

Original Article

CHARACTERIZATION OF DIFFERENT *SYZYGIUM CUMINI* SKEELS ACCESSIONS BASED ON PHYSICO-CHEMICAL ATTRIBUTES AND PHYTOCHEMICAL INVESTIGATIONS

JITENDRA P SINGH<sup>1\*</sup>, AK SINGH<sup>1</sup>, ANJU BAJPAI<sup>1</sup>, IFFAT ZAREEN AHMAD<sup>2</sup>

<sup>1</sup>Division of Crop Improvement and Biotechnology, Central Institute for Subtropical Horticulture, Rehmankhera, P. O. Kakori, Lucknow 226101 (U. P.) India, <sup>2</sup>Department of Bioengineering, Integral University, Kursi Road, Dasauli, Lucknow 226026, Uttar Pradesh, India  
Email: jeet.psingh.lko@gmail.com

Received: 11 Jan 2015 Revised and Accepted: 05 Feb 2015

ABSTRACT

**Objective:** To study variability in respect to physico-chemical and phytochemical characteristics of fruits.

**Methods:** The twelve *accessions* with uniform growth and vigour were selected to study physico-chemical characteristics of the fruits. Physical analysis was performed by different qualitative methods in the seed and pulp of fruits of different maturity stages.

**Results:** The selected accessions were characterized on the basis of physico-chemical screening by comparing presence of phytoconstituents at different stages of maturity in different parts of the fruits. The preliminary phytochemical screening in pulp and seed of fruits of different accessions clearly indicates the significant difference among all the accessions. The dendrogram was constructed to examine the variability in relation to physico-chemical attributes of different accessions. The cluster analysis was carried out based on physico-chemical attributes of the fruits of different accessions that grouped in to four major clusters. The dendrogram constructed on the basis of biochemical characteristics showed the same clustering of accessions as that of the grouping of accessions showed in dendrogram based on biochemical attributes. However, accessions J-51 and J-55 that grouped together in the dendrogram from different geographical regions. The grouping of different accessions may be based on their genetic makeup of plant however, some of the variation was also observed among accessions due to change in weather conditions during growth and development of fruits.

**Conclusion:** The significant variability was observed with respect to physico-chemical characteristics of fruits of different accessions. The *S. cumini* accessions grouped in to different clusters according to their physico-chemical properties that give useful insight into their genetic relationships.

**Keyword:** *S. cumini*, Phytochemical, Physico-chemical, Nutraceutical, Cluster analysis.

INTRODUCTION

*S. cumini* occurs larger parts of India from the Indo-Gangetic plains in the North to Tamil Nadu in the South India. The potential source of phytochemicals and natural antioxidants are secondary metabolites of plants [1] that are increasingly being used for health food, nutraceuticals and medicinal products [2]. Herbal products have reached widespread acceptability because of their readily availability, comparatively cheap, no side effects and there were well practiced knowledge for use of fruits as health food and phytomedicines [3, 4].

*Syzygium cumini* Skeels is one of such fruit plant that not only provides delicious fruits but also have higher medicinal properties. It plays an important role in primary healthcare for many centuries till date [5]. Recent research demonstrates that there are many "families" of phytochemicals obtained from *S. cumini* that can be used against many degenerative diseases. [6, 7]. It has a rich history of uses as edibles and as traditional medicines but many species are very poorly known and many more have not been described taxonomically [8]. *S. cumini* fruit, leaves, seeds and bark have a long traditional use as alternative medicine as well as can also be used to treat a range of ailments of mankind. [9, 10] Although, it is mostly known for its use in the treatment of diabetes as well as the source for natural antioxidants. [11-14]. The health-beneficial effects are mainly attributed to various phytoconstituents [15, 16] such as anthocyanins includes cyanidin, petunidin, malvidin [17] alkaloid contains jambosine, gallic acid, ellagic acid, corilagin, glycoside jambolin or antimellin, resin, albumin, chlorophyll, flavonoids, related tannin, 3, 6-hexahydroxydiphenylglucose and its isomer 4, 6-hexahydroxydiphenylglucose, 1-galloylglucose, 3-galloylglucose, quercetin [18] and a trace of pale yellow essential oil. The sourness of fruits may be due to presence of gallic acid and ellagic acid content. It has play role in diastatic conversion of starch into sugar so that seed extract minimize blood pressure [19, 20]. The fruits are rich in raffinose, glucose, fructose [21], mallic acid, vitamin C and

mineral salts such as zinc, chromium, vanadium, potassium. The color of the fruits might be due to the presence of anthocyanins. Many research studies have shown that the various extracts of Jamun possess a range of pharmacological properties such as antibacterial [22], antimicrobial [23], antiviral [24], anti-inflammatory [25], anti-ulcerogenic [26], cardioprotective [27], anti-allergic [28], anticancer [29], hepatoprotective [30], radioprotective [31], antioxidant [32-34], hypoglycemic and antidiabetic effects [35, 36].

*S. cumini* trees shows enormous variability in respect to morphology and physico-chemical characters of fruits. The present paper has been primed to describe the preliminary information of phytochemical constituents and physico-chemical properties of different parts of *S. cumini* for different accessions. There is still lack of promotion, less planting regions and low economic values of *S. cumini* in India. Characterization based on physico-chemical characters along with phytochemical screening are very useful for crop improvement, varietal selection for high yielding clones with higher medicinal and nutraceutical attributes. Secondly, the study provides practical information on *S. cumini* to herbal drugs manufactures [38].

MATERIALS AND METHODS

Collection of plant material

*Syzygium cumini* accessions from different geographical regions comprising Uttar Pradesh, Maharashtra, Gujarat and Tamil Nadu, maintained in the field gene bank at Central Institute for Subtropical Horticulture, Lucknow (U. P.) were used for the study of physico-chemical and phytochemical screening.

Physico-chemical characterization

For the study of variation in the physico-chemical attributes, 10 fruits from each selected accessions were collected randomly and analyzed. The fruit size, in terms of the two principal axial dimensions, that is the length and width was measured using a

digital vernier calliper. The weight of fruits, seeds, pulp content was determined by means of electronic balance (Sartorius, Germany). Total soluble solids (TSS) were estimated by using hand refractometer (Erma, Japan). Titrable acidity, total sugars, reducing sugar and ascorbic were determined according to A. O. A. C. method [39].

#### Preparation of extract

The fruits were picked, washed well with distilled water then the pulp and seeds were separated, dried at room temperature. The pulp was powdered in pestle-mortar and seeds were powdered in an electrical grinder and stored until further use. The powdered pulp and seeds were extracted in a soxhlet with methanol and water at a temperature of 50 °C for 48 h. After distillation with the solvents, the extracts were filtered using whatmann filter paper no. 2 and then centrifuged at 5000 rpm for 15 min [40]. The supernatant or extracts were subjected to various chemical tests to detect the presence of phytoconstituents present in different accessions at different stages of maturity of fruits.

#### Preliminary phytochemical screening

##### Screening of alkaloids

###### Mayer's test

1.36 gm of mercuric chloride dissolved in 60 ml and 5 gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To the test filtrates were treated with above Mayer's reagent (Potassium Mercuric Iodide) and the formation of pale yellow or white coloured precipitate showed the presence of alkaloids.

###### Hager's reagent

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating the presence of alkaloids.

###### Wagner's reagent

1.27 gm of iodine and 2 gm of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When the few drops of this reagent were added to the test filtrate, a brown flocculent precipitate was formed indicating the presence of alkaloids.

##### Screening of tannins

###### Ferric chloride reagent

A 5 % w/v solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a test filtrate. A dark green or blue colour precipitate indicated the presence of tannins.

###### Lead acetate test

In a test tube containing about 5 ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

##### Screening of sterols

###### Salkowski reaction

The few ml of test filtrate were dissolved in 2 ml of chloroform. Sulphuric acid was carefully added from the side of the test-tube to the lower layer. A reddish colour at the interface was an indicative of the presence of sterols.

##### Screening of saponins

**Foam test:** Extract was shaken vigorously with a small amount of sodium bicarbonate and 2 ml of water in a graduated cylinder. The formation of foam indicated that presence of saponins.

##### Screening of flavonoids

Two methods were used to determine the presence of flavonoids in the plant sample.

(a.) In a test tube containing 0.5 ml of an alcoholic extract of the samples, 5 to 10 drops of diluted HCl and the small amount of Zn or

Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

(b.) 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow colouration observed and yellow colouration disappeared on standing indicating a positive test for flavonoids.

#### Data analysis

The physico-chemical data were used for multivariate analysis through Darwin software (Dissimilarity Analysis and Representation for Windows, Version 5.0.148, <http://darwin.cirad.fr/darwin>). The Rogers-tanimoto dissimilarity coefficient used to study the variation based on physico-chemical properties. The dendrogram was constructed to examine relatedness among twelve *Syzygium cumini* accessions. The dissimilarity coefficients among all accessions were analyzed and clustering was carried out using neighbour joining (NJ) method. A cophenetic value matrix of the (NJ) clustering was used to test for the goodness-of-fit of the clustering to the dissimilarity matrix [41] on which it was based, by computing the cophenetic correlation (*r*) with 1000 permutations [42].

## RESULTS AND DISCUSSION

### Physicochemical parameters

The physicochemical traits serve as traditional markers for the discriminating different germ plasm or accessions. The enormous variability was observed with respect to morphology and physico-chemical attributes of fruits due to pre-dominance of seed propagation in *S. cumini* trees. The study of physicochemical attributes screening are very useful for the selection of high yielding accessions of *S. cumini*. The observations of physico-chemical parameters are important for characterization of *S. cumini* accessions. The data pertaining to physico-chemical quality attributes of fruits showed significant differences with respect to fruit weight, length, breadth, length: breadth ratio, pulp weight, pulp percent, seed weight, seed percent, pulp: seed ratio, TSS, acidity, TSS: acid ratio, total sugar, sugar: acid ratio and vitamin 'C' (table 1 & 2).

The fruit weight was recorded in the range of 11.65 to 20.74 g and the maximum fruit weight was observed in J-37 (20.74 g) followed by J-36 (19.65 g). While, the minimum fruit weight was found in J-51 (11.65 g). Fruit size and length: breadth ratio is a measure of fruit shape and higher length: breadth ratio indicated the oblong shape, while lower ratio suggested the oblong and round shape of the fruits [44]. There were wide variations in fruit size (length and breadth) observed among different accessions. The fruit length and breadth was recorded maximum in J-37 (3.97 cm & 2.91 cm) with big size fruit (11.83 cm<sup>2</sup>) whereas the length breadth ratio was found maximum in J-23 (1.48 cm) which exhibited oblong fruits shape. While minimum fruit length and breadth were recorded in accession J-49 (1.11 cm) which exhibited fruits towards the round shape. The maximum pulp weight was recorded in J-37 (17.09 g) however, highest pulp content was recorded in J-42 (93.75 %) followed by J-44 (92.94 %) and J-43 (92.66 %). The lowest pulp content was recorded (70.21 %) in accession J-49. Though the maximum weight of the fruit was observed in J-37 whereas, maximum pulp content was recorded with the accessions J-42, J-43 and J-44 which fruit weight was 13.28 g, 12.26 g and 13.46 g respectively, this is due to seedlessness of these accessions. The seed weight ranged from 2.79 to 4.10 g however, in case of seedless accessions J-42, J-43 and J-44 seed not found only the seed coat weight was recorded that ranged from 0.83 to 0.95 g. There was a wide variation also observed in seed weight and size and for an ideal variety lower weight and small size of seed is desirable character for table purpose of jamun fruits [45]. The maximum seed weight was recorded in J-34 (4.10 g) however, maximum seed length, breadth was found in J-51 (2.37 & 1.52 cm). Data revealed that pulp: seed ratio in various accessions ranged from 2.36 to 15.00 that showed the wide range of variability.

The different other studies have also been conducted to assess the variability of quality characters among best selected *S. cumini* accessions that reveals the wide range of variability for physico-

chemical traits [43]. The data presented in table-2, revealed wide variations in the biochemical composition of the fruits of all the accessions. The total soluble solids (TSS) content of the fruit is an important trait during selection of superior accessions and there were significant variations observed in different accessions selected for study [44]. The TSS was recorded in the ranged from 15.65 to 11.60 °Brix. TSS was found maximum in J-37 (15.65 °Brix) whereas, minimum TSS recorded in accession J-55 (11.60 °Brix). Titratable acidity content of the fruit varied from 0.90 to 1.08 %. Titratable acidity was found maximum in J-40 (1.08 %) followed by J-42 (1.06 %), while it was the lowest in accession J-51 (0.90 %). The TSS: acid ratio ranged from (16.13 to 11.15). The maximum ratio was noted in J-37 (16.13) and minimum in J-55 (11.15). A wide range of variability was also recorded in total sugar content among selected

accessions of *S. cumini* [45]. Total sugars were estimated to be highest in J-37 (16.57 %), whereas lowest sugar content recorded in J-55 (9.29 %). The reducing sugars were found to be maximum in J-37 (14.83 %) followed by J-23 (13.55 %) and it was observed minimum in J-55 (7.26 %) however, the non reducing sugar found maximum in accession J-49 (2.36 %) and lowest in accession J-42 (1.52 %). The sugar: acid ratio also showed considerable variability among accessions and was ranged from J-37 (17.08) to J-26 (8.90). There was a considerable variability in ascorbic acid content of fruit, which ranged from 25.74 to 37.68 mg/100g fruits among the accessions. The maximum ascorbic acid content was recorded in J-37 (37.68 mg/100g) followed by (35.65 mg/100 g) in J-23 and (34.85 mg/100g) in J-49 while, it was minimum (25.74 mg/100g) in J-26.

Table 1: Physical traits of fruits of the different *Syzygium* accessions

Accessions	Fruit Weight (g)	Fruit length (cm)	Fruit Breadth (cm)	Length: Breadth Ratio	Size (cm <sup>2</sup> )	Pulp Weight (g)	Seed Weight (g)	Seed Length (cm)	Seed Breadth (cm)	Pulp: Seed Ratio	Pulp Content (%)
J-37	20.73	3.97	2.93	1.35	11.63	17.06	3.67	2.27	1.42	4.65	82.30
J-36	19.63	3.76	2.89	1.30	10.87	15.68	3.95	1.87	1.03	3.97	79.88
J-34	18.74	3.74	2.91	1.29	10.88	14.61	4.13	2.18	1.43	3.54	77.96
J-49	12.57	2.93	2.66	1.10	7.79	9.2	3.37	1.97	1.12	2.73	73.19
J-51	11.63	3.62	2.73	1.33	9.88	8.16	3.47	2.37	1.52	2.35	70.16
J-40	15.83	3.59	2.76	1.30	9.91	11.97	3.86	1.87	1.03	3.10	75.62
J-26	13.47	2.53	1.96	1.29	4.96	10.55	2.92	1.76	1.12	3.61	78.32
J-23	16.24	3.94	2.67	1.48	10.52	13.01	3.23	1.96	1.13	4.03	80.11
J-55	12.67	2.6	1.92	1.35	4.99	9.91	2.76	1.67	1.19	3.59	78.22
J-42	13.26	3.49	2.53	1.38	8.83	12.43	0.83	0.11	0.11	14.98	93.74
J-43	12.67	2.94	2.03	1.45	5.97	11.74	0.93	0.11	0.11	12.62	92.66
J-44	13.46	3.43	2.96	1.16	10.15	12.5	0.96	0.22	0.23	13.02	92.87
CD at 5 %	0.051	0.051	0.051	0.115	0.852	1.77	0.051	0.118	0.122	1.10	5.59

Table 2: Biochemical properties of fruits of the different *Syzygium* accessions

Accessions	TSS (°B)	Acidity (%)	TSS Acidity Ratio	Total Sugars (%)	Reducing Sugars (%)	Non Reducing Sugars (%)	Sugar Acid Ratio	Ascorbic Acid (mg/100 g)
J-49	12.45	1.02	15.05	11.48	9.12	2.36	14.36	34.85
J-23	14.80	1.02	14.64	15.19	13.55	1.64	14.98	35.65
J-55	11.60	1.04	11.15	9.29	7.26	2.03	8.93	30.87
J-51	17.27	1.01	17.15	17.60	16.07	1.53	17.43	39.64
J-44	13.05	0.92	14.18	9.96	7.88	2.08	10.83	26.85
J-37	15.65	0.99	15.81	16.57	14.83	1.74	16.74	37.68
J-36	14.20	0.99	14.34	13.57	11.75	1.82	13.71	33.85
J-34	13.53	0.99	13.74	10.27	8.27	2.00	10.44	26.57
J-40	13.47	1.08	12.47	10.79	8.72	2.07	10.92	29.85
J-26	12.80	0.90	14.22	10.23	8.21	2.02	11.37	27.14
J-43	14.00	1.06	13.30	10.08	8.26	1.81	9.56	33.18
J-42	15.27	1.06	14.40	14.90	13.38	1.52	14.06	36.76
CD at 5 %	0.329	0.062	2.462	0.515	0.882	NS	0.952	1.403

### Preliminary phytochemical screening

The selected accessions were characterized on the basis of preliminary phytochemical screening by comparing presence of phytoconstituents at different stages of maturity in different parts of the fruits. The determination of phytochemical of the fruit seed clearly indicates the significant difference among all the accessions. The alkaloid content was highest in the seeds of fruits at different stage of maturity (ripe, semiripe and unripe) of J-23, J-26, J-37 and J-40. However, higher alkaloid content was in the seeds of ripe and semiripe fruit of accession J-37 and J-36. While, the seeds of ripe fruits showed higher alkaloid content J-36 and J-49. The estimation of alkaloid content in pulp of different maturity stages of fruits with the Mayer's reagent method clearly indicates that in most of the accessions, pulp of ripe fruits had higher alkaloids as compared to other stages of fruit pulp. The higher alkaloid content was noticed in the pulp of ripe fruits of J-37, J-36 and J-40. As per Wagner's reagent test the alkaloid content has higher in semiripe and ripe

pulp of fruits of the J-23 and J-40. Hager's reagent method did not show significant differences with respect to alkaloid content seed and pulp of fruit at different maturity stages among all the accession of *S. cumini*.

The earlier other studies on phytochemical investigation in different parts *S. cumini* were carried out to explore the potential as nutraceutical fruit with high medicinal values. The phytochemical screening studies revealed the presence of different phytoconstituents viz. flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid and tannins [46, 47, 48]. The estimation of tannins compound was concerned, FeCl<sub>3</sub> test showed higher tannins activity were found higher in seeds of ripe, semiripe and unripe fruits of J-37 and J-49 however, the higher tannins activity was also found higher in the seeds of semiripe and ripe fruit of accessions J-26, J-36 and J-49. Tannins with lead acetate method were observed higher in the seeds of unripe fruits of the accession J-37 and J-49 however, the semiripe and ripe seeds of accession J-36, J-

37, J-23, J-40 and J-49 also showed higher tannins content. As far as tannins content in fruit pulp of semiripe and ripe fruits the accessions J-37, J-40, J-42 and J-49 with ferric chloride (FeCl<sub>3</sub>) test however, The lead acetate method showed did not show considerable variation with respect to tannins content in all stages of pulp of all the accessions except J-37, J-49 and J-42 that showed higher tannins content in the pulp of semiripe and ripe pulp.

The estimation of saponins by foam test, it was quite clear that seeds at different maturity stages of all the accessions showed higher saponins content as compared to pulp. The ripe seeds of fruits

showed higher saponins content in accessions J-26, J-37, J-40 and J-49 however, the seeds of semiripe and unripe fruits have higher content in the accessions J-37, J-40 and J-49. The pulp of semiripe and ripe fruits showed higher activity of saponins content in the accessions J-37, J-26 and J-49. Mostly higher sterols content in the pulp and seeds of fruits was higher among all the accession as estimated by Salkowski reaction. The seeds and pulp of semiripe and ripe fruits showed higher sterols content as compared to the unripe seed and pulp of the fruits of different accessions however, in seeds and pulp of accessions J-37, J-26, J-40 and J-49 have higher sterol content than other accessions.

**Table 3: Screening for alkaloids in the seeds of different stage and accessions through different reagent**

Accessions	Alkaloids test								
	Unripe seed			Semi ripe seed			Ripe seeds		
	Hager's reagent	Mayer's reagent	Wagner's reagent	Hager's reagent	Mayer's reagent	Wagner's reagent	Hager's reagent	Mayer's reagent	Wagner's reagent
CISH J-37	+	++	+	+	+++	++	-	+++	+
CISH J-36	-	+	+	-	+	++	-	+	++
CISH J-34	-	-	+	-	+	+	-	++	+
CISH J-40	-	++	+	-	++	+	+	+++	+
CISH J-26	+	++	+	+	+++	+	-	+++	+
CISH J-23	-	++	+	-	++	+	+	+++	+
CISH J-49	--	-	+	-	-	+	-	+	++
CISH J-42	NA	NA	NA	NA	NA	NA	NA	NA	NA

**Table 4: Screening for alkaloids in the pulp of different stage and accessions through different reagent**

Accessions	Alkaloids test								
	Unripe pulp			Semi ripe pulp			Ripe pulp		
	Hager's reagent	Mayer's reagent	Wagner's reagent	Hager's reagent	Mayer's reagent	Wagner's reagent	Hager's reagent	Mayer's reagent	Wagner's reagent
CISH J-37	-	+	+	-	++	+	-	++	++
CISH J-36	-	+	+	+	+	+	+	++	+
CISH J-34	+	+	+	-	+	+	-	+	+
CISH J-40	-	+	+	-	+	++	+	+	+
CISH J-26	+	+	+	+	++	+	-	++	+
CISH J-23	+	+	+	-	+	+	-	+	++
CISH J-49	-	+	-	-	+	+	+	+	+
CISH J-42	+	-	+	-	+	+	-	+	+

**Table 5: Screening for Tannins in the seeds of different stage and accessions through different reagent**

Accessions	Tannins					
	Unripe seed		Semi ripe seed		Ripe seed	
	Ferric chloride reagent	Lead acetate test	Ferric chloride reagent	Lead acetate test	Ferric chloride reagent	Lead acetate test
CISH J-37	+++	++	+++	++	+++	++
CISH J-36	++	+	+++	+	+++	++
CISH J-34	++	+	++	+	++	+
CISH J-40	++	+	++	++	++	++
CISH J-26	++	+	+++	+	+++	+
CISH J-23	++	+	++	++	+++	++
CISH J-49	+++	++	+++	++	+++	++
CISH J-42	NA	NA	NA	NA	NA	NA

**Table 6: Screening for Tannins in the pulp of different stage and accessions through different reagent**

Accessions	Phenolics					
	Unripe pulp		Semi ripe pulp		Ripe pulp	
	Ferric chloride reagent	Lead acetate test	Ferric chloride reagent	Lead acetate test	Ferric chloride reagent	Lead acetate test
CISH J-37	++	+	+++	++	+++	++
CISH J-36	+	+	+	+	+	+
CISH J-34	+	+	+	+	+	+
CISH J-40	+	+	+	+	+++	+
CISH J-26	+	+	+	+	+	+
CISH J-23	+	+	+	+	++	+
CISH J-49	++	+	+++	++	+++	++
CISH J-42	+	+	+++	++	+++	++

Table 7: Screening for sterol and saponins in the seeds of different stage and accessions through different reagent

Accessions	Saponins			Sterols		
	Foam test			Salkowaski reaction		
	Unripe seed	Semi ripe seed	Ripe seed	Unripe seed	Semi ripe seed	Ripe Seed
CISH J-37	++	++	+++	++	+++	+++
CISH J-36	+	++	++	++	-	+++
CISH J-34	+	++	++	++	+++	-
CISH J-40	++	+++	+++	++	-	-
CISH J-26	++	+++	+++	++	++	++
CISH J-23	+	++	++	++	-	+++
CISH J-49	++	+++	+++	++	++	+++
CISH J-42	NA	NA	NA	NA	NA	NA

Table 8: Screening for sterol and saponins in the pulp of different stage and accessions through different reagent

Accessions	Saponins			Sterols		
	Foam test			Salkowaski Reaction		
	Unripe pulp	Semi ripe pulp	Ripe pulp	Unripe pulp	Semi ripe pulp	Ripe pulp
CISH J-37	+	++	++	++	+++	+++
CISH J-36	+	+	++	++	-	-
CISH J-34	+	+	+	++	-	-
CISH J-40	+	+	+	-	+++	+++
CISH J-26	+	++	++	++	+	+++
CISH J-23	+	+	+	++	+++	+++
CISH J-49	+	++	++	-	++	++
CISH J-42	+	+	++	++	+++	+++

### Characterization based on physico-chemical attributes

The *S. cumini* widely grown as it possesses diverse health benefits and nutraceutical properties. The trees showed enormous variability respect to tree and fruit morphology, fruit quality, maturity and productivity [44, 45]. There is good scope for breeding programs but very less information is available on improvement of this fruit crop. The characterizations based on physico-chemical attributes along with preliminary phyto chemical screening will be valuable for selecting of high yielding clones with higher medicinal and nutraceutical attributes that is important for the development of appropriate conservation strategies for *Syzygium cumini*. The purpose of present such study was to characterize different accessions through physico-chemical parameters that included attributes viz. fruit weight, length, breadth, length: breadth ratio, pulp weight, pulp percent, seed weight, seed percent, pulp: seed ratio, TSS, acidity, TSS: acid ratio, total sugar, sugar: acid ratio and vitamin C.

### Cluster analysis based on physical attributes

The accessions under study were broadly divided into four major clusters (fig. 2). Each cluster was representing the grouping of twelve *S. cumini* accessions according physical attributes. Cluster I included three accessions namely J-37, J-36 and J-34 that appears to distinct as compared to other clusters. All the accessions of this cluster grouped together according to their respective geographical origin as they are the selections from the Mohanlalganj, Lucknow (UP). These accessions have all most same physical characteristics. Cluster II has two accessions that include accessions J-23 and J-49 grouped in the same cluster as they having similar physical attributes. However, these accessions belong from different location of Lucknow (UP). The cluster III comprises two accessions J-42 and J-43 which grouped together according to their unique characteristics of seedlessness as well as they belong to same geographical origin from Varanasi, U. P. The accessions J-51 and J-55 were grouped together in a cluster III however, they related from different geographical regions. The accession J-51 from Godhra region of Gujrat and J-55 from Konkan region of Maharastra grouped in the same cluster because these accessions having similar morphological characteristics. Cluster IV contained accessions J-26 and J-40 were grouped together in the same cluster as they have similar physical characteristics as well as belong to the same geographical origin of Kankaha, Lucknow. These accessions are characterized by big canopies, producing small fruit, bold seeded, less pulp content, low

TSS, low sugar and vit C content. The accession J-44 are quite distinct and separated from other clusters as it is a seedless accession which was originating from Periyakulum region of Tamil Nadu. Bootstrap values above 60 are shown in dendrogram. The cophenetic correlation values for dendrogram were used to test for the goodness-of-fit of the clustering to the dissimilarity matrix by computing the cophenetic correlation ( $r$ ) with 1000 permutations. The cophenetic correlation of the dendrogram and the dissimilarity matrix revealed a good degree of fit ( $r = 0.977$ ;  $p < 0.001$ ) suggest the cluster analysis strongly represents the similarity matrix.

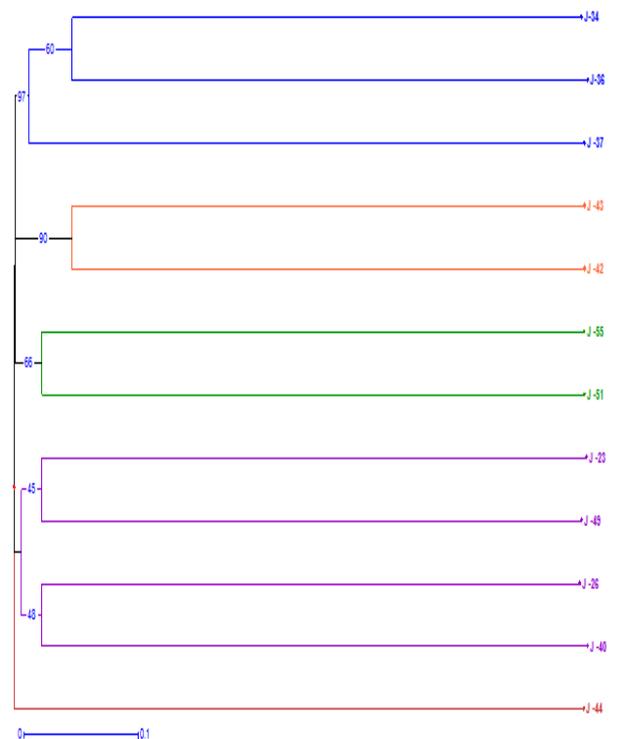


Fig. 1: Dendrogram depicting the cluster analysis based on physical characteristics

### Cluster analysis based on biochemical properties

The biochemical attributes of fruits of different accessions were also used to construct dendrogram with software Darwin 5 by calculating rogers-tanimoto dissimilarity coefficient and weighted neighbor joining (N) algorithm used for characterization of different accessions of *S. cumini* [40]. The dendrogram showed that clustering of different accessions may be due to their biochemical characteristics and the grouping of different accessions may be based on their genetic make up of plant [40, 50]. However, some of the seasonal variation were also observed among accessions due to change in weather conditions during growth and development of fruits. The dendrogram was constructed and the cluster analysis grouped different accessions in to four major clusters is presented in (fig. 2). Cluster I was observed to be the largest one with 3 accessions followed by cluster II (2 accessions), Cluster III (2 accessions) and Cluster IV (2 accessions) and each cluster represented the grouping of different accessions according biochemical characteristics. The dendrogram constructed were showed the clustering of different accessions similar to the clustering of accessions showed in dendrogram constructed based on physical characteristics. Except, accessions J-51 and J-55 that grouped together in the dendrogram made with physical characters however, biochemical attributes based dendrogram these accessions are separated because they related from different geographical regions of Gujrat and Maharashtra. The grouping of different accessions in a same cluster as the fruits of these accessions having similar biochemical properties as well as they belongs from similar geographical regions. A cophenetic was used to test for the goodness-of-fit of the clustering to the. The cophenetic correlation value ( $r$ ) of the dissimilarity matrix was determined with 1000 permutations revealed a good degree of fit ( $r = 0.987$ ;  $p < 0.001$ ). Thus different accessions of *S. cumini* grouped in to different clusters according to physical and biochemical that gives a useful insight into their genetic relationships that could be value for germ plasm accession management programs.

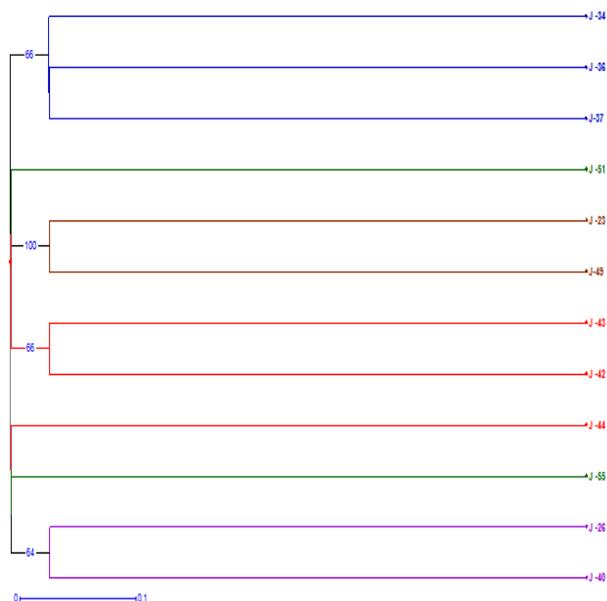


Fig. 2: Dendrogram depicting the cluster analysis based on biochemical properties

### CONCLUSION

The information obtained from preliminary phytochemical screening in pulp and seeds of different maturity stages among all accessions of will be useful for manufacturers to utilize superior accessions with higher content of phytoconstituents and selection of the raw material for drug production as well as in finding out the

genuity of the drug. The cluster analysis based on physical and biochemical attributes of fruits grouped different accessions in to four major clusters. The dendrogram showed grouping of similar accession either clustering based on physical or biochemical properties except few accessions that reveals the fact that clustering of different accessions may be due to their genetic make up. The significant variability has been observed with respect to physico-chemical characteristics of fruits of different accessions that could play an important role in characterization of these accessions selected for study along with preliminary phytochemical screening could provide information about varietal selection programme for breeding and crop improvement.

### CONFLICT OF INTERESTS

Declared None

### REFERENCES

1. Briskin DP. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol* 2000;124(2):507-14.
2. Sagar BPS, Zafar R, Panwar R. Herbal drug standardization. *Indian Pharm* 2005;4(35):19-22.
3. Kamboj VP. Herbal medicine. *Curr Sci Bangalore* 2000;78(1):35-8.
4. Choudhary N, Sekhon BS. An overview of advances in the standardization of herbal drugs. *J Pharm Educ Res* 2011;2(2):55-70.
5. Lim TK. *Syzygium cumini*. In: Edible medicinal and non medicinal plants. Springer Netherlands; 2012. p. 745-59.
6. Baliga MS, Bhat HP, Baliga BRV, Wilson R, Palatty PL. Phytochemistry, traditional uses and pharmacology of *Eugenia jambolana* Lam. (black plum): A review. *Food Res Int* 2011;44(7):1776-89.
7. Ayyanar M, Babu SP. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific J Trop Biomed* 2012;2(3):240-6.
8. Chase MW, Reveal JL. A phylogenetic classification of land plants to accompany APG III. *Bot J Linn Soc* 2009;161:122-7.
9. Jadhav VM, Kamble SS, Kadam VJ. Herbal medicine: *Syzygium cumini*: A Review. *J Pharm Res* 2009;2(8):1212-9.
10. Ayyanar M, Babu SP, Ignacimuthu S. *Syzygium cumini* (L.) Skeels A novel therapeutic agent for diabetes: Folk medicinal and pharmacological evidences. *Complementary Ther Med* 2013;21(3):232-43.
11. Helmstadter A. *Syzygium cumini* (L.) Skeels (Myrtaceae) against diabetes 125 years of research. *Pharm Int J Pharm Sci* 2008;63(2):91-101.
12. Baliga MS, Fernandes S, Thilakchand KR, D'souza P, Rao S. Scientific validation of the antidiabetic effects of *Syzygium jambolanum* DC (Black Plum), a traditional medicinal plant of India. *J Altern Complementary Med* 2013;19(3):191-7.
13. Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. *Food Chem* 2005;90(4):727-33.
14. Benherlal PS, Arumughan C. Chemical composition and *in-vitro* antioxidant studies on *Syzygium cumini* fruit. *J Sci Food Agric* 2007;87(14):2560-9.
15. Murti K, Paliwal D, Madan S, Kundu R, Kaushik M. Exploration of preliminary phytochemical studies of seed of *Syzygium cumini*. *Am J Pharm Toxic* 2012;7(1):12-4.
16. Chaudhary B, Mukhopadhyay K. *Syzygium cumini* (L.) Skeels: A potential source of nutraceuticals. *Int J Pharm Biol Sci* 2012;2(1):46-53.
17. Baliga MS, D'Souza JJ, Haniadka R, Arora R. Indian vegetarian diet and cancer prevention. *Bioactive foods and extracts: Can Treat and Pre*; 2010. p. 67-72.
18. Omar R, Li L, Yuan T, Seeram NP.  $\alpha$ -Glucosidase inhibitory hydrolyzable tannins from *Eugenia jambolana* seeds. *J Nat Prod* 2012;75(8):1505-9.
19. Wealth of India. Raw materials. CSIR: New Delhi; 1976.
20. Morton J. Fruits of warm climates. Miami: Julia Morton Winterville North Carolina; 1987. p. 375-8.
21. Noomrio MH, Dahot MU. Nutritive value of *Eugenia jambosa* fruit. *J Islam Acad Sci* 1996;9(1):9-12.

22. Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res 2010;2:1569-73.
23. Raheman F, Deshmukh S, Ingle A, Gade A, Rai M. Silver nanoparticles: novel antimicrobial agent synthesized from an endophytic fungus *Pestalotia* sp. isolated from leaves of *Syzygium cumini* (L.). Nano Biomed Eng 2011;3(3):174-8.
24. Sood R, Swarup D, Bhatia S, Kulkarni DD, Dey S, Saini M, et al. Antiviral activity of crude extracts of *Eugenia jambolana* Lam. against highly pathogenic avian influenza (H5N1) virus. Indian J Exp Biol 2012;179-86.
25. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Kumar RM, Aravindan P Krishan MRV. Anti-inflammatory activity of *Syzygium cumini* seed. Afr J Biotech 2008;7(8):941-3.
26. Bhargava S, Bhargava P, Jain UK. Evaluation of ulcer-protective and antimicrobial activity of *Syzygium cumini* (linn.) Skeels leaves. Pharmacologyonline 2009;3:266-74.
27. Mastan SK, Chaitanya G, Bhavya Latha T, Srikanth A, Sumalatha G, Eswar Kumar K. Cardioprotective effect of methanolic extract of *Syzygium cumini* seeds on isoproterenol-induced myocardial infarction in rats. Pharm Lett 2009;1(1):143-9.
28. Brito FA, Lima LA, Ramos MFS, Nakamura MJ, Cavalher-Machado SC, Siani AC Sampaio ALF. Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. Braz J Med Biol Res 2007;40(1):105-15.
29. Afify AMR, Fayed SA, Shalaby EA, El-Shemy HA. *Syzygium cumini* (pomposia) active principles exhibit potent anticancer and antioxidant activities. Afr J Pharm Pharmacol 2011;5:948-56.
30. Moresco RN, Sperotto RL, Bernardi AS, Cardoso RF, Gomes P. Effect of the aqueous extract of *Syzygium cumini* on carbon tetrachloride induced hepatotoxicity in rats. Phytother Res 2007;21(8):793-5.
31. Jagetia GC, Baliga MS. Evaluation of the radioprotective effect of the leaf extract of *Syzygium cumini* (Jamun) in mice exposed to a lethal dose of  $\gamma$ -irradiation. Food Nah 2003;47(3):181-5.
32. Banerjee J, Narendhirakannan RT. Biosynthesis of silver nanoparticles from *Syzygium cumini* (L.) Seed extract and evaluation of their *in vitro* antioxidant activities. Digest J Nanomater Biostructures 2011;6(3):961-8.
33. Banerjee A, Dasgupta N, De B. *In-vitro* study of antioxidant activity of *Syzygium cumini* fruit. Food Chem 2005;90(4):727-33.
34. Benherlal PS, Arumughan C. Chemical composition and *in-vitro* antioxidant studies on *Syzygium cumini* fruit. J Sci Food Agric 2007;87(14):2560-9.
35. Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Ravi Kumar A. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect *in vitro*. Evidence-Based Complement Alter Med 2011;2011:1-10.
36. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, et al. Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. J Med Plants Res 2008;2(9):246-9.
37. Benherlal, Palayyan Saraswathy, Chami A. Chemical composition and *in vitro* antioxidant studies on *Syzygium cumini* fruit. J Sci Food Agric 2007;87(14):2560-9.
38. Chaudhary B, Mukhopadhyay K. *Syzygium cumini* (L.) Skeels: A potential source of nutraceuticals. Int J Pharm Bio Sci 2012;2(1):46-53.
39. O. A. C. [Association of official Analytical Chemistry] Official methods of analysis of the association of official analytical chemists. 11<sup>th</sup> Edition. Washington D. C.; 1975.
40. Zaher H, Boulouha B, Baaziz M, Sikaoui L, Gaboun F, Udupa SM. Morphological and genetic diversity in olive (*Olea europaea* subsp. *europaea* L.) clones and varieties. Plant Omics J 2011;4(7):370-6.
41. Sneath PHA, Sokal RR. Numerical Taxonomy: the principles and practice of numerical classification. W. H. Freeman, San Francisco, USA; 1973.
42. Mantel NA. The detection of disease clustering and generalized regression approach. Cancer Res 1967;27:209-20.
43. Patel VB, Pandey SN, Singh SK, Das B. Variability in jamun (*Syzygium cumini* Skeels) accessions from Uttar Pradesh and Jharkhand. Indian J Hortic 2005;62(3):244-7.
44. Ghojage AH, Swamy GSK, Kanamadi VC, Jagdeesh RC, Kumar P, Patil CP, et al. Studies on variability among best selected genotypes of Jamun (*Syzygium cumini* Skeels.). Acta Hortic 2011;890:255-60.
45. Devi PS, Thangam M, Desai AR, Adsule PG. Studies on variability in physico-chemical characters of different jamun (*Syzygium cumini*) accessions from Goa. Indian J Hortic 2002;59(2):153-6.
46. Gowri, Shyamala S, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res 2010;2(2):1569-73.
47. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, et al. Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattappalayam, Erode district, Tamil Nadu, South India. Pak J Nutr 2009;8(1):83-5.
48. Ayyanar M, Babu SP. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pac J Trop Biomed 2012;2(3):240-6.
49. Prakash J, Maurya AN, Singh SP. Studies on variability in fruit characters of *Jamun*. Indian J Hortic 2010;67(4):63-6.