from sample weight loss after oven drying at 110 °C for 4 h. Ash content was calculated after heating the sample at 550 °C for 2 h. Carbohydrates was determined by difference. All samples were analyzed in triplicate.

2.4. Amino acid composition analysis

The powdered fruit sample of *L. acidissima* was hydrolyzed using 6 N HCl at 110 °C for 24 h. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Shimadzu LC-10 AD, Shimadzu Corporation, Kyoto, Japan). Samples were analyzed on Shimpack amino-Na type column (10 cm × 6.0 mm) obtained from Shimadzu Corporation. The post column samples were derivatized with *o*-phthaldialdehyde (OPA) and data were integrated using an integrator model C-R7A (Shimadzu chromatopac data processor).[19] The amount of each amino acid present in the sample was calculated as mg/100 g dry weight.

2.5. Total phenol content (TP)

Total phenolics content was determined by using Folin–Ciocalteu method Singleton et al (1999).[20] An aliquot of sample extract (0.1 ml) was mixed with distilled water (3 ml). To this 0.5 ml of Folin–Ciocalteu reagent was added. After 3 min 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath (100 °C) for exactly 1 min. It was then cooled and the absorbance was measured at 650 nm using spectrophotometer (Shimadzu, UV 2450) against the reagent blank. The results were expressed as milligram (mg) gallic acid equivalent (GAE) per gram sample dry weight.

2.6. Total flavonoid content (TF)

Total flavonoid content in the extract was determined based on the method described by Ordonez et al (2006).[21] A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm with UV–Visible spectrophotometer (Shimadzu, UV 2450). A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as milligram (mg) quercetin (QE) equivalent per gram sample dry weight.

2.7. 2, 2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging assay

The DPPH radical scavenging assay was determined according to Leong and Shui (2002).[22] Briefly, 2 ml of 0.15 mM DPPH (in methanol) was added to the different concentrations of the extract (1 ml). The reaction mixture was incubated for 30 min after which its absorbance was measured at 517 nm, methanol was used as both a blank and negative control. Results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) and defined as mg ascorbic acid equivalents (AA)/100 g of fresh weight basis.

2.8. Trolox equivalent antioxidant capacity (TEAC) assay

The method is based on the reduction of the ABTS radical cations ($ABTS^{\bullet+}$) by antioxidants present in extracts prescribed by Re et al (1999).[23] $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM aqueous ABTS with 2.45 mM potassium persulfate and kept in the dark at room temperature for 16 h. The blue–green solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The stock solution of sample extracts was diluted. After the addition of 1 ml of diluted $ABTS^{\bullet+}$ solution to 10 μl of antioxidant compounds or trolox standards in ethanol, it was incubated at 30 °C exactly 30 min. Appropriate solvent blanks were also run in each assay. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The unit of antioxidant activity was defined as the concentration of trolox having the equivalent antioxidant activity expressed as mm trolox equivalent per gram sample dry weight.