J. Hortic. Sci.
Vol. 19(1), 2024

https://doi.org/10.24154/jhs.v19i1.2837

Original Research Paper

Assessment of *in vitro alpha*-glucosidase inhibitory activity, phenolics, flavonoids and antioxidant potential of jamun (*Syzygium cumini*) seeds

Arivalagan M.*, Priyanka D.R., Rekha A. and Tripathi P.C.
ICAR-Indian Institute of Horticultural Research, Bengaluru - 560 089, India
*Corresponding author Email: arivalagan2100@gmail.com

ABSTRACT

Jamun (*Syzygium cumini* Linn. Syn. *Eugenia jambolana*) genotypes collected from different parts of the country were evaluated for *in vitro alpha*-glucosidase inhibitory activity, polyphenolic content and antioxidant potential along with fruit characters. Results indicated significant variation among genotypes for fruit characters, phenolics, flavonoids, antioxidant potential, and *alpha*-glucosidase inhibitory potential. Total phenolics and flavonoids in seeds varied between 15.5 mg to 98.7 mg GAE/g and 0.79 mg to 9.03 mg CE/g seeds, respectively. The seeds with a high content of polyphenolic compounds required a very less amount of sample to inhibit *alpha*-glucosidase enzyme. The genotype Selection 45 recorded the highest *alpha*-glucosidase inhibitory activity, and required only 50.3 µg of sample to inhibit 50 % of the enzyme activity. Both phenolics and flavonoids content positively correlated with antioxidant potential and negatively correlated with enzyme inhibitory potential. From cluster analysis, the genotypes AJG-85, Kaithanal, Selection-45, Collection-2, and Konkan Bahdoli were identified as superior genotypes having a considerable amount of seeds with *alpha*-glucosidase inhibitory compounds. These genotypes/collections may be effectively utilized for further studies to isolate and characterize the bioactive constituents with *alpha*-glucosidase inhibitory potential.

Keywords: *alpha*-glucosidase, antioxidant potential, diabetes, flavonoids, jamun, phenolics

INTRODUCTION

Jamun (*Syzygium cumini* Linn. Syn. *Eugenia jambolana*), otherwise called Indian blackberry, is being claimed to have a positive effect in reducing the plasma blood sugar level in diabetic patients and also can be utilized in conditions/complications related to diabetes (Katiyar et al., 2016). Its fruits and seeds are reported to have antidiabetic properties and are used in treating diabetic mellitus in folklore medicine. Jamun seeds act as diuretic and astringent and possess hypoglycemic, anti-inflammatory, antipyretic, hypolipidemic, and antioxidant properties (Bharia & Bajaj, 2005; Bushra et al. 2007).

Though, various parts of jamun fruits are reported to have numerous health-promoting activities, their action against controlling the blood glucose level in diabetic patients is attracting various health professionals and pharmaceutical industries. Mainly the jamun seed powder is extensively used in various formulations in ayurvedic medicine, and used to regulate blood glucose level. The presence of significant amount of antioxidant compounds such as phenolic acids, and flavonoids attribute to their medicinal values (Ayya et al., 2015). From the earlier reports based on *in vitro* and *in vivo* studies using animal models, it is well understood that jamun seed power is rich in antihyperglycemic agents which can effectively utilized in treatment of diabetes. India has rich variability in jamun germplasm for fruit and seed characters. Since the seeds are effectively used as antihyperglycemic agent, finding the collection or germplasm which is rich in phenolic and flavonoid compounds which are believed to have antihyperglycemic property will help in effective utilization of the genotype. Thus, the present study aimed to screen the seeds of jamun genotypes collected from different parts of Indian subcontinent for *alpha*-glucosidase inhibitory activity along with phenolic, flavonoid content and antioxidant potential.

MATERIALS AND METHODS

Sample collection and preparation

Matured fruit samples were collected from jamun trees grown in different parts of the country and brought to the lab with proper care. The basic information

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Table 1 : Basic information about the jamun genotypes used in the study

Sl. No.	Sample	Place of collection	State	IC Number	Tree type	Tree age**
1	Selection 45	Arabhavi, Belagavi	Karnataka	IC-0621954	Semi spreading, dense broad leaves	NA*
2	Madanapalli	Madanapalli, Chittor	Andhra Pradesh	NA	Spreading, sparse medium leaves	20 years
3	Selection 58	Arabhavi, Belagavi	Karnataka	IC-0621956	Spreading, dense broad leaves	NA*
4	Dhupdal	Arabhavi, Belagavi	Karnataka	IC-0621955	Semi spreading, dense broad leaves	NA*
5	KHA-3	Khanapur, Belagavi	Karnataka	IC-0631359	Upright, dense broad leaves	20-25 years
6	Savadatti	Arabhavi, Belagavi	Karnataka	IC-0621957	Semi spreading, dense broad leaves	NA*
7	Collection-2	Tegur, Dharwad	Karnataka	IC-0621961	Semi spreading, medium broad leaves	25-30 years
8	Kaithanal	Arabhavi, Belagavi	Karnataka	IC-0621952	Semi spreading, dense broad leaves	NA*
9	Konkan Bahdoli	Dapoli, Ratnagiri	Maharashtra	IC-0621958	Semi spreading, dense broad leaves	NA*
10	AJG85	Arabhavi, Belagavi	Karnataka	IC-0621953	Semi spreading, dense broad leaves	NA*
11	Collection-3a	Tegur, Dharwad	Karnataka	IC-0621962	Semi spreading, dense broad leaves	25-30 years
12	PGR-6	Erode	Tamil Nadu	NA	Spreading, dense broad leaves	NA***
13	PGR-7	Erode	Tamil Nadu	NA	Spreading, dense broad leaves	NA***
14	PGR-9	Erode	Tamil Nadu	NA	Spreading, dense broad leaves	NA***
15	MP-5	Kundam Forest, Jabalpur	Madhya Pradesh	IC-0621983	Upright, sparse narrow leaves	20-25 years
16	KHA-16	Khanapur, Belagavi	Karnataka	IC-0631360	Upright, dense broad leaves	25-30 years
17	BRH-1	BR Hills, Chamarajnagar	Karnataka	IC-0631375	Semi spreading sparse narrow leaves	>50 years
18	BRH-7	BR Hills, Chamarajnagar	Karnataka	IC-0631377	Spreading medium broad leaves	>30 years
19	Jabalpur-7	Bichiyya, Mandala	Madhya Pradesh	NA	Upright, Sparse narrow leaves	20-25 years
20	Jabalpur-8	Bichiyya, Mandala	Madhya Pradesh	NA	Semi spreading	30-35 years
21	Jabalpur-9	Bichiyya, Mandala	Madhya Pradesh	NA	Spreading, dense broad leaves	30-35 years
22	Varanasi-14	Kabirpur, Chanduli	Uttar Pradesh	IC-0631382	Spreading, dense broad leaves	>50 years
23	Varanasi-15	Kabirpur, Chanduli	Uttar Pradesh	NA	Spreading, dense broad leaves	30-35 years
24	Varanasi-25	BHU, Varanasi	Uttar Pradesh	IC-0631383	Semi spreading, dense broad leaves	>40 years

* Planted in germplasm block in 2013-14; **Based on local information; ***Planted between 2008-10; NA: Not available

pertaining to collected germplasm is presented in Table 1. Fruit characteristics such as fruit weight, fruit length, fruit width, pulp weight, seed weight, seed length, seed width, and seed-to-pulp ratio were recorded on 10 randomly selected fruits from each collection. After removal of pulp, the seeds were dried in a mechanical tray dryer at 50°C until the constant dry weight was achieved. After drying, the seed coats were carefully removed and the dried kernel was finely powdered using a ball mill to get uniform particle size and stored in vacuum desiccator until further analysis.

Sample extraction

About 250 mg of each sample was extracted with 10 mL of aqueous acetone (Acetone:water 80:20, v/v) for 1h in an ultra-sonic bath maintained at 60°C to get the optimum extract. The extracts were centrifuged and the supernatants were collected in the amber-coloured reagent bottle. The residue was re-extracted twice using the same solvent and the supernatants of three successive extractions were pooled. Acetone from the pooled extract was removed using a flash evaporator, and the acetone free extracts were re-suspended in 5 mL of water and centrifuged and then the clear supernatant collected was stored at -20°C until analysis.

Determination of total phenolics and flavonoids content

Total phenolics content (TPC) in the jamun extract was determined using Folin-Ciocalteu assay (Singleton et al., 2011). Gallic acid was used as standard and the phenolics content was expressed as mg gallic acid equivalent (GAE), per g dry weight of jamun seed kernel. Total flavonoid content (TFC) was determined according to Zhishen et al. (1999), and catechin (0–50 µg) was used as a standard and the results were expressed as mg of catechin equivalent (CE) per g dry weight of jamun seed kernel.

Determination of antioxidant potential

DPPH (1,1'-diphenyl-12-picrylhydrazyl) free radical scavenging activity was assessed by the method described by Brand-Williams et al. (1995) using trolox as a positive control and the results were expressed as mM trolox equivalent/ g dry jamun seed powder, obtained from a trolox solution having a free radical scavenging activity (IC_{50}) equivalent to that of sample. The FRAP assay was done according to Benzie & Strain (1996). Trolox served as a positive control and results were expressed as mM trolox equivalent/g dry jamun seed powder, obtained from a trolox solution having reducing power equivalent to that of the sample.

Determination of *alpha*-glucisidase inhibitory activity

Alpha-glucisidase inhibitory activity of jamun seed extract was measured according to the method described by Arivalagan et al. (2021). Individual sample extracts with different concentrations were make up to 1 mL with phosphate buffer (pH 6.9) and about 100 µL of *alpha*-glucosidase enzyme (containing 25 µg of enzyme) was added and incubated at 25°C for 5 min. After the pre-incubation, about 100 µL of *p*-nitrophenyl-α-*D*-glucopyranoside (25µg) was added, mixed well and the reaction mixture was incubated for 20 min at 25°C. The reaction was terminated by the addition of 1 N Na_2CO_3. After 10 min, the yellow colour developed was measured at 405 nm and the *alpha*-glucosidase inhibition % (IC %) was calculated. From the results, IC_{50} values were calculated. IC_{50} value signifies the amount of sample (in µg) required to inhibit 50% of the enzyme *alpha*-glucosidase (25 µg) activity (IC_{50}) in the presence of substrate *p*-nitro phenyl glucopyranoside (25 µg). Less value indicates better inhibitory activity.

Statistical analysis

All the experiments were conducted on triplicate samples. Variability among the jamun collections for *alpha*-glucosidase inhibitory activity, total phenolics, flavonoids, and antioxidant potential was evaluated by ANOVA using SAS software package version 9.3 (SAS Institute Inc., Cary, NC, USA) (SAS, 2011). Values were expressed as means ±standard deviation. Pearson's linear correlation was performed to measure the correlation and strength of the relationship between the parameters studied, and cluster analysis was carried out using JMP 10 Pro (JMP, 2012).

RESULTS AND DISCUSSION

A study was conducted to determine the variability among the seed kernel of jamun genotypes collected from different parts of the country for *alpha*-glucosidase inhibitory activity, total phenolic, flavonoid content and antioxidant potential. Fruit characters also studied to identify the superior genotypes with potential *alpha*-glucosidase inhibitory activity. The fruit characters such as fruit weight, fruit length, fruit width, pulp weight, seed weight, seed length, seed width, and pulp-to-seed ratio are presented in Table 2.

Table 2 : Fruit and seed characters of 24 jamun genotypes

Genotype	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Pulp weight (g)	Seed weight (g)	Seed length (cm)	Seed width (cm)	Seed-pulp ratio
Selection 45	8.96±0.90	3.76±0.13	3.00±0.12	6.92±0.58	2.04±0.38	3.10±0.12	1.80±0.19	0.29±0.04
Madanapalli	10.48±0.82	3.50±0.22	2.92±0.18	8.20±1.05	2.28±0.39	2.16±0.21	1.58±0.22	0.29±0.09
Selection 58	11.08±0.74	3.64±0.17	3.00±0.16	8.88±0.99	2.20±0.47	1.34±0.37	1.16±0.05	0.25±0.08
Dhupdal	10.24±1.61	3.94±0.13	3.14±0.11	8.00±1.44	2.24±0.57	2.90±0.19	1.90±0.14	0.29±0.09
KHA-3	1.27±0.07	1.48±0.19	1.08±0.04	1.01±0.05	0.26±0.01	0.96±0.17	0.50±0.07	0.26±0.00
Savadatti	9.64±1.39	3.92±0.36	3.02±0.08	7.32±1.49	2.32±0.58	2.70±0.16	1.92±0.22	0.33±0.12
Collection-2	4.56±0.52	3.18±0.13	2.64±0.11	3.24±0.65	1.32±0.18	2.92±0.11	1.96±0.15	0.43±0.15
Kaithanal	7.20±2.10	3.52±0.47	2.84±0.21	5.32±2.03	1.88±0.30	2.96±0.27	1.78±0.04	0.39±0.13
Konkan Bahdoli	10.60±1.65	3.88±0.25	3.32±0.16	6.32±0.98	4.28±0.67	3.18±0.11	2.46±0.19	0.68±0.02
AJG85	9.92±0.66	3.38±0.54	2.94±0.21	7.40±0.80	2.52±0.33	2.98±0.16	1.84±0.17	0.35±0.08
Collection-3a	3.88±0.33	2.96±0.11	2.54±0.11	2.04±0.22	1.84±0.17	1.34±0.37	0.94±0.05	0.91±0.09
PGR-6	17.76±1.51	3.84±0.23	2.68±0.18	10.24±0.87	7.52±0.64	2.90±0.12	1.98±0.04	0.73±0.02
PGR-7	16.60±3.69	3.82±0.47	2.84±0.62	9.56±2.13	7.04±1.57	2.78±0.24	1.70±0.24	0.74±0.03
PGR-9	16.08±1.92	3.30±0.38	2.58±0.11	8.62±1.03	7.46±0.89	2.22±0.18	1.46±0.13	0.87±0.07
MP-5	1.16±0.09	0.76±0.09	0.66±0.11	0.86±0.17	0.30±0.10	1.66±0.27	1.18±0.30	0.38±0.20
KHA-16	3.78±0.85	2.08±0.19	1.60±0.19	2.21±0.49	1.57±0.35	1.38±0.28	0.96±0.05	0.71±0.03
BRH-1	0.96±0.08	2.06±0.13	1.31±0.07	0.60±0.09	0.29±0.04	1.89±0.04	1.25±0.11	0.48±0.08
BRH-7	0.95±0.05	2.12±0.10	1.37±0.03	0.34±0.07	0.44±0.04	1.88±0.05	1.27±0.12	1.30±0.07
Jabalpur-7	1.06±0.40	1.20±0.07	1.10±0.00	0.44±0.11	0.30±0.07	0.74±0.09	0.62±0.13	0.70±0.19
Jabalpur-8	12.78±1.27	3.10±0.10	2.66±0.21	7.48±0.95	3.64±0.67	2.34±0.11	1.48±0.18	0.49±0.09
Jabalpur-9	11.28±0.95	2.84±0.27	2.62±0.35	6.20±0.93	2.86±0.26	2.28±0.28	2.00±0.29	0.47±0.06
Varanasi-14	7.14±1.09	2.28±0.16	2.12±0.11	4.24±0.59	2.06±0.44	1.62±0.16	1.26±0.09	0.49±0.10
Varanasi-15	7.44±1.74	2.50±0.16	2.14±0.23	5.04±1.30	1.96±0.75	1.86±0.24	1.24±0.15	0.39±0.11
Varanasi-25	9.34±1.38	2.64±0.25	2.26±0.15	6.38±1.08	2.20±0.37	1.90±0.17	1.20±0.07	0.29±0.04

Significant differences were observed among the genotypes studied for all the fruit characters. Fruit weight ranged from 0.90 g (MP-5) to 16 g (PGR-6). Out of 24 collections studied, 10 collections recorded >10 g fruit weight, 6 recorded between 5-10 g fruit weight, and 4 recorded 1-5 g fruit weight, while, genotypes MP-5, BRH-7, BRH-1, and Jabalpur-7 recorded <1.0 g fruit weight. Fruit weight is a primary selection character to get the required amount of seeds. Fruit length and width varied from 1.2 cm to 4.46 cm and 1.08 cm and 2.94 cm, respectively. Out of 24 genotypes studied, 10 collections recorded fruit width between 1.0-2.0 cm, while, 14 recorded 2.01 to 2.94 cm.

As expected, fruit weight had a significant positive correlation with fruit length (r=0.933) and fruit width (r=0.876) (Table 4). Pulp weight ranged from 0.34 g to 12.3 g. Seed weight ranged between 0.19 g to 3.76 g and eight lines namely, PGR-6 (3.76 g), PGR-9 (3.72 g), Jabalpur-8 (3.58 g), PGR-7 (3.52 g), Konkan Bahdoli (3.05g), Madanapalli (2.36 g), Varanasi-25 (2.16 g), and Jabalpur-9 (2 g) recorded high seed weight. Since the seed kernel exhibits higher biological activity, fruits with a high seed weight are considered as a key desirable for selecting superior genotypes. Seed length and width ranged from 0.74 cm (Japalpur-7) to 2.89 cm (PGR-6), and 0.5 cm (KHB-3) to 2.0 cm (Japalpur-9), respectively. Out of 24 collections studied, 12 genotypes recorded >2.0 cm seed length, and 12 recorded <2.0 cm. Ghosh et al. (2017) reported similar results in jamun samples collected from Odisha region.

Total phenolics and flavonoids content

Total phenolic content varied significantly among the jamun genotypes studied and varied between 15.5 mg to 98.7 mg/g jamun sample (Table 3). Highest total phenolic content was recorded in genotype BRH-1 (98.7) and Selection-45 (98.7) followed by Collection-2 (96.0 mg), Konkan Bahdoli (88.5 mg), PGR-9 (84.0 mg), AJG-85 (82.0), and Kaithanal (81.1 mg). The genotypes Madanapalli, Dhupdal, BRH -7, Jabalpur-7, Jabalpur-9, Varanasi-15, Jabalpur-8, Collection-3A, PGR-6, Selection-58 recorded phenolic content between 67 to 41 mg, while, 'Varanasi 25'recorded significantly least total phenolic content (15.5 mg). Total flavonoid content in the jamun genotypes varied between 0.79 mg to 9.03 mg CE/g seed kernel. Among the genotypes, selection-45 had

Table 3 : Linear correlation (*r*) between fruit morphology, seed traits, polyphenolics, antioxidant potential and *alpha*-glucosidase inhibitory activity

Traits	Fruit length	Fruit width	Seed weight	Seed length	Seed width	Pulp weight	Pulp: seed	TPC	TFC	FRAP	DPPH	α-GI
Fruit weight	0.933*	0.876*	0.897*	0.833*	0.667*	0.980*	0.487*	0.020	0.048	0.073	0.001	-0.001
Fruit length		0.768*	0.845*	0.947*	0.757*	0.919*	0.401	0.145	0.149	0.198	0.012	-0.117
Fruit width			0.841*	0.700*	0.663*	0.849*	0.332	-0.062	-0.023	-0.014	-0.039	0.234
Seed weight				0.755*	0.718*	0.906*	0.236	0.030	0.032	0.105	0.071	0.072
Seed length					0.825*	0.839*	0.389	0.227	0.213	0.243	0.054	-0.158
Seed width						0.697*	0.256	0.177	0.166	0.095	0.119	-0.056
Pulp weight							0.547*	0.101	0.141	0.113	0.122	-0.102
Pulp:seed								0.198	0.303	0.138	0.071	-0.204
TPC									0.968*	0.898*	0.770*	-0.612*
TFC										0.829*	0.782*	-0.609*
FRAP											0.488*	-0.486*
DPPH												-0.567*

TPC: Total phenolic content; TFC: Total flavonoid content; α-GI: *alpha*-glucosidase inhibitory activity; **Correlation is significant at the 0.01 level; *Significant correlation at 0.05 level

highest flavonoid content (9.03 mg) followed by Collection-2 (9.02mg), BRH-1 (8.46 mg), Kaithanal (8.41 mg), and Konkan Bahdoli (8.12 mg), whereas, it was lowest in Varanasi-25. Ghosh et al (2017) reported that the jamun seeds collected from Odisha had about 386 mg phenolics per g sample, which was higher than the present study.

Antioxidant potential

Since fruits and vegetables contain a wide range of polyphenolic compounds with varying antioxidant potentials, a single method is insufficient to assess the antioxidant potential of a given fruit or vegetable (Arivalagan et al., 2018). Therefore, in this study, the antioxidant potential of the jamun collections was studied both in terms of radical scavenging activity using DPPH radical and reducing power using the FRAP method, and the results were expressed as µM trolox equivalent/g kernel sample (Table 3). Total antioxidant potential varied between 0.20 to 1.44 µM for FRAP method and 0.21 to 1.79 µM for the DPPH method. Genotypes PGR-9, Collection-2, BRH-1, Selection-45, Konkan Bahdoli and AJG-85 recorded highest antioxidant potential in terms of both DPPH and FRAP methods, while genotypes Varanasi-25, KHB-3, Savadatti, Varanasi-14, PGR-7, KHA-16, MP-5, Slection-58, PGR-6, and Collection-3a had lowest antioxidant potential as measured by DPPH and FRAP methods. Correlation studied indicated that the antioxidant potential measured by DPPH and

Table 4 : *Alpha*-glucosidase inhibitory activity, phenolics, flavonoids and antioxidant potential of 24 jamun genotypes

Genotype	Q-glucosidase Inhibitory activity	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	Antioxidant activity (mM TE/g)	
				FRAP	DPPH
Selection 45	50.3±4.9	98.7±1.73	9.03±0.19	1.08±0.02	1.36±0.03
Madanapalli	85.6±19.1	66.6±3.61	6.71±0.37	0.81±0.04	0.94±0.02
Selection 58	146.7±23.6	41.1±4.46	4.19±0.49	0.49±0.05	0.50±0.02
Dhupdal	69.1±2.9	63.2±2.10	6.12±0.19	0.74±0.02	0.82±0.06
KHA-3	242.7±10.9	29.1±0.45	3.06±0.08	0.36±0.01	0.38±0.02
Savadatti	159.3±33.9	27.2±2.05	2.57±0.20	0.32±0.02	0.39±0.01
Collection-2	58.1±21.1	96.0±4.86	9.02±0.45	1.26±0.06	1.34±0.08
Kaithanal	88.8±13.2	81.1±2.65	8.41±0.23	0.98±0.03	1.07±0.01
Konkan Bahdoli	92.9±17.2	88.5±0.42	8.12±0.00	1.07±0.01	1.25±0.06
AJG85	74.6±1.4	82.0±3.08	7.04±0.24	1.01±0.04	1.12±0.01
Collection-3a	151.7±50.4	44.4±0.96	3.36±0.05	0.53±0.01	0.55±0.03
PGR-6	257.0±56.9	44.0±0.27	3.18±0.04	0.49±0.00	0.53±0.05
PGR-7	164.6±35.5	35.9±0.52	2.60±0.05	0.43±0.01	0.46±0.00
PGR-9	63.7±14.4	84.0±4.08	6.74±0.33	1.44±0.07	1.14±0.08
MP-5	186.1±1.7	38.3±3.81	2.90±0.29	0.45±0.04	0.47±0.00
KHA-16	224.6±49.9	37.1±2.32	2.56±0.15	0.46±0.03	0.47±0.00
BRH-1	58.2±14.0	98.7±3.31	8.46±0.25	1.11±0.04	1.37±0.07
BRH-7	128.3±9.7	62.1±0.62	4.52±0.05	0.76±0.01	0.84±0.08
Jabalpur-7	95.1±10.0	59.6±0.99	5.38±0.18	0.33±0.01	1.79±0.47
Jabalpur-8	115.8±11.0	50.0±1.21	5.04±0.14	0.30±0.01	1.61±0.39
Jabalpur-9	100.1±1.2	55.6±2.67	5.69±0.35	0.33±0.02	1.11±0.22
Varanasi-14	752.1±8.7	36.1±1.02	3.54±0.14	0.43±0.01	0.45±0.02
Varanasi-15	169.3±4.6	54.1±1.53	5.39±0.19	0.62±0.02	0.71±0.06
Varanasi-25	906.2±82.0	15.5±0.73	0.79±0.05	0.20±0.01	0.21±0.01

GAE: gallic acid equivalent; CE: catechin equivalent, TE: trolox equivalent

FRAP had significant positive correlation with total phenolic content (r=0.898 for FRAP, 0.770 for DPPH) and flavonoid content (r=0.828 for FRAP, 0.782 for DPPH) and negatively correlated with the amount sample required to inhibit the *alpha*-glucosidase inhibitory activity (r=-0.486 for FRAP and -0.567 for DPPH) (Table 4).

Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was measured using standard procedure (Table 3). The amount of sample required to inhibit 50% of the enzyme *alpha*-glucosidase activity varied between 50.3 µg (Selection 45) to 906.9 µg (Varanasi-25), lesser the amount required is better the inhibitory activity. Out of 24 jamun collections analyzed, 10 genotypes required only about 50 to 100 µg of sample to inhibit the 50% of the enzyme activity, 9 genotypes required about 100 to 200 µg of sample to inhibit the 50% of the enzyme activity and remaining 5 genotypes require more than 200µg of sample to inhibit the 50% of the enzyme activity. The amount of seed material required to inhibit the *alpha*-glucosidase enzyme had significant negative correlation with total phenolic content (r=-0.612), total flavonoid content (r=-0.609), and antioxidant potential measured by FRAP (r=-0.486), and DPPH method (r=-0.567) (Table 4). This clearly indicates that higher the levels of TPC, TFC and antioxidant potential, lesser the amount required to inhibit the enzyme activity.

Selection of superior genotypes for *alpha*-glucosidase inhibitory activity

Significant variations were observed among jamun genotypes studied for fruit characteristics, *alpha*-glucosidase inhibitory activity as well as total phenolic and flavonoid content and antioxidant potential. The genotype BRH-1is an excellent source of *alpha*-glucosidase inhibitory activity and phenolics, flavonoids and antioxidant potential, but the fruit and seed weight in very less (0.96 and 0.29 g, respectively), thus it is not economically viable source for *alpha*-glucosidase inhibitory compounds. On the other hand, PGR-6 and PGR-7 are good sources of seeds, with higher seed weight (>3.5 g), and fruit weight (>15 g), but both are poor sources of *alpha*-glucosidase inhibitory activity and phenolics, flavonoids and antioxidant potential, thus they also not a viable source for *alpha*-glucosidase inhibitory compounds. Thus,a holistic approach is needed to identify the genotypes which possess a considerable amount of seeds with *alpha*-glucosidase inhibitory compounds. Thus, two-way cluster analysis was carried out to identify the suitable genotype that can be a viable source for *alpha*-glucosidase inhibitory compounds, which can be used in pharmaceutical industries to develop anti-diabetic drugs.

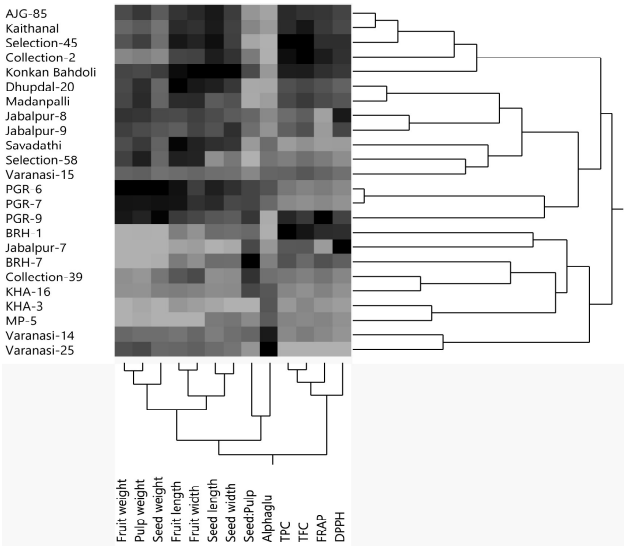

Fig. 1 : Two-way hierarchal cluster of jamun genotypes constructed using Ward method

Cluster analysis (Fig.1), grouped 24 jamun genotypes into two major clusters. Genotypes with high amounts of phenolics, flavonoids, and antioxidant potential and fruits with high pulp and seed content grouped together in cluster-1, while, the fruits with less pulp and seed content along with low amounts of phenolics, flavonoids and antioxidant potential grouped together in cluster 2. As already stated, the *alpha*-glucosidase inhibitory potential was negatively correlated with polyphenolic content; the genotypes grouped in cluster -1 required less amount of sample to inhibit the enzyme. The cluster-1 was further divided into three sub-clusters, and the genotypes AJG-85, Kaithanal, selection-45, collection-2 and Konkan Bahdoli with high polyphenolics, antioxidant potential, and a moderate amount of seed weight grouped together in sub-cluster. These genotypes may be effectively utilized for further studies to isolate and characterize the bioactive constituents with *alpha*-glucosidase inhibitory potential.

CONCLUSION

Jamun genotypes exhibited significant diversity for fruit characters, phenolics, flavonoids, antioxidant

potential, and *alpha*-glucosidase inhibitory potential. Seeds with a high content of polyphenolic compounds required a minimal amount of sample to effectively inhibit the enzyme. From cluster analysis, the genotypes AJG-85, Kaithanal, selection-45, collection-2, and Konkan Bahdoli were identified as superior genotypes having considerable amount of seeds with *alpha*-glucosidase inhibitory compounds. These genotypes can be effectively utilized for further studies to isolate and characterize the bioactive constituents with *alpha*-glucosidase inhibitory potential.

ACKNOWLEDGEMENT

Authors are grateful to the Director, ICAR-IIHR, Bengaluru, Karnataka, for constant support and encouragement in conducting this study.

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(Received : 22.4.2024; Revised : 25.6.2024; Accepted : 28.6.2024)