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EVALUATION OF ACTUAL ANTIOXIDANT CAPACITY OF "JAMUN" *(SYZYGIUM CUMINI)* USING THE *IN VITRO* GASTROINTESTINAL MODEL

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ABSTRACT

Many scientific evidences have proven that diets rich in antioxidant compounds which occur particularly in plant foods lower the risk of developing lifestyle diseases. Jamun (Syzygium cumini) is a good source of anthocyanin and other phenolic compounds. The pulp of the Jamun berry contains antioxidants like anthocyanins, delphinidin, petunidin and malvidin-diglucosides, which impart the fruit its bright purple color. Abundant literature exists on the content of different phenolic compounds present in fruits and vegetables and their antioxidant capacities measured from the extracts using different polarity solvents based on *in vitro* methods. These features may differ quantitatively and qualitatively when fruits and vegetables are digested in the human gastrointestinal tract. Also, other antioxidant compounds may be adversely or positively affected by the pH, temperature, and other factors during the digestion process. The present study involves the measurement of the antioxidant potential of the fruit "jamun" using the in vitro digestion model.

Keywords: *Syzygium cumini, Antioxidant activity, Phenolic compounds, Anthocyanin*

INTRODUCTION

Free radicals have been implicated in the etiology of a large number of major diseases. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to disease conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels. There are epidemiological evidences correlating higher intake of foods with antioxidant abilities to lower the incidence of various human morbidities or mortalities. Dietary and other components of plants form major sources of antioxidants. Phenolic compounds such as catechins, phenolic acids, flavonoids, proanthocyanidins and anthocyanins have exhibited a range of biological effects including antibacterial, antiviral, anti-inflammatory, antithrombotic and vasodilatory actions [1-3]. They also exert pronounced antioxidant and free radical-scavenging activities [4-8]. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. Higher intake of foods with functional attributes including a high level of antioxidants in functional foods is one strategy that is gaining importance in advanced countries and is making its appearance in our country.

Jamun fruit (Syzygium cumini) is a good source of phenolic compounds and anthocyanin. Jamun contains the widest variety of anthocyanidin groups: delphinidin, cyanidin, petunidin, peonidin, and malvidin, all present as diglucosides [9-11]. These impart a bright purple color to the fruit. The anthocyanin rich edible part of Jamun is comparable with that of blueberry, blackberry and blackcurrant, whose nutraceutical properties are well documented, suggesting the potential nutraceutical value of Jamun fruit. Anthocyanins in these fruits are reported to be powerful antioxidants and stability studies showed that they are stable up to 6 months in dry pulps [12]. Anthocyanins (cyanidin glucosides] have been shown to protect cell membrane lipids from oxidation [13]. Also, hypoglycemia [14], anti-inflammatory [15] neuropsychopharmacological [16], anti- bacterial [17], anti-HIV [18] and anti-diarrhoeal [19] effects of Jamun plant has been reported earlier. Some cyanidins are many times more powerful antioxidants than tocopherols [20]. Bertuglia et al, [21] showed that anthocyanin supplements effectively inhibited inflammation and subsequent blood vessel damage and maintained the integrity of vascular micro capillaries in the animal model. Health benefits associated with anthocyanin intake include reduced risk of coronary heart disease [22], protection against obesity and hypoglycemia [23], memory enhancement [24] and protection of fetal brain tissue [25].

Abundant literature exists on the content of different phenolic compounds and anthocyanin present in fruits and vegetables and their antioxidant capacities measured from these extracts using different polarity solvents. These features may differ quantitatively and qualitatively when fruits and vegetables are actually digested in the human gastrointestinal tract. Also, antioxidant compounds may be adversely or positively affected by the pH, temperature, and other factors during the digestion process. According to the literature reviewed, no study has reported the antioxidant potential of Jamun using the *in vitro* digestion model so far. The present study was thus carried out to compare the antioxidant activity of Jamun based on chemical and physiological extracts.

MATERIALS AND METHODS

Materials: Wholesome, ripe Jamun were selected and purchased from the local market of Anand and were used for chemical and *in vitro* enzyme based digestive (physiological) extraction.

Chemicals : Pepsin (P-7000), Pancreatin (P-1750), Lipase (L-3126), Bile Extract Porcine (B-8631), α-Amylase (A-3176), Amyloglucosidase (A-7095), ABTS [2,2 Azinobis (3–ethylbenzothiazolin-6-sulfonic acid) diammonium, salt] (A-1888), DPPH (2,2-Diphenyl-1-picryl-hydrazyl) (D-9132), Catechin (C-1251), Vanillin (V-2375), Rutin (R-5143), Gallic acid (G-7384) and TPTZ (2,4,6–Tris (2-pyridyl)–s–triazine) (T-1253) were purchased from Sigma Aldrich-Germany and Trolox (6-Hydroxy-578-tetra methyl-chromane-2 carboxylic acid) – 56510 was purchased from Fluka.

Sample preparation: Jamuns were washed thoroughly, air dried and the edible portion was collected and homogenized for pulp preparation.

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Extraction:

Extraction of jamun was done by two methods (a) chemical (methanolic) extraction (ME) and (b) *in vitro* enzyme based digestive (physiological) extraction which involved a control (PC) and an enzymatic extract (PE). Control (PC) was extracted in similar buffers as enzymatic extraction as mentioned below but without the addition of enzymes.

(a) **Chemical extraction:** About 900 mg of pulp was extracted in 80% methanol which was acidified to pH 2.0 with 1N HCl by shaking at room temperature for 45 minutes. Supernatants were centrifuged and volume was made up to 30 ml with the solvent. These samples were stored at -20°C for antioxidant activity determination.

(b) **Extraction using in vitro gastrointestinal digestion:** About 900 mg of Jamun pulp was used for in vitro gastrointestinal digestion. The digestive enzymatic extraction was carried out by using the in vitro procedure previously described by Serrano et al, [26]. Samples were successively incubated with digestive enzymes to simulate digestion in the small intestine. About 900 mg of sample was incubated with pepsin (0.6 ml of a 300 mg/ml solution in a buffer of 0.2 M HCl–KCl, pH 1.5, 40 °C, 1 h, Merck 7190), pancreatin (3 ml of a 5 mg/ ml solution in 0.1 M phosphate buffer, pH 7.5, 37°C, 6h, Sigma P-1750), lipase (6 ml of a 7 mg/ml solution in 0.1 M phosphate buffer, pH 7.5, 37°C, 6 h, Sigma L-3126), bile extract porcine (6 ml of a 17.5 mg/ml solution in 0.1 M phosphate buffer, pH 7.5, 37°C, 6 h, Sigma B-8631) and α-amylase (3 ml of a 120 mg/ ml solution in 0.1 M tris-maleate buffer, pH 6.9, 37°C, 16 h, Sigma A-3176). Also, all samples were digested with similar buffers without enzyme addition which was referred as control (PC) so as to evaluate the effect of enzyme addition.

Then, the samples were centrifuged (15 min, 6000 rpm) and supernatants were removed. Residues were washed twice with 5 ml of distilled water, and all supernatants were combined. Each supernatant was incubated with 300 µl of amyloglucosidase (Sigma A-7095) for 45 min at 60 °C.

Supernatants were filtered and centrifuged and volume was made up to 30 ml. All samples were transferred to eppendorf tubes and stored at -20°C for antioxidant determination. Both chemical and digestive enzymatic extracts were used as test samples to determine the antioxidant capacity and polyphenol content.

Total Phenol: Folin – Ciocalteu method [27] was used to determine the total phenol content of the chemical and physiological extracts. Different aliquots of known concentration of gallic acid were taken as standard.

Flavonoid: Different aliquots of concentrated sample were taken and volume was made up to 5 ml with distilled water [28]. Different aliquots of known concentration of Rutin was treated as standard.

Anthocyanin: Anthocyanin was measured by the pH differential method suggested by Rapisarda, 2000 [29]. Concentration of anthocyanin was calculated by the equation considering the molecular mass and molar absorptivity of cyanidin-3-glucoside chloride after calculation of the difference of absorbance at 510 nm between pH 1 and pH 4.5 solutions.

Flavonol: Different aliquots of concentrated samples were taken and volume was made up to 500 µl with methanol [30]. Catechin was treated as standard.

Determination of Antioxidant Capacity: The antioxidant capacity was measured by four different methods namely FRAP, DPPHRSA, ABTSRSA and RPA. For each method, a calibrated Trolox curve was standardized and results were expressed in terms of TEAC (mg of Trolox equivalents/100g.) The antioxidant activity of the extracts to scavenge the stable DPPH radical was determined by the method described by Brand-Williams et al. [31]. The radical scavenging capacity of different wheat extracts was determined using the modified ABTS radical decolorization assay [32]. FRAP and RPA were determined by using the method of Benzie and Strain [33] and Oyaizu [34] respectively.

Statistical analysis: Experiment was done in duplicate batches with two separate purchases in the same season. Four observations of two different experiments were analyzed statistically. Differences between variables were tested for significance based on a one-way analysis of variance, DUNCAN based on level of significance ($p \leq 0.05$) by using SPSS 15.0.

RESULTS AND DISCUSSION

There are very limited studies conducted on Jamun fruit for its chemical composition and biological activities. Antioxidant capacity of different Jamun fruit parts has been reported by Benherlal and Arumughan, [35] by ethanolic extraction but no reports have been found on the effect of gastrointestinal digestion on the antioxidant property of Jamun. However, the effect of digestion on the anthocyanin levels and antioxidant levels of wine and raspberries have been reported by Mc Dougall et al, [36] and on chokeberry by Bermudez-Soto et al, [37]. So, in the present study, the investigators compared the antioxidant activity of Jamun fruit by the chemical method based on methanolic extraction and the in vitro method based on gastrointestinal model referred to here as the enzymatic method.

The total phenolic content (TPC) of the methanolic extract (ME) was found to be 206.07 mg GAE/100g. Kaur and Kapoor, [38] found 215mg/100g of TPC in Jamun whereas Benherlal and Arumughan, [35] found 390 mg/100g of TPC in Jamun pulp extracted in ethanol. Luximon-Ramma et al, [39] extracted Jamun in acetone/water (70:30 v/v) which showed a TPC of 235.9 mg gallic acid/100g fresh weight. The physiologically digested extracts varied significantly among themselves. Control Jamun (digested without enzyme addition) (PC) had 206.23 mg GAE/100g whereas the enzymatic extract (PE) had 158.8 % higher TPC than the chemical extract. This rise may be due to the release of phenolic compounds from carbohydrates, proteins and other molecules as a result of enzymatic action during digestion.

The flavonol content followed a similar pattern as the TPC which is shown in Figure 1. The ME showed a 102.21 µg catechin eq/g of flavonol. In physiologically digested samples, PC showed 45.65 µg catechin eq/g while the PE showed 254.52 µg catechin eq/g i.e. 149% higher flavonol than the chemical extracts. TPC and flavonol content of different jamun extracts is depicted in Figure 1.

Figure 2 depicts the flavonoid and anthocyanin content of different Jamun extracts. The flavonoid content of the ME was found to be 68.53 mg rutin eq/100g. Luximon-Ramma et al, [39] found 13.5 mg quercetin/ 100g in acetone /water extracts of Jamun. In the physiological extracts, the flavonoid content was found to

decrease significantly. The PC (−79.18%) and PE (−47.86%) had lesser flavonoid content than the ME i.e. 35.73 mg rutin eq/100g. The decrease in flavonoid content may be a result of decrease in the anthocyanin content.

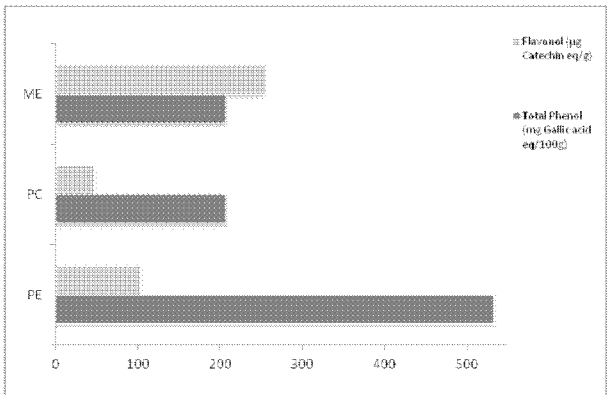

Fig 1: Total Phenol and Flavonol content of different Jamun extracts

7 mg/100g. Anthocyanin content of the PC in the present study was 2.93 mg cyanidin- 3 –glycoside eq/100g which was lower by −34.21% and the PE was 3.93 mg cyanidin-3 –glycoside eq/100g which was lower by −11.87 % when compared to the ME. The decrease in anthocyanin levels after digestion may be due to the increase in pH on pancreatin addition. The pancreatin is dissolved in phosphate buffer with pH 7.5 for 6 hours. Anthocyanins exist in equilibrium as four molecular species; the colored basic flavylium cation and three secondary structures; the quinoidal bases, the carbinol pseudobase and the chalcone pseudobase forms. At pH 2 or below, the flavylium cation form predominates but as the pH is raised towards neutrality; the colorless chalcone pseudobase begins to dominate. Chalcone formation is also favored by elevated temperatures and prolonged exposure may enhance degradation between the B and C rings resulting in the destruction of the anthocyanin chromophore [40, 41].

Strack and Wray, [40] reported that anthocyanins in red wine were stable to gastric conditions whereas there was a small loss in the TPC. However, after pancreatic digestion, the total anthocyanins were very poorly recovered compared to the bulk phenols. The pH shift to >7.5 in the pancreatic/small intestine digestion was the main factor in the irreversible breakdown of the anthocyanins. Mc Dougall et al, [36] studied the stability

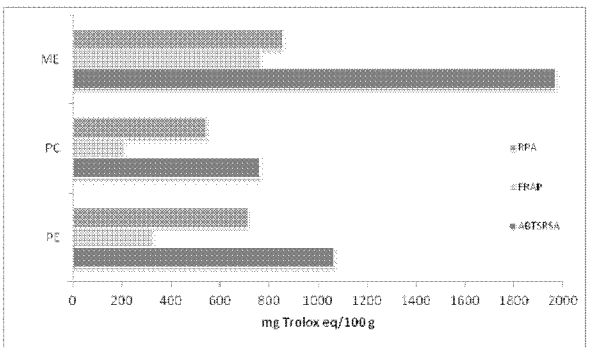

Fig 3: Ferric Reducing Antioxidant Power, ABTS Radical Scavenging Ability and Reducing Power Assay of different Jamun extracts

The ME in the present study was found to have 4.45 mg cyanidin- 3 –glycoside eq/100g of anthocyanin content whereas Benherlal and Arumughan, [35] reported the anthocyanin content in the Jamun pulp to be

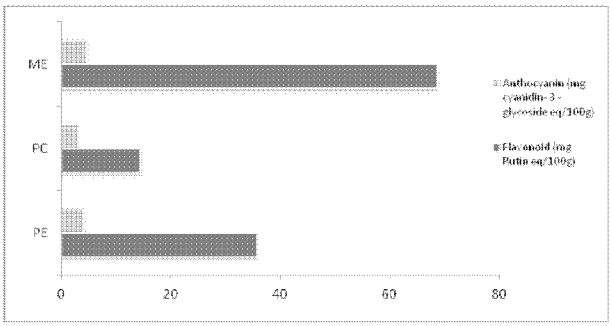

Fig 2: Flavonoid content and Anthocyanin content of different Jamun extracts

of anthocyanin from red wine and found that anthocyanins were stable to gastric digestion but after the pancreatic digestion, the total anthocyanin were negatively affected due to the increase in pH. Other studies also support the breakdown of anthocyanins after pancreatic digestion. Bermudez-Soto et al, [37] studied the stability of polyphenols in chokeberry subjected to in vitro gastric and pancreatic digestion and found a loss in antioxidant capacity after in vitro digestion. Ryan et al, [42] reported a similar degradation of anthocyanin and consequential loss of antioxidant capacity of commercially available fruit juices of cranberry, red grape and pomegranate when subjected to an in vitro digestion. The presence of bile salts does not interfere with the total polyphenol and flavonoid assays but interferes with the assay used to quantify the total anthocyanin concentration in the digested samples [36]. Anthocyanins can form insoluble complexes with particulates and can also bind to components of the pancreatin/bile salts mixture [43].

The antioxidant capacity of Jamun measured by FRAP, ABTSRSA and RPA is represented in Figure 3. FRAP value of the chemical extract was found to be 771.42 mg TE/100g. Luximon-Ramma [39] found the FRAP value of Jamun to be 1.6 mmol Fe (II) /100g. On physiological digestion, reduction was seen. PC extract showed −73.08% lesser FRAP value than the ME extract

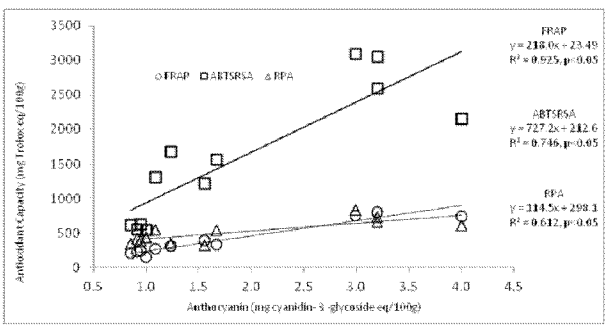

Fig 4: Relationship between Anthocyanin content and Total Antioxidant Capacity of different Jamun extracts

while the PE showed −57.79% lesser FRAP value than the ME. Luximon-Ramma, [39] measured the ABTSRSA of Jamun and found 1.5 mmol TE/100g whereas in the present study the ME had 1972.60 mg TE/100g which is equal to 7.88 mmol TE/100g. The PC had 763.75 mg TE/100g and PE had 1067.78 mg TE/100g which was −61.28% and −45.87% lower respectively than the chemical extract (ME) of Jamun. The Reducing Power of ME of Jamun was found to be 856.38 mg TE/100g. It was reduced in both PC (-36.17%) and PE (-16.51%) as compared to the ME. Benherlal and Arumughan [35] reported lower Reducing Power of Jamun as compared to ascorbic acid. Figure 4 shows the relationship between Anthocyanin content and Total Antioxidant Capacity of different Jamun extracts. Best correlation was found between anthocyanin and FRAP although all parameters showed positive and strong correlation with anthocyanin. The reduction in the antioxidant capacity of physiological extract measured by the three different methods may be a result of the decrease in anthocyanin content of Jamun and this is confirmed in the present study.

CONCLUSION

From the results, it is concluded that the chemical extraction method does not imply the actual antioxidant capacity of Jamun and also that the antioxidant capacity of Jamun is dependent on the flavonoid especially anthocyanin content of Jamun.

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