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Sugar profile, total phenolic and antioxidant potential of anthocyanins rich *Syzygium cumini* fruit

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ABSTRACT

Fruits of *Syzygium cumini* (Jamun) are well known for its nutritional, therapeutic properties and as a rich source of dark purple colored anthocyanins. Anthocyanins rich jamun pulp was extracted with acidified ethanol. The total anthocyanins content 209 mg/100 g fruit on a dry weight basis was determined by pH differential method. The antioxidant activity was investigated with FRAP and DPPH assay which indicates that jamun extract have significant antioxidant activity. Folin-Ciocalteau method was used to determine the total phenolic content which found to be 285 mg GAE100 g^{-1}. The sugar composition of jamun fruit was investigated by GC-MS. Glucose (88.28%) was found to be the main sugar while other sugars like fructose (6.4%) and sorbose (4.1%) were also present. β-D-galactofuranose was also present in trace while no trace of sucrose was found. The results revealed that jamun may serve as dietary source of natural antioxidants and sugar for health promoting effects.

KEYWORDS

Anthocyanins;
pH-differential method;
Antioxidant activity;
GC-MS.

INTRODUCTION

Free radicals generated in the redox processes, may cause cancer, cardiovascular diseases and neurodegenerative diseases by inducing oxidative damage to biomolecules[1,2,3].Antioxidants play an important role in biological systems by suppressing the formation/scavenging of reactive free radicals (OH^-, ROO^-, O_2^-)[4-6]. Bioactive compounds, such as carotenoids and phenolics exhibit strong antioxidant activity and help prevent oxidative damage. Phenolic compounds are secondary metabolites and important component of fruits and vegetables. They not only influence the sensory qualities but also regulate the biological activities[7,8]. They are potent antioxidants with redox properties and act as reducing agents, hydrogen donators and singlet oxygen quenchers[9-11]. Due to their potent antioxidant properties they are widely used in therapeutic and nutritional supplements[12]. Anthocyanins are naturally occurring poly-phenolics that impart orange, red, purple and/or blue colour to many fruits, vegetables, flowers and plants. Such compounds are known for their strong antioxidant capacity[13,14] and health-protecting effects such as reduced risk of coronary heart disease, prevention of cancer and neurodegenerative diseases[15-17]. Sugars are main energy source in human diet[18] and determined the fruit quality and the sweetness. Sugars play various roles from diet to cardiovascular diseases, diabetes and dental decay in the human body[19].

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Syzygium cumini (Jamun) is evergreen medicinal plants belong to Myrtaceae family is native to tropical/ sub-tropical regions especially India, Southeast Asia and Australia. The berry is oblong, ovoid and shinning crimson black when fully ripe. Ripe fruits are very juicy, almost odorless, with a pleasant and astringent taste[20]. Jamun fruits are universally accepted to be very good for medicinal purposes especially for curing diabetes[21]. The *in vitro* antioxidant activity of jamun fruit anthocyanins had been proved by many researchers[22,23]. The anti-proliferative and pro-apoptotic effects of jamun fruit against breast cancer cells was reported[24,25]. Due to high phytochemical constituents, jamun fruits are well known for its nutritional and therapeutic properties[26]. The present study is therefore focused on determination of sugar, total phenolic, anthocyanin content as well as overall antioxidant potential of jamun fruit.

MATERIALS AND METHODS

Reagents and chemicals

2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) and 1,1-diphenyl-2-picrylhydrazyl (DPPH)were purchased from Sigma. Folin's Ciocalteau phenol reagent (Merck), gallic acid (Loba chemie), ascorbic acid (Merck) were purchased. Hexanefor GC-MS analysis and analytical grade methanol and hydrochloric acid were procured from Merck® (India).

Plant material

Fully ripe berries of *Syzygium cumini* were collected from farms of Indian Agricultural Research Institute, New Delhi. The fruit pulp (20 g) was accurately weighed and placed in a conical flask and 40 mL of acidified 80% ethanol (0.1% HCl) was added. The flask was sonicated for 10 min and the material were centrifuged for 5 min. Residue was again extracted with 40 ml of acidified 80% ethanolic solution and centrifuged. The pooled supernatants were concentrated under vacuo in a rotary evaporator till dryness at 39± 1°C to get ethanolic concentrate. Ethanolic concentrate was kept in deep freezer for further analysis of phenolics, sugars and antioxidant activity. For the determination of anthocyanins content, ethanolic extract was partitioned with hexane and ethyl acetate to remove other phenolic and carotenoid compounds and then concentrated under vacuo (39 ± 1 °C) in a rotary evaporator to get anthocyanins rich ethanolic concentrate.

Determination of total anthocyanins by pH-differential method

Anthocyanin content was determined by using the pH differential method[27] and expressed as cyanidin-3-glucoside equivalent (molar extinction coefficient (ε)26900, mol. mass 449.2 g mol^{-1}). 500 µl of the extract was diluted with buffers of pH 1.0 and 4.5. The reaction mixtures were allowed to equilibrate at room temperature for 15 min and their absorption was measured at 520 nm and 700 nm. The difference in absorbance is equivalent to anthocyanin content and calculated by using the formula:

Total anthocyanins = (Absorbance × Mol. mass × dil factor)/ εL

Where, Absorbance (A) = $(Abs_{510} - Abs_{700})$ pH1.0 - $(Abs_{510} - Abs_{700})$ pH4.5

Determination of total phenolic content

Total phenolics content was estimated spectrophotometrically using Foline-Ciocalteu reagent[28]. An aliquot (100 µL) of extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 2.9 mL of de-ionized water and 2.0 mL of 20% Na_2CO_3 solution. The mixture was allowed to stand for 60 min and the absorbance was measured at 760 nm against a reagent blank. Calculations were performed using the standard calibration curve of gallic acid. Results were expressed as gallic acid equivalent (mg GAE/100 g dw). All the samples were analyzed in triplicates

Determination of antioxidant activity

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of ethanolic concentrate was evaluated by FRAP assay according to the standard procedures with some modification[30,31]. The FRAP assays measure the reducing power of the extracts in terms of reduction of ferric (Fe^{3+}) to ferrous form (Fe^{2+}). The FRAP reagent comprise 300mM acetate buffer (pH3.6),10mM TPTZ in 40 mM HCl and 20mM $FeCl_3$ in the ratio 10:1:1(v:v:v). FRAP reagent (10 ml) was mixed with 1ml (100 µg ml^{-1}) of sample extract in a test tube and vortexed. After 4min, a UV-absorbance reading was recorded at 593nm wavelength. Results were

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expressed in terms of μmolml^{-1}using the equation: y = 0.0014x + 0.0649, R^2 = 0.9885.

DPPH radical scavenging assay

DPPH (2,2 -diphenyl-1-picrylhydrazyl) radical scavenging assay was used for the evaluation of antioxidant potential of jamun extract with minor modification[32,31]. This assay is based on the radical scavenging ability of antioxidant towards stable DPPH radical. A 0.004% of DPPH solution was prepared in methanol. A 3.0 mL aliquot of DPPH solution was added to 1.0 mL of methanolic solution (1to 500μg ml^{-1}) of concentrate and mixture was incubated in dark for 30 min. Absorbance was measure at 517 nm by keeping methanol as a blank.

The percentage inhibition was calculated by using formula:

% Inhibition = ($A_{control}$ –– A_{test} / $A_{control}$) X 100

IC_{50} of extract was calculated by plotting % inhibition *vs* concentration.

Estimation of sugars by GC-MS

The ethanolic extract was reacted with acetic anhydride in the presence of triethyl amine and kept overnight in dark. The extract was poured onto ice cold water to obtain the precipitate which was filtered, dried and dissolved in hexane for GC-MS analysis. Gas chromatographic-mass spectrometric analysis of sugars was performed on Agilent 7890 GC equipped with Agilent 5975 mass quadrupole detector (Agilent Technologies, Palo Alto, CA, USA) and a 30m × 0.25mm × 0.25mm HP-5MS fused-silica capillary column (Agilent, USA). Helium gas was used at a flow rate of 1 mlmin^{-1}. The oven temperature was kept at 90 °C for 1 min and then programmed at 30°Cmin^{-1} to 200 °C for 10 min then 5°Cmin^{-1} to 280°C. The mass-spectrometer interface temperature was set at 280 °C. The temperature of the ion source was 250 °C, electron energy 70 eV, and quadrupole temperature 150 °C. The chromatographic plot (Figure 1) was obtained by total ion current (TIC) mode: the acquired mass ranges were 50 amu up to 550 amu.

RESULTS AND DISCUSSION

Jamun fruit is rich source of anthocyanins and its content almost equivalent to the other nutraceutical commodities like blue/black berries and black current[23]. Spectrophotometric analysis of anthocyanins from jamun fruit revealed 209 mg100 g^{-1} fruit on a dry weight basis. Our results are in agreementwith the earlier reports[33,23]. Phenol and flavonoids are known for their wide range of biological activities including antioxidant activity. These compounds are especially associated with decreased risk of diseases such as cardiovascular diseases, cancer, ageing etc.[34,35]. Jamun fruit contains 285 mg GAE100 g^{-1} of phenolic content. In literature, variable results were observed which were probably due to the variability of raw material or methodology used[20,23,35]. Evaluation of antioxidant activity of jamun fruit is necessary for determining the nutritional, functional quality and health promoting properties. In this study *in vitro* FRAP and DPPH assays were used for analysing the antioxidant activity. The scavenging ability of antioxidant towards the stable DPPH radical was determined by decrease in absorbance along with change in color from purple to yellow. Results shows that the ethanolic extract with IC_{50} values of 195 μg ml^{-1} exhibited strong DPPH scavenging activity (Figure 1). Similar antioxidant activity of anthocyanins was reported by previous workers[20,22]. The FRAP assay provided the reducing potential of antioxidant substances and is widely used in the evaluation of antioxidant potential of polyphenols. It was concluded that total antioxidant of jamun extract was equivalent to 9.3μmol Ascorbic acidg^{-1}. This explains anthocyanins-rich extract of *S. cumini* fruit have high antioxidant potential and can be used as nutraceutical components.

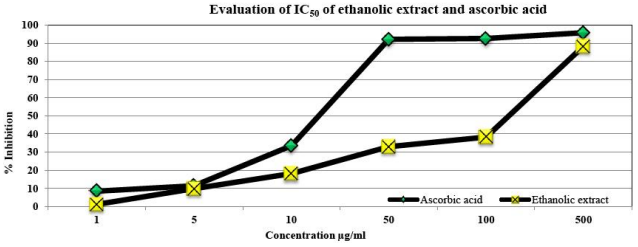


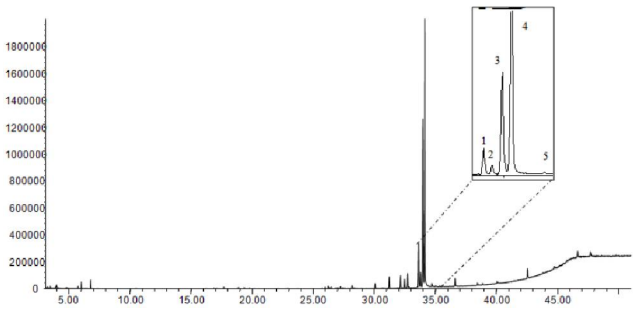
Figure 1 : Evaluation of IC 50 of ethanolic extract of jamun fruit pulp

A representative gas chromatogram of a jamun fruit pulp using GC-MS is shown in Figure 2. Significant variations were observed in sugar composition (TABLE 1). Glucose (88.28%) was found to be the main sugar while other sugars like fructose (6.4%) and sorbose

(4.1%) were also present in small quantities. β-D-Galactofuranose was also present in trace. No sucrose was present in jamun fruit pulp. Our results are in agreement with the earlier reports that glucose and fructose as the main sugar in jamun fruit[36,37,38].

TABLE 1 : Sugars in jamun fruit pulp by GC-MS

Peak Number	Compound Name	% Area	RT
1	Fructose	6.94	33.64
2	Sorbose	4.13	33.79
3	α-D-Glucose	28.26	33.97
4	β-D-Glucose	60.02	34.14
5	β-D-Galactofuranose	0.65	34.73



(1.Fructose; 2.Sorbose; 3.α-D-Glucose; 4. β-D-Glucose; 5. β-D-Galactofuranose)

Figure 2 : Representative TIC of sugars in Jamun Fruit Pulp estimated by GC-MS

CONCLUSIONS

The results demonstrate that *Syzygium cumini* is a rich source of anthocyanins, sugar and other phenolic like other nutraceutical commodities hence may be used as nutraceuticals and functional foods. It is a healthy fruit with minimum calories as glucose and fructose, the main source of sweeteners in the ripe fruits with absolutely no trace of sucrose. Anthocyanins enrich extract have strong antioxidant activities and it may serve as dietary source of natural antioxidants for disease prevention and health promotions.

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