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Genetic diversity analysis of *Phyllanthus acidus* Skeels of north-east India: Insights from multivariate analysis

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Phyllanthus **is the largest genera of Phyllanthaceae mostly distributed in tropical and subtropical region of southeast Asia.** ***Phyllanthus acidus*** **(star gooseberry) is one important species of** ***Phyllanthus*** **predominantly found in north-eastern region of India having medicinal significance. The present investigation was focused to assess the genetic variability of 20 accessions of** ***Phyllanthus acidus*** **and to identify the elite types based on various physico-biochemical attributes. The findings underscored a remarkable range of diversity among the accessions. Correlation coefficients unveiled meaningful positive and negative correlations among the traits under scrutiny. Notably, these correlations predominantly manifested fruit quality attributes. Principal component and cluster analysis performed based on 37 physico-biochemical characteristics. The PCA revealed significant portion of the total variability, explaining 86.66% of cumulative variance contribution rate across the initial 5 PCs extracted. The PC was highly contributed by positive loading with fruit characters viz. fruit weight, fruit volume, pulp weight, leaf length, leaf area, leaf perimeter, ascorbic acid, total sugar, total flavonoid and TSS of the fruit. However, negative correlation was observed with duration of flowering, moisture, acidity and seed weight. The accessions were clustered into 3 major clusters using wards method employing Euclidian distance. The cluster I included 6 accessions, the second illustrated 9 accessions and third cluster reflected 5 accessions. The biplot revealed 7 distinct accessions viz. PAS-14, PAS-9, PAS-6, PAS-1, PAS-20, PAS-3, PAS-18 with maximum variability and desired characteristics which can be considered as elite types and potential parent for quality breeding initiatives.**

Keywords *Phyllanthus acidus*, Genetic diversity, Multivariate analysis, Principal component analysis, Cluster analysis, Northeast India

Among the different families of flowering plants, phyllanthaceae is one of the largest family, which consists of 59 genera, 10 tribes, 2 subfamilies and around 2000 species[1]. This family was segregated from another family euphorbiaceae[2,3]. Among the different genera of phyllanthaceae, *Phyllanthus* is one of the largest genera of the Phyllanthaceae family, represented worldwide by some 700 well-known species, mainly distributed in the tropics and subtropics[4]. *Phyllanthus acidus* (star gooseberry) is an annual, erect, branched herb, distributed widely in south-east Asia in a wide variety of soils and climatic conditions without commercial cultivation.

The plant is small to medium, height is below 5 m with a spreading crown. The branches are arranged alternatively and each branch contains around 25–40 leaves. The leaves are bear in clusters, shape of the leaves are either ovate or ovate-lanceolate and the tips of the leaves are pointed. The colour of the leaves is green or dark green. Flowers are small, light pink in colour, and bear in both new and old shoots. Fruits are usually star-shaped with 6 to 8 lobs, colour is greenish yellow when young and light yellow when ripe. The taste of the fruit is usually sour with slight sweetness and contains one stony seed. *Phyllanthus* species have a wide range of medicinal properties such as anti-viral, anti-bacterial, antipyretic, anti-inflammatory, anti-hepatotoxic, anti-oxidant, and analgesic activities[5]. It contains many bioactive compounds including flavonoids, phenolics, tannins, alkaloids, kaempferol, gallic acid, and quercetin and also has good antioxidant properties. In Manipur north-east India, there is a rich diversity of star gooseberry trees and the indigenous people of the state have used the different parts of *Phyllanthus acidus* in the traditional system of medicines. Since there are no standard commercial methods

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of vegetative propagation, the farmers have been propagating the plant from the seeds, and due to this, there is large variability among the existing populations in morphology, plant shape, fruit colour, size of fruits as well as the chemical constituents of the fruits. But till now, there is no systematic research has been conducted to study the genetic diversity in wild populations to identify the elite types with horticulturally important fruit qualities.

In any breeding programme, exploration of available germplasm and identification of suitable genotypes is very crucial. To assist the breeders of *Phyllanthus acidus* fruits of north-east India, this present investigation was aimed to elucidate the phenotypic diversity among the natural populations and to select the elite types which may have very important horticultural characteristuics and may be useful for the breeders in developing new cultivars by using them as one of the parents in the genetic improvement programme. The goals of this study were to characterize and quantify the genetic variability of *Phyllanthus acidus* accessions using principal component and cluster analysis. It will be crucial in developing an effective breeding strategy for genetic improvement. The study aimed to provide theoretical references for selecting elite accessions and to guide the breeders and other stakeholders in mainstreaming the lesser-known fruit of biodiversity hot spots.

Materials and methods

In order to find out the diversity of *Phyllanthus acidus* Skeels in the natural populations in wild and semi-wild stage, and to identify the most promising one from them, this research study was conducted during 2020–2021 at Manipur, north-east India (Fig. 1).

Survey of germplasm

During the fruiting season of 2020–2021, a field survey was conducted in different locations of Manipur, north-east India comprising of 5 districts viz., Imphal east, Imphal west, Kakching, Bishnupur and Thoubal. From the preliminary survey, finally, 20 accessions have been selected in their natural population. Table 1 depicts the various accessions collected from different locations throughout the state of Manipur, north-east India along with their latitude, longitude and elevation. The samples comprised of leaves, flowers and fruits were brought to the post-harvest laboratory of the department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl, Mizoram, India for analysis of their physical parameters, chemical constituents, anti-oxidants and other bioactive compounds present therein (Figs. 2, 3, 4, 5, 6, 7, 8).

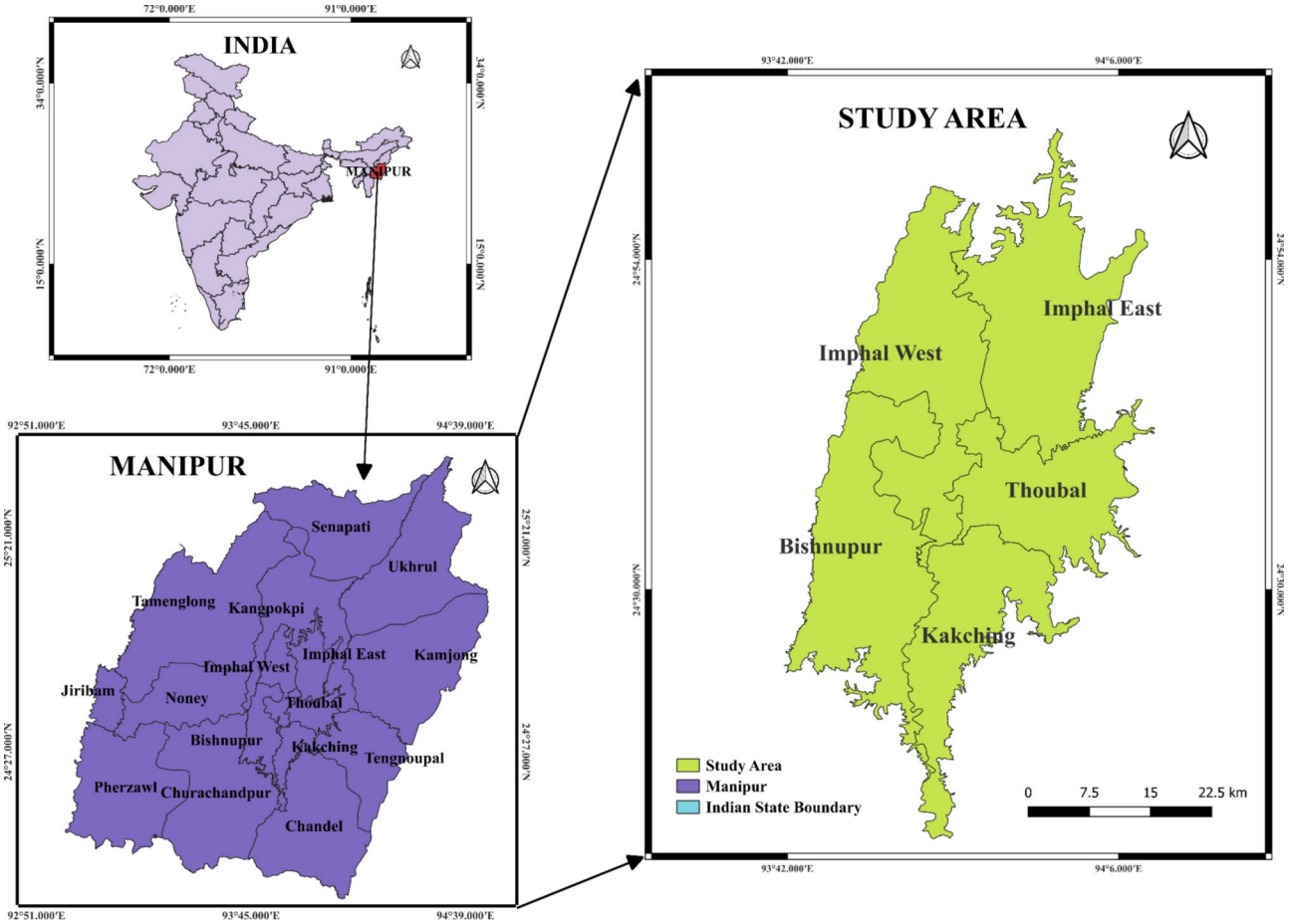

Fig. 1. Map of the experimental site.

Sl. no	Accession Code	Accession	Location	Latitude	Longitude	Elevation amsl (m)
1	PAS-1	MZU-HAMP-PAS-1	Nambol Kakyai	24°40′29.8″N	93°53′16.3″E	803
2	PAS-2	MZU-HAMP-PAS-2	Canchipur Kha Naorem Leikai	24°42′45.2″N	93°52′32.3″E	791
3	PAS-3	MZU-HAMP-PAS-3	Kairang mamang Leikai	24°45′19.4″N	93°56′12.9″E	757
4	PAS-4	MZU-HAMP-PAS-4	Kairang maning Leikai	24°50′15.4″N	93°57′52.4″E	772
5	PAS-5	MZU-HAMP-PAS-5	Khurai	24°50′33.5″N	93°57′33.7″E	782
6	PAS-6	MZU-HAMP-PAS-6	Lamlong	24°50′46.1″N	93°57′20.5″E	785
7	PAS-7	MZU-HAMP-PAS-7	Kairang Awang Leikai	24°50′45.3″N	93°57′20.2E”	771
8	PAS-8	MZU-HAMP-PAS-8	Canchipur Maniur University campus	24°50′15.5″N	93°57′44.8″E	801
9	PAS-9	MZU-HAMP-PAS-9	Haobam marak	24°50′15.5″N	93°57′44.8″E	771
10	PAS-10	MZU-HAMP-PAS-10	Wangkhei	24°46′39.5″N	93°55′50.7″E	764
11	PAS-11	MZU-HAMP-PAS-11	Irengbam Mamang Leikai	23°44′12.1″N	92°39′47.4″E	821
12	PAS-12	MZU-HAMP-PAS-12	Kha Sanjenbam	24°40′42″N	93°52′48″E	822.18
13	PAS-13	MZU-HAMP-PAS-13	Khongman Mangjil	24°46′54.4″N	93°56′55.9″E	730
14	PAS-14	MZU-HAMP-PAS-14	Sanjenthong	24°47′35.5″N	93°56′15.07″E	426
15	PAS-15	MZU-HAMP-PAS-15	Singjamei Chingamathak	24°46′54.28″N	93°56′56.07″E	778.11
16	PAS-16	MZU-HAMP-PAS-16	Keinou Thongkha	24°26′16.2″N	93°48′06.9″E	816
17	PAS-17	MZU-HAMP-PAS-17	Keinou Thongthak	24°26′16.2″N	93°48′06.9″E	815
18	PAS-18	MZU-HAMP-PAS-18	Keinou Maning leikai	24°40′12.8″N	93°47′24.6″E	785
19	PAS-19	MZU-HAMP-PAS-19	Tentha	24°41′38.1″N	93°47′28.2″E	790
20	PAS-20	MZU-HAMP-PAS-20	Kakching Turel Wangma Ningthou Pareng	24°29′53″N	93°58′52″E	787

Table 1. Accessions of *Phyllanthus acidus* Skeels. and their sources.

Fig. 2. *Phyllanthus acidus* tree.

Fig. 3. *Phyllanthus acidus* phyllotaxy.

Fig. 4. *Phyllanthus acidus* inflorescence.

Fig. 5. *Phyllanthus acidus* leaves.

Fig. 6. *Phyllanthus acidus* fruits on tree.

Morphological characterization

From each location, randomly 20 numbers of samples were collected for analysis of the morphological characteristics of the leaves, flowers, and fruits. Standard protocols were followed for the estimation of morphological parameters. For measurement of the length and breadth of the leaves, inflorescence, flowers, fruits and seeds, digital vernier callipers were used. Systronics-211 leaf area meter (CID Bioscience, USA) was used for measuring the area of the leaves.

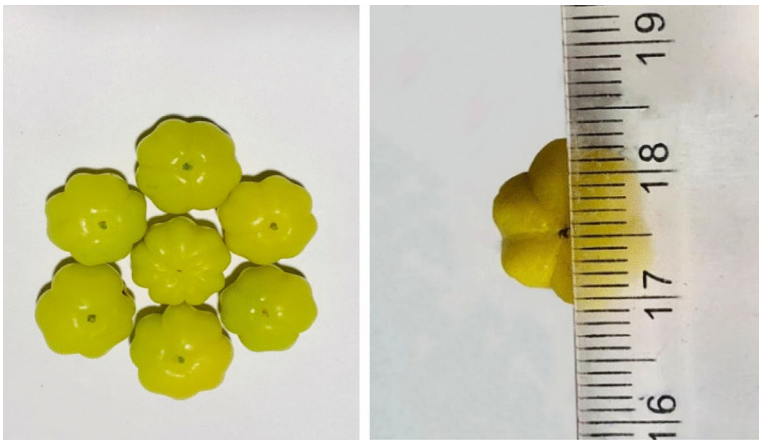

Fig. 7. *Phyllanthus acidus* fruit.

Fig. 8. *Phyllanthus acidus* cross-section.

Biochemical characterization

For analyzing the biochemical characteristics of the ripened fruits of *Phyllanthus acidus*, the standard protocols were used. The total soluble solids were measured by using a Zeiss hand refractometer. AOAC[6] standard protocols were followed for measuring the titratable acidity, reducing, non-reducing and total sugars of the fruit juices. The carbohydrate content of the fruits was estimated by using Anthrone's reagent, while protein content was estimated by using Lowry's method. Folin Ciocalteau standard protocols were followed for the estimation of phenols in the fruits. UV VIS spectrophotometer was used for estimation of the total chlorophyll present in the leaves of the plant. Molyneux[7] standard protocol was used for estimation of the antioxidant activity of the fruits by the free radical scavenging ability of DPPH (1, 1-diphenyl-2- 95 picrylhydrazyl).

Data analysis

One-way analysis of variance (ANOVA) was made to determine the significance of the means of average data in each replication by using Duncan's multiple range test (DMRT) using the software OPSTAT. Principal component analysis (PCA) was performed to determine the interrelations among the accessions using Origin 2024 learner's edition. The open-source Geographic Information System (GIS) software, QGIS version 3.38.3, was utilized to plot geographical coordinates and construct the study area map.

Results and discussion

Physical characteristics

The physical characteristics of the leaves, flowers, fruits and seeds of *Phyllanthus acidus* are depicted in Tables 3 and 4 respectively with significance at $P < 0.05$. From the data presented in Table 3, it is observed that different leaf morphological characters showed significant variation across the accessions. As depicted in Table 2, among the different accessions of *Phyllanthus acidus*, the leaf length ranged between 4.07 and 5.27 cm with a coefficient of variation (CV) and standard deviation (SD) of 7.65 and 0.37 respectively. Among all the accessions, PAS-9 recorded the maximum value for leaf length (5.27 cm). Similarly, the highest leaf breadth was recorded in PAS-14

Characters	Abbreviation	Min	Max	Mean	SD	CV
Leaf length (cm)	LL	4.07	5.27	4.78	0.37	7.65
Leaf breadth (cm)	LB	2.08	2.60	2.34	0.16	6.65
Leaf area (cm^2)	LA	6.33	10.89	8.48	1.35	15.96
Leaf perimeter (cm)	LP	5.22	9.39	6.86	1.36	19.75
Total chlorophyll (mg g^{-1})	TC	1.06	1.67	1.30	0.20	15.61
Duration of 50% flowering (days)	DoF_{50}	7.33	14.67	11.19	2.41	21.51
Duration of flowering (days)	DoF	23.67	33.67	30.02	3.38	11.26
Flower length (cm)	FoL	0.15	0.25	0.19	0.03	14.30
Flower breadth (cm)	FoB	0.40	0.56	0.47	0.04	9.42
Fruit weight (g)	FrW	3.27	5.12	3.93	0.59	14.96
Specific gravity (g/cc)	SP	0.88	0.97	0.93	0.02	2.24
Fruit diameter (mm)	FrD	20.21	24.75	21.66	1.30	6.01
Fruit length (mm)	FrL	15.06	19.28	16.31	1.13	6.95
Fruit volume (cc)	FrV	3.68	5.40	4.21	0.55	12.94
Pulp weight (g)	PW	2.81	4.64	3.36	0.59	17.48
Edible portion (%)	EP	83.22	90.51	85.34	2.05	2.40
Seed length (mm)	SL	5.28	6.82	6.00	0.43	7.23
Seed diameter (mm)	SD	6.65	8.22	7.35	0.45	6.15
Seed weight (g)	SW	0.49	0.63	0.57	0.04	6.76
Seed volume (cc)	SV	0.85	1.03	0.92	0.06	6.05
Pulp: seed ratio	PSR	4.97	9.55	5.97	1.27	21.32
Moisture (%)	Mo	81.13	91.65	86.05	3.34	3.88
Juice (%)	Ju	25.25	46.11	34.32	7.12	20.75
TSS (%)	-	6.76	9.26	7.54	0.78	10.39
Acidity (%)	Ac	1.20	1.81	1.54	0.19	12.12
Ascorbic Acid (mg100 g^{-1})	AA	29.30	44.70	37.24	4.62	12.40
Total sugars (%)	TS	3.45	5.37	4.47	0.56	12.58
Reducing sugars (%)	RS	2.45	4.01	3.37	0.44	13.05
Non-reducing sugars (%)	NRS	1.04	1.75	1.26	0.20	15.79
Sugar: acid ratio	SAR	2.00	4.34	2.98	0.70	23.31
TSS: acid ratio	TAR	4.01	7.59	5.03	1.15	22.84
Total Carbohydrates (g 100 g^{-1})	TCHO	5.07	6.59	5.78	0.52	9.01
Total Protein (g 100 g^{-1})	TPr	1.55	3.57	2.66	0.54	20.34
Carotenoids (µg 100 g^{-1})	Crtds	2111.96	2488.35	2264.78	111.68	4.93
Total phenolics (mg GAE g^{-1})	TPh	25.30	34.82	29.95	2.66	8.89
Total flavonoids (mg GAE g^{-1})	TFn	13.94	24.68	18.76	3.75	19.96
DPPH (% inhibition)	DPPH	75.52	90.54	84.20	5.12	6.08

Table 2. Descriptive statistics of quantitative traits of *P. acidus* Skeels accessions.

(2.60 cm) and the lowest was in PAS-16 (2.08 cm) with SD of 0.16 and a moderate CV of 6.65. The leaf perimeter ranged from 5.22 (PAS-10) to 9.39 cm (PAS-14) with an average of 6.86 cm and greater CV of 19.75 (Table 2).

Chlorophyll, often associated primarily with photosynthesis and overshadowed by yellow/orange carotenoid pigments, may have significant but overlooked physiological effects in disease prevention[8]. Abundant in green fruits and vegetables that are part of our diet, chlorophylls and their derivatives show therapeutic potential, offering antioxidant, antimutagenic, anti-cancer, and anti-obesogenic benefits[9]. A study by Zhuo et al.[10] revealed that chlorophylline 6–mediated PDT induces apoptosis in human bladder cancer cells, possibly by inhibiting superoxide dismutase activity and generating reactive oxygen species. In our present investigation, the highest total chlorophyll was recorded in PAS-6 (1.67 mg g^{-1}) followed by PAS-9 (1.65 mg g^{-1}), PAS-14 (1.61 mg g^{-1}), and PAS-20 (1.56 mg g^{-1}), while the lowest was recorded in PAS-10 (1.06 mg g^{-1}) (Table 3). The variation in leaf characters among the accession may be due to several factors which include differences in the age of the plants, variation in the nutrient availability and differences in the agro-climatic conditions in the location where it grown. Our results are in close conformity with the studies of Murali[11] who reported that the variation in leaf characters could have been a result of differences in the environment. In addition, the amount of nutrient availability, light intensity perceived, and amount of water absorption are different in each environment and may also impact the morphological parameters of the plant[11].

Floral qualities are distinctive to each accession and can serve as important morphological markers in breeding. The duration of 50% flowering was observed in the range of 7.33 to 14.67 days, while the duration of flowering was in the range of 23.67 to 33.67 days (Table 3) with the CV value of 21.51 and 11.26 respectively.

Accessions	LL	LB	LA	LP	TC	DoF50	DoF	FoL	FoB
PAS-1	5.15a	2.5abc	10.03abc	8.21ab	1.51ab	7.33g	24h	0.21abcde	0.53a
PAS-2	4.57defg	2.37bcdef	8.08defg	6.06def	1.11fghi	10.33de	29.67de	0.17cde	0.47bc
PAS-3	4.47efgh	2.3cdefg	7.51defgh	5.66ef	1.14efghi	12.67bc	33.67a	0.2abcde	0.45cd
PAS-4	4.4fgh	2.55ab	8.48def	6.66cde	1.32cd	11.17cd	30.67cd	0.19abcde	0.44cd
PAS-5	4.9abcd	2.2efgh	8.06defg	6.32cdef	1.08hi	12.33bc	32abc	0.2abcde	0.43cd
PAS-6	5.13ab	2.45abcd	10.27ab	8.41ab	1.67a	8.67fg	23.67h	0.23abc	0.54a
PAS-7	4.47efgh	2.13gh	7.17efgh	5.46ef	1.29cde	14.67a	33a	0.2abcde	0.46bcd
PAS-8	5.03abc	2.18fgh	8.07defg	6.36cdef	1.08hi	13.67ab	33.67a	0.17cde	0.42cd
PAS-9	5.27a	2.47abcd	10.47a	9.33a	1.65a	9efg	25.67gh	0.24ab	0.56a
PAS-10	4.07h	2.19efgh	6.33h	5.22f	1.06i	13.33ab	32.67ab	0.16de	0.46bcd
PAS-11	4.47efgh	2.31cdefg	6.84gh	5.67ef	1.17defghi	8g	28.67ef	0.15e	0.45cd
PAS-12	5.23a	2.22efgh	8.75cde	6.97cd	1.23defghi	12.67bc	33a	0.16de	0.4d
PAS-13	4.93abcd	2.27defgh	8.38defg	6.7cde	1.4bc	13.67ab	33.67a	0.19abcde	0.45cd
PAS-14	5.19a	2.6a	10.89a	9.39a	1.61a	8.67fg	25.33gh	0.22abcd	0.55a
PAS-15	4.55defg	2.21efgh	6.98fgh	5.37f	1.28cdef	9.67def	31bcd	0.18bcde	0.45cd
PAS-16	4.67cdef	2.08h	7.34defgh	5.7ef	1.1ghi	10.33de	29.77de	0.19abcde	0.46bcd
PAS-17	4.2gh	2.4abcde	7.74defgh	6.23cdef	1.16defghi	13b	33a	0.17cde	0.46bcd
PAS-18	4.75bcdef	2.43abcd	8.88bcd	7.37bc	1.25cdefgh	14.67a	28ef	0.17cde	0.46bcd
PAS-19	4.87abcde	2.28defgh	8.62cde	7.01cd	1.27cdefg	12.33bc	32.33abc	0.19abcde	0.47bc
PAS-20	5.2a	2.58a	10.61a	9.13a	1.56a	7.67g	27fg	0.25a	0.52ab
S.Em (±)	0.17	0.09	0.68	0.54	0.07	0.70	0.82	0.01	0.03
$CD_{0.05}$	0.35	0.19	1.43	1.12	0.15	1.47	1.71	0.03	0.06

Table 3. Leaf and Flower physical characteristics of the *P. acidus* Skeels accessions. Values with same letter within each column demonstrate a lack of significant difference as determined by DMRT ($p \leq 0.05$).

Barua[12] reported that the variation in the time of flowering may be due to the difference in soil nutrient status and changes in climatic conditions especially temperature and rainfall patterns. The flower length ranged between 0.15–0.25 cm and among all the accessions PAS-20 recorded the maximum flower length (0.25 cm) while PAS-11 recorded the minimum (0.15 cm) with CV of 14.30 (Table 2). Similarly, flower breadth ranged between 0.40 and 0.56 cm having a mean of 0.47 cm with SD 0.04 and CV of 9.42. Among all the accessions, PAS-9 recorded the highest (0.56 cm) and PAS-12 recorded the lowest (0.40 cm). Our study is in line of conformity with the studies of Barua[12] who also reported similar results for flower length and breadth in *Phyllanthus acidus*.

The quality of fruit is a multifaceted character that relies on several factors. Table 4 shows the significant variation among the fruit's physical parameters. Among the studied accessions of *Phyllanthus acidus,* the fruit weight ranged from 3.27 to 5.12 g with SD of 0.59 and CV 14.96 (Table 2) and PAS-20 recorded the significantly maximum value (5.12 g) for fruit weight, but it was statistically *at par* with PAS-1 (5.10 g) and PAS-6 (4.87 g). Among the various factors responsible for the increase of fruit weight, the biogenesis of naturally occurring growth-promoting substances viz. auxins, gibberellins and cytokinins plays a profound role[13]. The rich variation in fruit weight could be due to the highly heterozygous and diverse genetic backgrounds of parents. Since all the plants are of seed origin, there might be differences in the genetic makeup of the plants, which might have contributed the variations in fruit weight among the genotypes. In our study, the maximum weight in PAS-20 might be due to more synthesis of growth-promoting substances in the fruits of this accession. Similarly, among the accessions of *Phyllanthus acidus,* PAS-20 recorded the statistically maximum value for fruit length (19.28 mm) and fruit diameter (24.75 mm), while, PAS-16 recorded the minimum value for fruit length (15.06 mm) and fruit diameter (20.21 mm). The variation in fruit length and diameter may be attributed to differences in genetic features of the individual genotypes and soil and climatic conditions. Hazariks et al.[14,15] and Rozar et al.[16] in their study also observed statistical differences in fruit morphological characters among several accessions. It is observed from the data presented in Tables 2 and 4 that among the different accessions of *Phyllanthus acidus,* there were significant differences in the volume of the fruits. The fruit volume ranged between 3.68 and 5.40 cc with SD and CV values of 0.55 and 12.94 respectively. Among all the accessions, the maximum fruit volume was observed in PAS-20 (5.40 cc), while the minimum was recorded in PAS-15 (3.68 cc). The variation in fruit volume among star gooseberry genotypes may be due to differences in their genetic makeup and prevailing agro-climatic conditions, i.e. nutrients, soil, light, water and altitude under which the plants are growing. Singh et al.[17], Hazarika et al.[18] and Singh and Singh[19] also reported variation in fruit volume among aonla genotypes from north-east India. Our study is in line of conformity with the findings of Hazarika and Ngurthankhumi[20] in *Phyllanthus acidus* from north-east India. The accessions did not differ significantly with respect to the specific gravity of the fruits. However, among all the accessions, PAS-6 was recorded as highest (0.97 g/cc) and PAS-16 recorded the lowest value (0.88 g/cc). The pulp weight ranged between 2.81 and 4.64 g with SD and CV of 0.59 and 17.48 respectively (Table 2), which confirmed the presence of higher variation in the trait. Among the studied accessions, PAS-20 recorded the maximum value for pulp weight (4.64 g), while, the lowest was observed in PAS-16 (2.81 g). The accessions varied significantly for the edible portions and it ranged between 83.22 and

Accessions	FrW	SP	FrD	FrL	FrV	PW	EP	SL	SD	SW	SV	PSR
PAS-1	5.10a	0.96	23.68b	18.39b	5.33a	4.59a	90.07a	6.78ab	8.15ab	0.51fg	0.99abc	9.07a
PAS-2	3.77cd	0.93	20.62def	16.4c	4.07cd	3.21d	85.02cdefg	5.7ghij	7.05efg	0.57abcdef	0.86f	5.63efg
PAS-3	3.67cde	0.94	20.9def	15.8cde	3.9cde	3.13de	85.25cdef	5.97efghi	7.18defg	0.54defg	0.9def	5.8def
PAS-4	3.61cdef	0.93	21.26cde	16.29cd	3.91cde	3.01def	83.36g	5.82efghij	7.22defg	0.6abcd	0.92cdef	5.01g
PAS-5	3.65cde	0.91	21.07cde	15.81cde	4.03cd	3.13de	85.76cde	6.01defghi	7.51cde	0.52efg	0.89def	6.01cde
PAS-6	4.87ab	0.97	23.51b	17.71b	5.02b	4.27b	87.68b	6.5abcd	7.83abc	0.6abcd	1.03a	7.17b
PAS-7	3.86c	0.93	21.37cd	15.87cde	4.13c	3.27d	84.91cdefg	6.14cdefg	7.34cdef	0.62ab	0.96abcde	5.28fg
PAS-8	3.62cdef	0.92	23.24b	16.29cd	3.93cde	3.04def	83.98fg	6.32bcde	7.33cdef	0.58abcde	0.92cdef	5.26fg
PAS-9	4.73b	0.96	23.46b	17.79b	4.91b	4.1bc	86.61bc	6.23cdef	7.67bcd	0.63a	1.01ab	6.47c
PAS-10	3.58cdef	0.92	21def	15.71cde	3.87cde	3.02def	84.27efg	5.69ghij	7.19defg	0.56bcdef	0.93bcdef	5.37efg
PAS-11	3.67cde	0.94	21.29cde	16.04cde	3.9cde	3.12de	85.01cdefg	6.11cdefgh	7.5cde	0.55cdefg	0.88ef	5.68defg
PAS-12	3.52cdef	0.92	21.02def	15.34de	3.81cde	2.98def	84.81defg	5.69ghij	6.88fg	0.54defg	0.85f	5.54efg
PAS-13	3.66cde	0.94	21.15cde	15.69cde	3.9cde	3.06def	83.74fg	5.78fghij	7.24def	0.61abc	0.87f	5.02g
PAS-14	4.55b	0.95	21.86c	16.47c	4.81b	3.93c	86.32bcd	6.6abc	8.1ab	0.62ab	0.97abcd	6.34cd
PAS-15	3.39ef	0.92	20.47ef	15.11e	3.68e	2.82f	83.28g	5.28j	6.65g	0.57abcdef	0.86f	5g
PAS-16	3.27f	0.88	20.21f	15.06e	3.82cde	2.81f	83.93fg	5.49ij	6.66g	0.54defg	0.87f	5.22fg
PAS-17	3.73cde	0.93	20.76def	15.57cde	4.02cd	3.16de	84.85defg	5.6hij	7.22defg	0.56bcdef	0.88ef	5.64efg
PAS-18	3.46def	0.91	20.56def	15.26e	3.79de	2.87ef	83.22g	5.6hij	7.03efg	0.58abcde	0.87f	4.97g
PAS-19	3.68cde	0.92	21.05def	16.41c	4.02cd	3.1def	84.28efg	5.81efghij	7.09efg	0.58abcde	0.9def	5.38efg
PAS-20	5.12a	0.95	24.75a	19.28a	5.4a	4.64a	90.51a	6.82a	8.22a	0.49g	0.99abc	9.55a
S.Em (±)	0.15	-	0.34	0.43	0.13	0.13	0.73	0.21	0.26	0.02	0.04	0.30
$CD_{0.05}$	0.32	NS	0.71	0.90	0.28	0.26	1.53	0.45	0.55	0.05	0.09	0.64

Table 4. Fruit and Seed physical characteristics of the *P. acidus* Skeels accessions. Values with same letter within each column demonstrate a lack of significant difference as determined by DMRT ($p \leq 0.05$).

90.51% with SD and CV of 2.05 and 2.40 respectively. Among all the accessions, the maximum edible portion was observed in PAS-20 (90.51%), while the lowest was recorded in PAS-18 (83.22%). Our study is in line of conformity with the studies of Rozar et al.[16] where they reported variation in edible portion percentage among a number of aonla accessions from north-east India.

There was significant variation among the accessions with respect to seed parameters. The seed length ranged between 5.28–6.82 mm with SD and CV of 0.43 and 7.23 respectively. Among all the accessions, the statistically maximum seed length was recorded in PAS-20 (6.82 mm) and the minimum was in PAS-15 (5.28 mm) (Table 2). The seed diameter ranged between 6.65 and 8.22 mm with SD and CV of 0.45 and 6.15. Our study is in line of conformity with the studies of Barua[12] who also reported similar results for the seed length of *Phyllanthus acidus*. Similar to our results, Hazarika et al.[18] and Sharma[21] also reported seed diameter of aonla in the range of 7.50–11.00 mm, and 9.27–12.34 mm respectively. The data presented in Table 4 depicts that the seed weight ranged between 0.49 and 0.63 g and the maximum was recorded in PAS-9 (0.63 g), but it was statistically *at par* with PAS-7 (0.62 g) and PAS-14 (0.62 g), while the minimum was recorded in PAS-20 (0.49 g). For an ideal variety, lower weight and small size of seed are the desirable characters. These observations revealed a positive correlation among pulp weight, seed weight and fruit weight. The genotypes produced higher fruit weight may be due to higher pulp weight and less seed weight. This clearly indicated that, during the selection of any genotype based on fruit, the breeder should emphasize on fruit pulp content rather than fruit weight alone[20]. The seed volume ranged from 0.85 to 1.03 cc with SD and CV of 0.06 and 6.05 respectively. The pulp: seed ratio ranged between 4.97–9.55 and exhibited higher variability with CV value of 21.32 and among all the accessions, PAS-20 recorded the significantly highest value (9.55) and PAS-18 recorded the lowest (4.97). The Pulp-to-stone ratio is a vital factor in identifying a superior genotype by breeders. These results align with previous studies conducted by Hazarika and Laltluangkimi[22] and Chandra et al.[23].

Biochemical characteristics

It is obvious from the data presented in Table 5 that *Phyllanthus acidus* accessions varied significantly with respect to biochemical parameters of the fruits. The moisture content ranged between 81.13 and 91.65% with coefficient of variation of (3.88) (Table 2). Among all the accessions, the significantly maximum moisture content was recorded in PAS-10 (91.65%), while the lowest was in PAS-20 (81.13%). The variation in moisture content may be due to the fact that all accessions have grown in different soil and climatic conditions, having variations in stage of maturity and time of harvest which as a whole impacted the moisture content of the fruits. Our study is in line of agreement with the study of Jahan et al.[24] who reported that the variation in the moisture content among the accessions might be due to several factors such as the rainfall received,available soil moisture level as well as the type of the soil which together greatly influence the quantity of moisture present in the fruits. The data presented in Table 5 revealed that there was significant variation among the accessions with respect to juice content of the fruits. The juice content of the fruits ranged between 25.25–46.11% with SD and CV of 7.12 and 20.75 respectively. The highest juice per cent was recorded in PAS-1 (46.11%), while, the lowest was recorded in

Accessions	Mo	Ju	TSS	Ac	AA	TS	RS	NRS	SAR	TAR	TCHO	TPr	Crtds	TPh	TFn	DPPH
PAS-1	82.83ghi	46.11a	8.89a	1.46cdefgh	42.62a	5.37a	3.81abc	1.75	3.68abc	6.08c	6.33ab	3.45a	2488.35a	34.67a	24.44a	90.54a
PAS-2	84.62fgh	26.62e	7.17c	1.68abcd	35.9def	4.19efg	3.25fghi	1.10	2.49ef	4.26de	6.06bc	2.24e	2111.96k	30.17def	15.06fg	77.34ij
PAS-3	87.48cdef	40.15abc	7.27c	1.73abc	29.3i	3.45h	2.45l	1.12	2f	4.21de	5.62cde	2.44de	2236.12fgh	27.39fgh	15.99efg	86.72cde
PAS-4	85.93efg	31.49de	7.1c	1.76ab	34.39efg	4.38cdef	3.36efg	1.19	2.49ef	4.04de	5.61cdef	1.6f	2265.52fg	27.25gh	14.55fg	79.77hi
PAS-5	83.02ghi	36.39cd	7.37c	1.73abc	36.99cde	4.43cdef	3.5cdef	1.10	2.56ef	4.28de	6.07bc	1.55f	2134.01jk	27.56fgh	13.94g	82.03gh
PAS-6	82.14hi	42.41abc	9.26a	1.28fgh	44.3a	5.17ab	3.73abcd	1.63	4.04ab	7.24a	6.59a	3.57a	2363.9cd	32.2abcd	23.23ab	90.49ab
PAS-7	90.86ab	28.03e	7.08c	1.57abcde	36.13cdef	4.46cdef	3.4efg	1.23	2.9de	4.59de	6.1abc	2.37de	2188.77hij	29.04efg	17.53def	87.9abcde
PAS-8	83.4ghi	27.57e	7.23c	1.43defgh	36.99cde	4.84abcd	3.62bcde	1.40	3.4bcd	5.1d	6.03bc	2.45de	2255.16fg	30.43cde	15.17fg	80.45h
PAS-9	82.86ghi	42.92ab	8.97a	1.2h	44.7a	4.92abc	3.95a	1.17	4.18a	7.59a	6.54ab	3.33ab	2367.67cd	33.49ab	24.19a	89.02abcd
PAS-10	91.65a	39.94abc	7.33c	1.81a	31.39hi	4.59cdef	3.57cdef	1.20	2.56ef	4.07de	5.18def	2.7cde	2127.2jk	29.34efg	17.02efg	89.3abc
PAS-11	87.29def	37.81bc	7.42c	1.54abcdef	34.69efg	3.67gh	2.74kl	1.06	2.39ef	4.83de	5.7cd	2.88bcd	2268.42efg	27.52fgh	18.71cde	87.63bcde
PAS-12	88.35bcde	29.19e	6.88c	1.73abc	38.13bcd	4.31cdef	3.25fgh	1.22	2.52ef	4.01e	5.45def	2.68cde	2171.93hijk	31.22bcde	16.14efg	81.55gh
PAS-13	85.72efg	29.01e	7.32c	1.53abcdef	33.45fgh	3.5h	2.58l	1.04	2.3ef	4.78de	5.24def	2.83cd	2213.09ghi	28.78efg	14.95fg	76.56j
PAS-14	82.05hi	43.75ab	8.67ab	1.23gh	44.23a	5.35a	4.01a	1.54	4.34a	7.04ab	6.5ab	3.37a	2471.45ab	34.82a	24.68a	88.37abcde
PAS-15	85.69efg	29.89e	6.76c	1.53abcdef	32.9gh	4.23defg	3.29efg	1.10	2.86de	4.51de	5.07f	2.68cde	2187.59hij	28.4efg	20.9bc	85.98ef
PAS-16	90.41abc	26.45e	6.79c	1.45cdefgh	39.67b	4.35cdef	3.11ghij	1.39	3.01cde	4.68de	5.5def	2.31e	2262fg	27.46fgh	22.91ab	76.21j
PAS-17	90.19abcd	30.13e	7.14c	1.5bcdefg	38.61bc	3.98fgh	2.94h jk	1.19	2.67def	4.78de	5.1ef	2.54de	2151.45ijk	25.3h	19.91cd	83.52fg
PAS-18	90.15abcd	29.06e	6.93c	1.62abcd	34.22fg	4.61bcde	3.53cdef	1.26	2.86de	4.28de	5.31def	2.56de	2284.69ef	31.11bcde	16.55efg	75.52j
PAS-19	85.25fg	25.25e	7.12c	1.77ab	32.93gh	4.41cdef	3.46def	1.12	2.49ef	4.03e	5.2def	2.58de	2329.42de	29.73def	16.6efg	86.2de
PAS-20	81.13i	44.22ab	8.17b	1.32efgh	43.34a	5.18ab	3.92ab	1.46	3.94ab	6.22bc	6.37ab	3.14abc	2416.79bc	33.05abc	22.75ab	88.85abcd
S.Em (±)	1.33	2.80	0.31	0.12	1.19	0.25	0.14	-	0.33	0.43	0.21	0.21	30.92	1.18	1.27	1.22
CD0.05	2.77	5.83	0.64	0.25	2.48	0.53	0.29	NS	0.68	0.90	0.45	0.44	64.50	2.47	2.65	2.54

Table 5. Biochemical characteristics of the *P. acidus* Skeels accessions. Values with same letter within each column demonstrate a lack of significant difference as determined by DMRT ($p \leq 0.05$).

PAS-19 (25.25%). The rich variation in moisture and juice could also be due to highly heterozygous and diverse genetic backgrounds of the parents[20].

Total soluble solids in fruits are critical for evaluating ripeness, quality, flavour, nutrition, and suitability for processing. TSS monitoring ensures that fruits and their products align with consumer expectations and industry standards[25]. Among all the accessions, the highest TSS (%) was recorded in PAS-6 (9.26%) and the lowest was in PAS-15 (6.76%). Breeders should also prioritize selecting elite varieties based on the TSS content of the fruits as it acts as the major factor in organoleptic acceptability. Singh et al.[17], Hazarika et al.[18], Sharma[21] and Rozar et al.[16] observed differences in TSS among aonla genotypes from north-east India. Mishra et al.[13] also reported that with the advancement of fruit maturity, there is an increase in the TSS content of the fruits due to the breakdown of polysaccharides and the formation of monosaccharides and simple sugars. The variation in TSS among the genotypes may be due to different genetic make-up of the individual genotypes and agro-climatic conditions. The fruits growing in arid regions with limited availability of water tend to accumulate more, and thus had the higher TSS in fruits[26], as also observed in the present study. The breeders during the selection of superior genotypes should emphasize total soluble solids content of the fruit.

The titratable acidity in fruits enhances their flavour, aids in preservation, boosts nutritional value, and potentially offers health benefits. This characteristic defines their culinary appeal, making them valuable in diverse cuisines and traditional remedies[25]. Titratable acidity exhibited significant variability in the range of 1.2–1.81% with a moderate CV of 12.12 (Table 2). Our results are in close conformity with the studies of Shukla et al.[27] who also reported variation in acidity among different germplasm of aonla. In our study, since all *Phyllanthus acidus* accessions have been grown in different agro-climatic conditions there might be variation in titratable acidity among the accessions. Mishra et al.[13] reported that the accessions, where there is higher synthesis of organic acids always have higher titratable acidity, and the accessions where there is more bioconversion of organic acids to sugars always have lower in titratable acidity. Our study is in line of conformity with the studies of Rozar et al.[16] where they reported variation in titratable acidity among several aonla accessions from north-east India.

Vitamin C plays a pivotal role in maintaining overall health and well-being due to its diverse range of benefits. With its antioxidant properties, immune-boosting effects, involvement in collagen formation, and various other functions, it is a vital nutrient that significantly contributes to the myriad health advantages associated with the consumption of fruits[25]. It is noteworthy that high-dose consumption of vitamin C is associated with reduced risks of developing cancers in the oral cavity, stomach, oesophagus, pancreas, cervix, breast, and rectum[28]. Additionally, high-dose vitamin C has the potential to alleviate cancer-related pain and enhance the overall quality of life for cancer patients[29]. The significantly highest ascorbic acid was observed in PAS-9 (44.70 mg/100 g) and the lowest was in PAS-3 (29.3 mg/100 g). The accessions may vary in their ascorbic acid content due to genetic differences among them. The accessions where there is more supply of hexose sugars in photosynthetic activity may always attributed to higher ascorbic acids[13]. It is a fact that, if TSS increases, the ascorbic acid also increases because the precursor of ascorbic acid is glucose- 6-phosphate[30], which was also confirmed in our study. Our study is in line of conformity with the studies of Rozar et al.[16] where they reported variation in ascorbic acid content among a number of aonla accessions from north-east India.

Significant variation was exhibited among the accessions with respect to total sugars of the fruits. The total sugars of the fruits ranged between 3.45–5.37% with SD and CV of 0.56 and 12.58 respectively (Table 2). The significantly highest total sugars were recorded in PAS-1(5.37%), while it was lowest in PAS-3(3.45%). Hazarika et al.[18] and Singh et al.[17] also reported variation in total sugars of aonla accessions in the range of 5.57–12.15 and 7.94–13.15% respectively. The reducing sugar of fruits ranged from 2.45 to 4.01% (Table 5) with a higher variability of CV 13.05. Among all the accessions, PAS-14 and PAS-3 recorded the highest (4.01%) and lowest values (2.45%) for reducing sugars respectively. Sharma[21] also reported variations in reducing sugars. There was no significant variation among the accessions with respect to non-reducing sugars. However, among all the accessions, PAS-13 and PAS-1 recorded the highest (1.75%) and lowest value for non-reducing sugars (1.04%). Our study is in line of conformity with the studies of Barua[12] who also reported similar values of non-reducing sugars in *Phyllanthus acidus*. Significant variability was exhibited among the accessions for sugar: acid ratio and TSS: acid ratio of the fruits and ranged between 2.00–4.34 and 4.01–7.59 with CV of 23.31% and 22.84% respectively (Table 2). Among all the accessions, PAS-14 exhibited the highest sugar: acid ratio (4.34) and PAS-9 recorded the highest TSS: acid ratio (7.59) (Table 5). Debbarma and Hazarika[31],Singh et al.[17] and Singh and Singh[19], reported significant variations in sugar: acid ratio and TSS: acid ratio among different accessions of bael and aonla from north-east India.

The carbohydrate content found in fruits serves multiple vital functions, including providing energy, supporting digestive well-being, managing blood sugar levels, and facilitating nutrient absorption. This component is a cornerstone of a well-rounded diet, enhancing both the overall nutritional profile and culinary versatility of fruits[25]. The data presented in Table 5 revealed the variation in total carbohydrates among the *Phyllanthus acidus* accessions. The total carbohydrates of the accessions ranged between 5.07–6.59 g 100 g^{-1} with the SD and CV values of 0.52 and 9.01 respectively. Among the studied accessions, the highest value was recorded in PAS-6 (6.59 g 100 g^{-1}) while the lowest was in PAS-15 (5.05 g 100 g^{-1}). Suriyavathana and Subha[32] and Barua[12] also reported similar results for the carbohydrate content of *Phyllanthus acidus* fruits.

Proteins serve as fundamental structural and functional elements within all living cells. Approximately half of the protein content within our body is found in muscle tissue, with the remainder distributed among bones, cartilage, and skin[33]. Similarly, the protein content of the accessions ranged between 1.55–3.57 g 100 g^{-1} with the SD and CV values of 0.54 and 20.34 respectively. Among all the accessions, the significantly highest total protein content was recorded in PAS-6 (3.57 g 100 g^{-1}), while it was lowest in PAS-5 (1.55 g 100 g^{-1}). Our results are in line of conformity with the findings of Rozar et al.[16] where they reported variation in protein content among a number of aonla accessions from north-east India.

Carotenoids have demonstrated their efficacy in both preventing and treating various diseases, owing to their non-toxic properties, as highlighted by Zare et al.[34]. Numerous studies have proposed that carotenoids can exert their anti-cancer effects through a range of mechanisms. These include acting as antioxidants and pro-oxidants, mitigating inflammation, inhibiting angiogenesis, modulating the immune system, promoting cell differentiation, and curbing cell proliferation[35,36]. The carotenoid content of the fruits varied significantly among the accessions and it ranged between 2111.94–2488.35 µg 100 g^{-1}. Among all the accessions, PAS-1 and PAS-2 exhibited the highest (2488.35 µg 100 g^{-1}) and lowest values respectively (2111.96 µg 100 g^{-1}). Fitriansyah[37] reported that the strong yellow to orange colour of aonla fruits is due to the presence of carotenoids.

Among the studied accessions, the total phenols ranged between 25.30 and 34.82 mg GAE g^{-1} with a moderate CV of 8.89. Among all the accessions, PAS-14 exhibited the highest (34.82 mg GAE g^{-1}) and PAS-17 recorded the lowest value (25.30 mg GAE g^{-1}) for total phenols. The variation in the total phenol content of the *Phyllanthus acidus* fruits may be due to differences in ripening stage and agro-climatic conditions. Our results are in line of conformity with the findings of Rozar et al.[16] and Barua[12] where they reported variation in protein content among several aonla and *Phyllanthus acidus* accessions from north-east India.

The data displayed in Table 5 revealed that the accessions varied significantly with respect to total flavonoids of the fruits. The flavonoids of the fruits ranged between 13.94–24.68 mg GAE g^{-1} with SD and CV values of 3.75 and 19.96 respectively. Among all the accessions, the significantly highest flavonoids was recorded in PAS-14 (24.68 mg GAE g^{-1}), while it was lowest in PAS-5 (13.94 mg GAE g^{-1}). Our study is in line of conformity with the findings of Foyzun et al.[38] who reported similar results in total flavonoid content of *Phyllanthus acidus* fruits. The highest DPPH antioxidant activity of fruits was recorded in PAS-1 (90.54%) and the lowest was in PAS-18 (75.52%). Pradeep et al.[39] reported that fruit extract of *Phyllanthus acidus* exhibited more than 90% of inhibition. Our results are in line of conformity with the studies of[32] who reported that the DPPH inhibition percentage ranged from 87.89 per cent to 90.78 per cent among *Phyllanthus* genotypes and can be recommended as a source of natural antioxidants to fight against vulnerable diseases.

Correlation among different physico-biochemical characters

The information about the important characters among several accessions can be obtained by analyzing the correlation coefficient. Pearson correlation analysis revealed substantial ($p < 0.05$) positive and negative relationships between the characters. The results of the correlation analysis between different physico-biochemical parameters are displayed in Fig. 9. In the current study the correlation coefficient ranged between -0.82 to 0.98 (Fig. 9). A highly significant correlation was observed between leaf length with leaf area (r = 0.82), leaf perimeter (r = 0.80), total flavonoids (r = 0.77) and highly negative correlation with moisture content (r = -0.73). However, leaf length didn't show any correlation with seed weight (r = 0.00). Leaf breadth exhibited a highly positive correlation with leaf area (r = 0.77) and leaf perimeter (r = 0.78). Leaf area exhibited a highly positive correlation with leaf perimeter (r = 0.98), total chlorophyll (r = 0.86), fruit weight (r = 0.82), pulp weight (r = 0.8), ascorbic acid content (r = 0.79), total phenols (r = 0.8) and total flavonoids (r = 0.82). As displayed in Fig. 9, it is observed that there was a substantial correlation among the fruit physico-biochemical characters. Fruit weight was positively correlated with fruit diameter (r = 0.87), fruit length (r = 0.93), pulp weight (1.00), seed length (r = 0.87) and TSS (r = 0.92). TSS was positively correlated with leaf area (r = 0.79), total chlorophyll (0.83), fruit weight (r = 0.92), seed length (0.81), total carbohydrates (r = 0.80) and total proteins (0.75). Dangi et al.[42] also reported similar correlation coefficients among different quantitative traits of sweet cherry cultivars.[40] also observed positive correlations of the leaf length with leaf width,leaf area, and TSS and negative correlation with acidity. In the present study, the fruit weight and diameter of the fruit have a positive correlation with

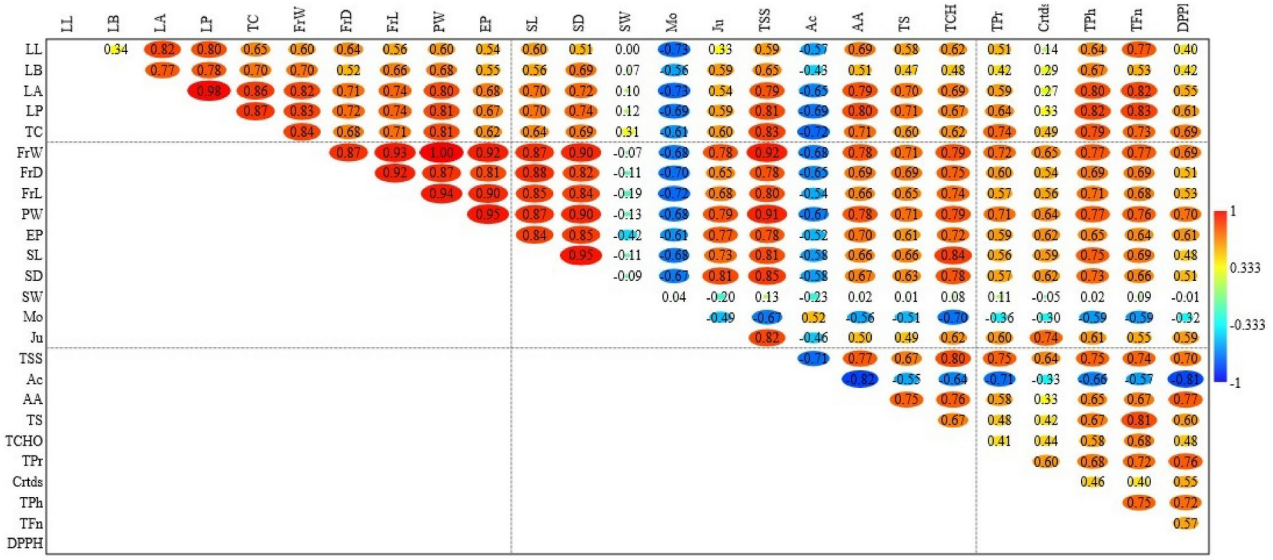

Fig. 9. Correlation matrix among different physical and biochemical characteristics.

length of the fruit, suggesting that larger fruits typically have larger dimensions. The highly positive significant correlation of fruit weight with fruit dimension indicates that fruit weight is highly influenced by the length and width of the fruit[41]. Our study is in agreement with the findings of Dangi et al.[42] who reported a highly positive correlation of fruit weight with fruit length and diameter. As illustrated in the current investigation, Ganopoulos et al.[43], Khadvi et al.[44], and Srivastava et al.[45] also reported a highly positive correlation of fruit weight with fruit length and diameter.

Carotenoids showed positive associations with the majority of fruit characteristics, suggesting that specific biochemical traits could be linked to the dimensions of fruits and seeds. Certain fruit quality indicators, such as total sugar and TSS, have positive stimuli with one another, indicating that their levels may be influenced by one another. Moisture content and acidity show highly negative correlations with various characteristics indicating an inverse association with several fruit characteristics. Some variables, like seed weight and juice content, exhibited weak correlations with most of the other fruit characteristics, suggesting less pronounced relationships with other fruit characteristics. The correlations between fruit characteristics and biochemical properties suggest complex interrelationships that may be influenced by genetic factors, environmental conditions, and cultivation practices. Certain traits, such as fruit weight and size, appear to be closely related, indicating potential genetic control over these attributes. Understanding these correlations can help the breeders about the fruit quality improvement.

Principal component analysis (PCA) of different physico-biochemical characteristics

Principal component analysis (PCA) is used to determine the association between different qualities within the sub-sets and the link between genotypes[46]. Principal component analysis (PCA) stands out as a vital tool for assessing and categorizing genotypes by identifying and quantifying crucial traits, as it simplifies complex physico-biochemical data, providing insights in understanding the variables. PCA permits us to achieve a dimensionality reduction, data exploration for finding relationships between objects, an estimation of the correlation structure of the variables and an investigation of how many components (a linear combination of original features) are necessary to explain the greater part of variance with a minimum loss of information[47]. It has been used to evaluate various comprehensive traits of pomegranates, bael, sweet cherry, *Prunus species* and *Garcinia pedunculata*[31,47–51].

In our study, based on PCA analysis 20 principal components (PC) were formed as shown in Fig. 10. The 5 PCs having eigenvalue of more than 1 were considered for interpretation as the cumulative contribution rate of the 5 PCs concluded 86.66% (Table 6) that explains most of the physico-biochemical characteristics of the *Phyllanthus acidus* accessions. Among the 5 PCs, PC1 accounted for 67.16% of the variability, PC2 for 6.68%, PC3 for 5.05%, PC4 for 4.29% and PC5 for 3.48% of the variability. The results illustrated that PC1 exhibited a significant and positive correlation with most of the physico-biochemical characters. It showed the highest positive loading with fruit characters such as fruit weight (0.974), fruit volume (0.975), pulp weight (0.968) and TSS (0.940) of the fruit. However, a negative correlation was observed with duration of flowering, moisture content and acidity. PC2 loaded significantly positive correlation with leaf length, leaf area, leaf perimeter, ascorbic acid, total sugar,

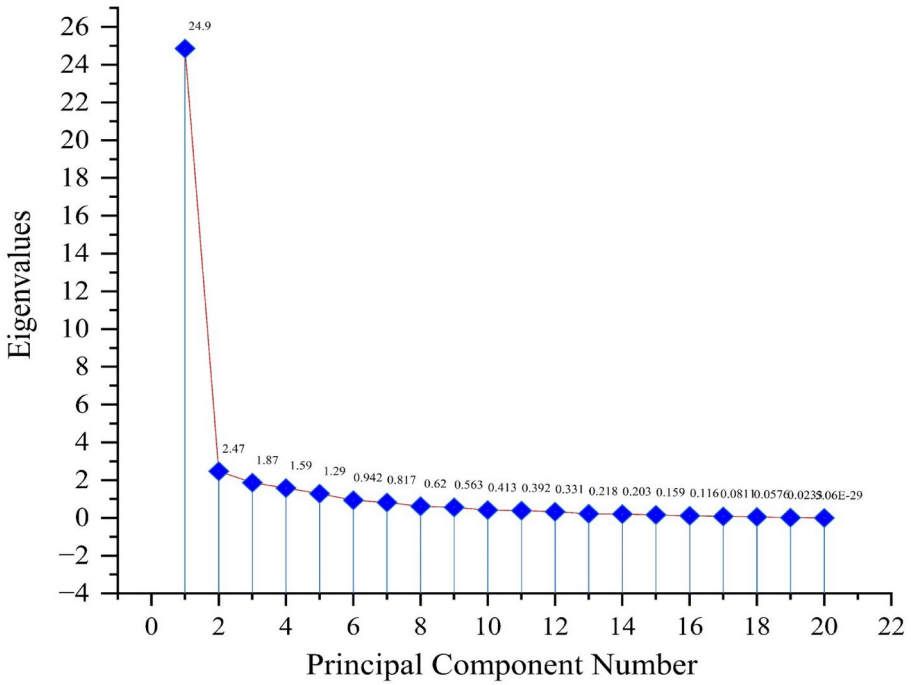

Fig. 10. Scree plot showing the Eigen values and Principal component (PC) numbers resulting from the PC analysis.

Characters	PC 1	PC 2	PC 3	PC 4	PC 5
LL	0.688	0.259	− 0.424	0.183	− 0.192
LB	0.702	− 0.006	0.083	0.284	− 0.294
LA	0.870	0.235	− 0.209	0.233	− 0.198
LP	0.886	0.246	− 0.136	0.185	− 0.176
TC	0.868	0.252	0.208	0.167	− 0.124
DoF50	− 0.708	0.186	− 0.117	0.252	0.410
DoF	− 0.843	− 0.134	− 0.061	0.215	0.214
FoL	0.813	0.030	0.001	0.168	0.018
FoB	0.885	0.154	0.284	− 0.085	− 0.036
FrW	0.974	− 0.156	0.049	0.059	0.021
SP	0.764	− 0.163	0.428	0.360	− 0.011
FrD	0.870	− 0.169	− 0.145	0.124	0.166
FrL	0.888	− 0.292	− 0.090	0.152	0.013
FrV	0.975	− 0.149	− 0.028	− 0.003	0.008
PW	0.968	− 0.200	0.015	0.020	0.002
EP	0.865	− 0.442	− 0.110	− 0.066	− 0.041
SL	0.869	− 0.244	− 0.104	0.158	0.190
SD	0.884	− 0.256	0.012	0.176	0.110
SW	0.001	0.758	0.442	0.356	0.270
SV	0.878	− 0.021	0.160	0.048	0.397
PSR	0.842	− 0.463	− 0.169	− 0.103	− 0.100
Mo	− 0.723	0.036	0.239	− 0.377	0.189
Ju	0.774	− 0.365	0.256	− 0.042	0.037
TSS	0.940	0.008	0.193	0.065	0.048
Ac	− 0.768	− 0.361	− 0.143	0.171	0.111
AA	0.845	0.233	− 0.167	− 0.190	− 0.064
TS	0.786	0.201	− 0.351	− 0.227	0.319
RS	0.704	0.256	− 0.353	− 0.127	0.367
NRS	0.746	0.026	− 0.246	− 0.372	0.134
TCHO	0.824	0.003	− 0.131	0.152	0.191
TPr	0.746	0.109	0.346	− 0.217	− 0.108
Crtds	0.609	− 0.358	0.439	− 0.141	0.347
TPh	0.847	0.102	− 0.022	− 0.064	− 0.158
TFn	0.827	0.213	− 0.203	0.000	0.053
DPPH	0.758	0.154	0.240	− 0.537	− 0.154
Eigenvalue	**24.85**	**2.47**	**1.87**	**1.59**	**1.29**
% of Variance	**67.16%**	**6.68%**	**5.05%**	**4.29%**	**3.48%**
Cumulative %	**67.16%**	**73.85%**	**78.89%**	**83.18%**	**86.66%**

Table 6. Principal components (PC) loadings of the *P. acidus* Skeels accessions. PCs with Eigenvalues > 1.0 was selected for the interpretation covering 86.66% of cumulative variance.

total flavonoid and seed weight which was not prominently featured in PC1 whereas it exhibited a negative correlation with fruit and seed physical characters, pulp seed ratio, acidity and carotenoids contents. The biochemical characters of *Phyllanthus acidus* viz. total proteins, carotenoids and antioxidant activity were highly positively linked in PC3 with a variance of 5.05%. PC4 explained 4.29% of the total variance captured by the PC analysis. Among the total variance, leaf breath, leaf area, specific gravity, seed weight showed higher positive loadings and moisture, total sugar, total protein and antioxidant activity exhibited higher negative loadings. PC5 was positively loaded with duration of flowerings, seed weight, seed volume, total sugars and carotenoids. Former studies utilized PCA as a tool for characterizing and evaluating germplasms[40,44,52]. In our earlier research, we also reported similar variability in *Aegle marmelos* and *Garcinia pedunculata*, which is in consistent with the results of the present study[31,47]. Dangi et al.[42] also observed variation among sweet cherry accessions which was illustrated by scree plots, scatter plots along PCA biplots explaining 40% of the total variation. Integrating characteristics such as leaf area, fruit weight, pulp weight, TSS, pulp seed ratio and others as reflected within the PCs highlights their importance as essential quantitative variables. Furthermore, their inclusion within the PCs emphasizes their crucial role in capturing the variability and enhancing our understanding of the characteristics and quality of *Phyllanthus acidus* accessions.

The scatter biplot (Fig. 11) segregates the accessions and characters in PC1 and PC2. It is employed to recognize and contrast accessions with significantly diverse traits, potentially serving as valuable parental candidates in

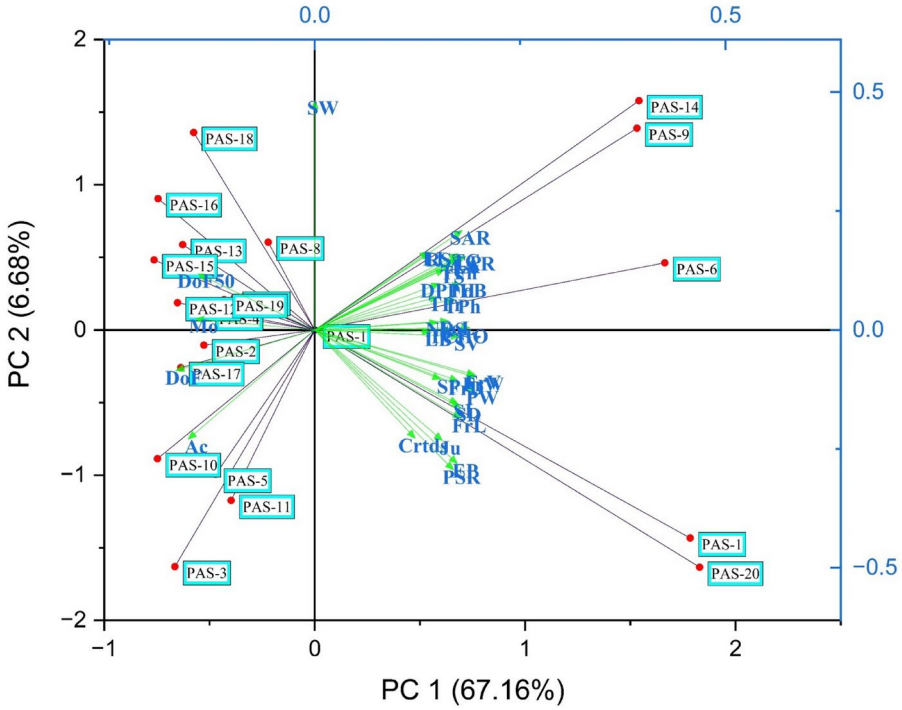

Fig. 11. Scatter biplot of the accessions on the first and second principal components based on physico-biochemical characteristics.

breeding initiatives. The biplot illustrates that accessions PAS-6 and PAS-15 had the highest positive scores while PAS-10 had the largest negative score in PC1 axis. PAS-14 exhibited the highest positive score which is opposed by PAS-3 on PC2 axis. In Fig. 11, there were 3 accessions with both positive PC1 and PC2 values namely PAS-14, PAS-9 and PAS-6, showing the distinctiveness as the variability is proportional to the vectors. Moreover, these accessions are associated with the weight of the fruit, length of the fruit, fruit volume, pulp: seed ratio and TSS of the fruits. In the negative PC1 and positive PC2 quadrant, 9 accessions were depicted. Six accessions, namely PAS-3, PAS-11, PAS-5, PAS-10, PAS-17, and PAS-2, were illustrated in both the negative PC1 and PC2 planes. In regions where PC1 is positive and PC2 is negative, PAS-1 and PAS-20 are notably distinct from other observations.

Cluster analysis

The process of grouping a set of data into clusters based on how similar or dissimilar individuals are to one another is known as cluster analysis. Using cluster analysis, comparable groupings were sought out. In contrast to PCA, all attributes are used equally in this analysis. Cluster analysis assists as a key tool in discerning genetic diversity within a crop population, facilitating the identification of distinct groups based on genetic similarities. In the present investigation, the cluster analysis was performed among the accessions using the Wards method based on euclidean distance using Origin 2024 learner edition (Fig. 12). The resultant dendrogram unveils a plethora of clusters denoting differences among the accessions, adeptly capturing the comprehensive spectrum of variations inherent within the *Phyllanthus acidus* population. It divided the accessions into 3 major clusters (I, II and III). The first major cluster I (PAS-1, PAS-14, PAS-20, PAS-6, PAS-9, PAS-19) includes 6 accessions. The second major cluster II facilitated 9 accessions in two smaller clusters of 4 and 5 accessions viz. PAS-2, PAS-5, PAS-10, PAS-17 and PAS-3, PAS-13, 7, 15, PAS-12, respectively. The third major cluster III reflected 5 accessions (PAS-4, PAS-11, PAS-8, PAS-16, PAS-18) under its category. Rai and Misra[53] and Debbarma and Hazarika[31] intriguingly classified bael genotypes into three clusters, attributing such patterns to variances in the genetic constitution and enduring environmental influences. Studies on pomegranate[48] and walnut[54] have advocated for the application of cluster analysis to assess variability among the accessions. Exploiting the cluster dendrogram as a foundation, it becomes apparent that the populations manifested notable variability. Concurrently, through the analysis in the current investigation, variability was discerned across all physico-biochemical traits, indicating a substantial degree of phenotypic polymorphism among the accessions. This observation underscores the existence of diverse morphotypes at the individual accession level, presenting abundant opportunities for obtaining desirable trait combinations while developing specific cultivars of *Phyllanthus acidus*. Such findings hold pivotal importance in the breeding program, to mainstream this lesser-known fruit of north-east India and increase the fruit basket for the consumers.

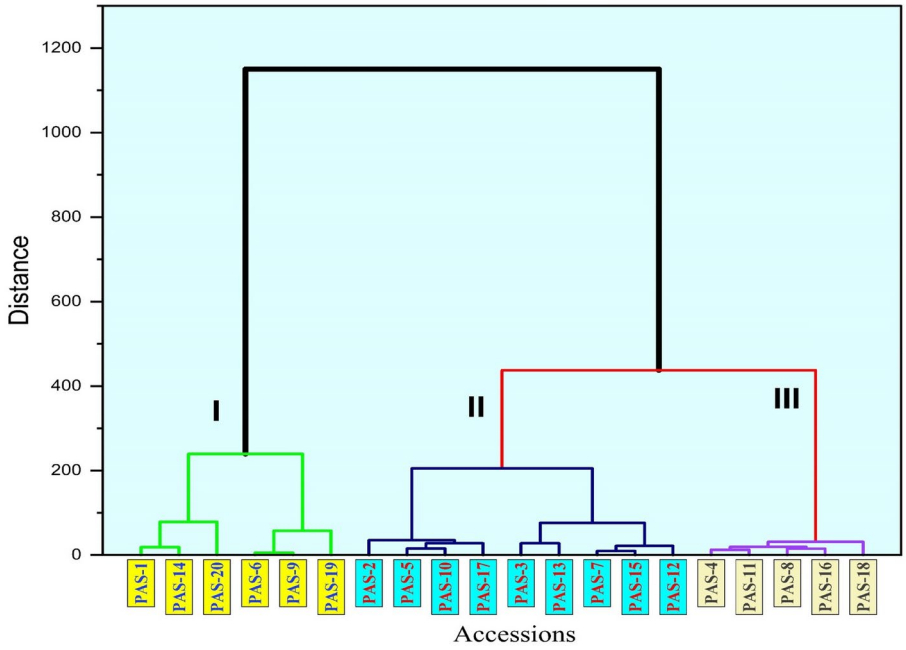

Fig. 12. Cluster map of *Phyllanthus acidus* Skeels accessions using wards method based on Euclidean distance.

Conclusion

A genetic variability study is an initial step aiming to characterizing and conservation of the precious germplasm for future breeding programs. Identification of promising *Phyllanthus acidus* accessions based on genetic variability, and phenological and pomological characteristics will be useful to harness the economic advantage associated with this valuable crop. Consumer preferences for fruits with maximum fruit weight, length and breadth, maximum pulp weight, pulp-to-peel ratio, juice content, ascorbic acid, TSS, low acidity, higher antioxidant activity and sugar acid ratio. The present investigation on *Phyllanthus acidus* accessions exhibited significant variability in the studied physico-biochemical characters, thereby offering a valuable resource for including them in breeding programmes and mainstreaming them. From the results of the present investigation, it can be concluded that among all the studied accessions 7 accessions have been revealed with greater variability with all the desired horticultural characteristics viz. PAS-14, PAS-9, PAS-6, PAS-1, PAS-20, PAS-3, PAS-18 and can be considered as elite accessions and be used as potential parents for future breeding Programmes. Expanding the cultivation of these *Phyllanthus acidus* accessions holds great promise for enhancing local nutrition and well-being, with a particular focus on their unique potential in traditional healthcare. However, further studies are necessary to understand the underlying molecular mechanisms that control these morphological characters. Such studies could facilitate the identification of key genes involved in morphological traits and enable the development of molecular markers for selecting desirable genotypes. Additionally, further research could investigate the relationship between morphological traits and fruit quality attributes.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

LN and LSD: Conducted the field and laboratory experiment TKH: Conceptualized the research, finalized the manuscript PD: Conducted the Statistical Analysis MDM: Prepared the draft PL: Conducted the Laboratory Analysis.

Competing interests

The authors declare no competing interests.

Additional information

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