Research Article

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Phytochemical profiling and antioxidant potential of *Pandanus leram (Pandanaceae)* fruit pulp: A component crop of coconut ecosystem in Nicobar Islands, India.

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Abstract

Pandanus leram (family*Pandanaceae*) is an important tree typically found in the tropical islands of Andaman and Nicobar. It is found as a component crop in the coconut groves. The fruits, fruitlets, seed and leaves of the tree are widely utilized as staple food or therapeutic applications by the local community. Nevertheless, biochemical composition of the fruit pulp is not known and hence this study was designed to determine the presence of various bioactive components, and to evaluate their antioxidant potential. Three distinct accessions of *Pandanus leram* were collected from the Nicobar Islands and the accessions were curated in ICAR-NBPGR under IC numbers: IC 0646202 (Galathea), IC 0646205 (Kinyuka), and IC 0646206 (Kamorta). Biochemical evaluation of the fruit pulp showed that IC 0646202 exhibited high carbohydrate and total fat whereas Accession 2 (IC 0646206) showed high crude fibre. Nevertheless, accession 3 (IC 0646205) displayed a relatively high content of protein (4.4±0.122 g/100g). Analysis of the antioxidant potential of the accessions revealed the accession IC0646206 exhibited highest hydroxyl radical scavenging activity compared to other two accessions. Further analysis involved phytochemical screening and gas chromatography–mass spectrometry (GC–MS) of methanolic extracts from the fruit pulp of these accessions.The results unveiled the presence of 34, 32, and 32 bioactive compounds in Accessions IC 0646202, IC 0646205, and IC 0646206, respectively.This comprehensive study sheds light on the biochemical composition and antioxidant capabilities of *Pandanus leram*, offering valuable insights into its potential applications in nutrition and health.

Key words: *Pandanus leram*fruit pulp, bioactives, GC-MS profiling, antioxidant potential.

Introduction

Pandanus leram (family *Pandanaceae*) is a species indigenous to the Nicobar and Andaman Islands, as well as the south coasts of Sumatra and western Java (Lim 2012). It is also known as Nicobar-Breadfruit and PandanWong. In the Nicobar Islands, its vernacular names include *mukung* (Shompen) and *larohm*. Botanically, the tree is an erect, evergreen reaching approximately 15 m in height, with vigorous aerial basal prop roots. The leaves are clustered at the shoot apex, either linear or strap-shaped, wider at the proximal end and tapering to a sharp point at the distal apex.The midrib and margin of the leaves are characterized by short but fine prickles (Lim 2012).

The ripe fruit serves as the staple food or daily bread for the Shompen people in the Nicobar Islands. Furthermore,*P. lerum* is described as a large orange-colored fruit and constitutes the staple food of the Nicobarese and Shompen tribes of the islands (Srivastava *et al*., 2000; Bhargava 1981).The raw wedge-shaped fruitlets are consumed after cooking in water, and the pulp, after boiling, is separated and mixed with other food items (pig fat and sugar) to prepare local dishes (Lim 2012).Nicobari tribes extract flour from the fruits, from which a cake is prepared (Sankaran and Dinesh 2020).

A preliminary study on the nutritional composition of the fruit pulp and seeds of *P. leram* found the following: Moisture 75.8% and 57.1%;

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Mineral content 0.6% and 0.9%; Protein 0.4% and 7.1%; Fiber 8.1% and 3.3%; Lipids 0.5% and 23.7%; Non-fiber carbohydrates 14.6% and 7.9%, respectively (Katiyar *et al*., 1989). Though seeds constitute only 3% of the fruit, they are found to be nutritionally rich compared to the fruit pulp.

to the ICAR-National Bureau of Plant Genetic Resources, New Delhi (110012), resulting in the assignment of IC numbers: IC 0646202 (Galathea), IC 0646205 (Kinyuka), and IC 0646206 (Kamorta). The particulars of these accessions are presented in Table 1. The locations

Table 1. Passport data of *P. lerum* accessions collected for biochemical analysis.

Sl. No	Accession	Botanical name	Fruit Morphology	Geographical co-ordinates			Place of collection and Island group	Unique accession Number
				Latitude (N)	Longitude (E)	Altitude (mMSL)		
1.	Acc 1	*P. lerum*	Big round fruit with high pulp recovery	$06^{0}49'20.17''$	$93^{o}51'1.76''$	6	Galathea Great Nicobar, India	IC 0646202
2.	Acc 2	*P. lerum*	Big, oval shaped fruit with pink colouredpulp	$08^{0}02'38.33''$	$93^{0}32'26.41''$	5	KamortaNancowry, India	IC 0646206
3.	Acc 3	*P. lerum*	Big oval shaped fruit with yellow coloured pulp	$09^{0}10'30.37''$	$92^{0}48'54.85''$	15	Kinyuka Car Nicobar	IC 0646205

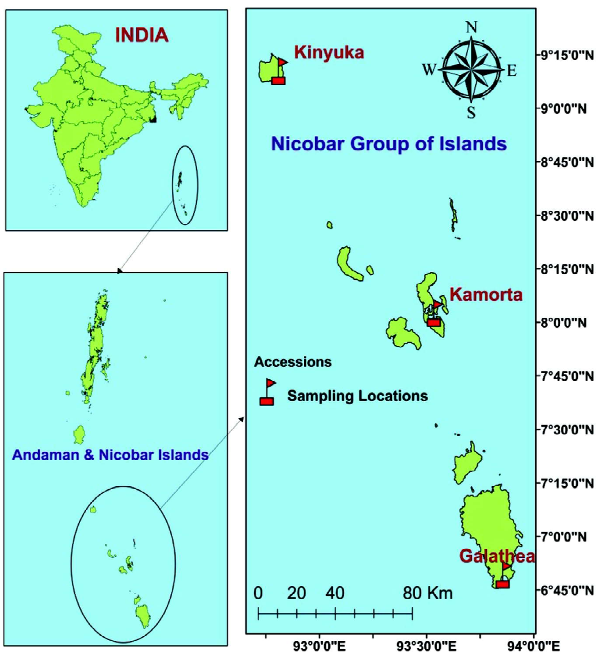
Singh *et al*. (2016) have highlighted the efforts and challenges in the conservation and sustainable utilization of neglected and underutilized horticultural resources for the development of the Andaman and Nicobar archipelago in the era of changing climate. Despite the multiple uses of the fruit, fruitlets, and leaves of *P. leram* as food and in ethnomedicine, little is known about the biochemical composition of the fruit and its active ingredients, hindering efforts to upscale or conserve this biodiversity for future generations. Hence, this study was conducted with a view to analyze the biochemical properties of fruit pulp of three distinct accessions of *P. leram*collected from the Islands of Nicobar and to identify novel phytocompounds with potential nutritional or health benefits.

Materials and Methods

Plant Collection and Identification

In October 2022, samples of*P. leram* growing in their natural habitat were systematically collected from three distinct locations in the Andaman and Nicobar Islands. These collected specimens were then submitted

Fig 1. Map depicting the locations of collection *P. leram*

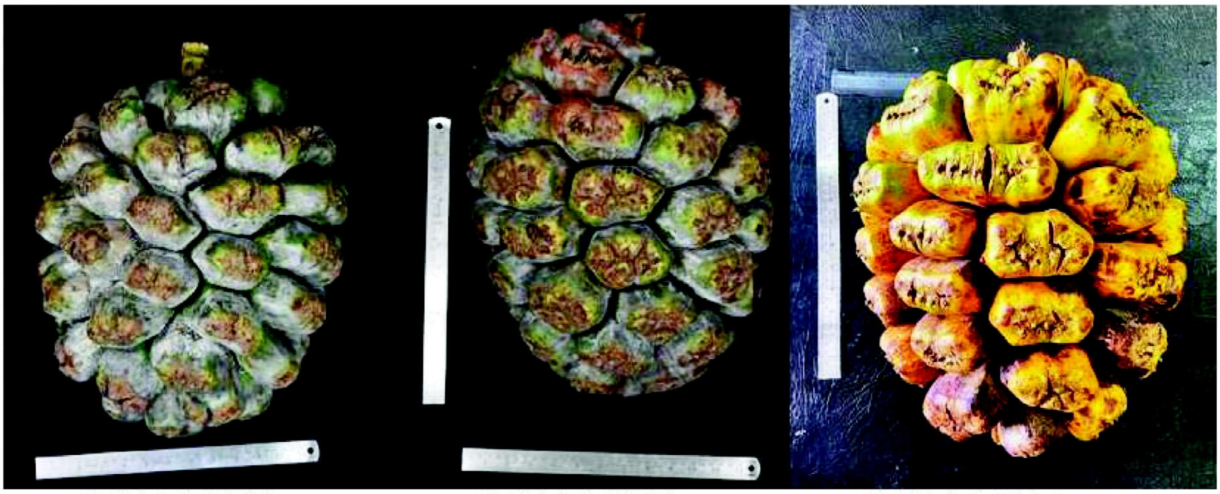


where the samples were collected are illustrated in the study area map, presented in Fig 1. Utilizing ArcGIS 10.8 (ESRI., 2021) and the QGIS (v3.10) platform, an open-source GIS software, the study area map was created.

Preparation and processing of *P. leram* fruit to pulp

The collected fruit samples of *P. leram* underwent a thorough cleaning process in running water to eliminate any dust and foreign particles adhered to the surface (Fig.2). Subsequently, the pulp was meticulously extracted from the fruit keys using specialized knives. Following this, the samples were left to air dry in the shade at room temperature and then ground into a coarse powder. For analysis, both dry samples and methanolic extracts (prepared at a ratio of 1:20 w/v) of the fruit pulp were generated. To achieve this, approximately 50 g of sample powder was subjected to extraction with 1000 mL of methanol in a Soxhlet apparatus until the color of the extracted material in the siphon tube became less intense. The resulting extract was then cooled to room temperature and filtered through Whatman No.1 filter paper (Abubakar and Haque, 2020). The filtrates obtained were subsequently used for analysis, with measurements conducted in triplicate.

Fig 2. Fruits of *Pandanus leram* accessions collected from the islands of Nicobar for biochemical analysis.
The fruit characteristic features of three accessions (IC 0646202; IC 0646206 an IC0646205) collected for phytochemical analysis are presented herein.



IC 0646202 IC 0646206 IC 0646205

Biochemical characterization

The biochemical features of *P.leram* pulp were analyzed using the methanolic extracts of fresh samples. Proximate analysis involved determinations of total carbohydrates, total protein and ascorbic acid contents following standard AOAC (1990)method with necessary modifications. Hence, the quality parameters namely total carbohydrate Content (Albalasmeh, *et al*., 2013) protein Content (Bonjoch 2001) and ascorbic acid content (Al-Alimi *et al*., 2017) were determined by sulfuric Acid – UV method, Bradford method and titration methods, respectively.

Besides, characteristic features such as total phenol content, total flavonoid content, total tannin content (Chandran and Indira, 2016), total saponin content (Makkar, *et al*.,2007) and total oxalate content (Mishra, 2017) were analyzed in methanolic extracts of dry pulp samples of *P. leram.*

Determination of antioxidant activity

The dry fruit pulp samples of *P. leram* methanolic extracts were subjected to antioxidant analysis *viz*., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity (Yu, *et al*., 2019), 2,22 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity are expressed as a % radical scavenging activity (%RSA) and Ferric Reducing Antioxidant Power (FRAP) (Rajurkar and Hande, 2011) is expressed as a µM Trolox equivalents (TE) per gram dry weight.

Identification of biochemical compounds using Clarus SQ 8C Gas Chromatography - Mass Spectrometer

Phytochemical profiling of the methanolic extracts was performed in the Clarus SQ 8C Gas Chromatography - Mass Spectrometer (Perkin Elmer). The instrument conditions were set as follows, Injector port temperature set to 220° C, Interface temperature set as 250° C, source kept at 220°C.The oven temperature programmed as available, 75° C for 2 mins, 150°C @ 10°C/ min, up to 250° C@ 10°C/min. Split ratio set as1:12 and the injector used was split less mode. The DB-5 MS capillary standard non - polar column was used whose dimensions were 0.25mm OD x 0.25µm ID x 30 meters length procured from Agilent Co., USA. Helium was used as a carrier gas at 1 mL/min. The MS was set to scan from 50 to 550 Da. The source was maintained at 220°C and 4.5e^{-6} m torr vacuum pressure.The ionization energy was -70eV.The MS was also having inbuilt pre-filter which reduced the neutral particles.

To analyse the mass spectra and chromatograms software Turbo mass was adopted. The data system has inbuilt libraries for searching and matching the spectrum.NIST(National Institute Standard and Technology) MS Search 2.2v contains more than five lakh references as well as literature values. Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST14).The spectrum of the known component was compared with that of the known components in the inbuilt library.

Statistical Analysis

All the experiments were conducted on triplicate samples. Values are expressed as means ± standard deviation. The statistical analysis performed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used to determine the significant differences ($p < 0.05$) between the means of parameters analyzed ($p<0.05$).

Results

Proximate composition of pulp of *P. leram* accessions

Table 2. Illustrates the proximate composition of fruit pulp of *P. leram* accessions collected from Andaman and Nicobar Islands. Significant variations were observed in the total carbohydrates, total protein, total fat, and moisture and ash contents of *P. leram* pulp among the different accessions. Accession 1 (IC 0646202) exhibited notably high levels of carbohydrates (84.76±0.501g /100g) and total fat (7.73±0.05 g/ 100g) contents, while Accession 2 (IC 0646206)

Table 2. Proximate composition of pulp of *Pandanus lerum* accessions from Andaman and Nicobar Islands.

Parameters	IC 0646202	IC 0646206	IC 0646205
Moisture (g/100g)	9.39±0.078	10.88±0.329	14.46±0.535
Total ash (g/100g)	3.22±0.101	6.21±0.146	9.63±0.379
Protein (g/100g)	0.79±0.040	2.64±0.180	4.4±0.122
Total fat (g/100g)	7.73±0.05	4.77±0.36	7.48±0.146
Crude fiber (g/100g)	12.75±0.111	21.63±0.14	18.14±0.205
Carbohydrates (g/100g)	84.76±0.501	77.45±1.068	71.36±0.904
Energy (Kcal/100g)	359.74±4.352	340.89±4.852	318.35±1.042

Three independent experiments were performed and data are presented as mean ± SD CD- Critical Difference at 5% level of significance; Different letters in each column after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT).

showed relatively high content of crude fibre (21.63±0.14 g/100g) among the accessions studied. However, the accession 3 (IC 0646205) showed a relatively high contents of protein (4.4±0.122 g/100g) moisture (14.46±0.535 g/100g) and total ash (9.63±0.379 g/100g). Nevertheless accessions 1 and 3 show comparable levels of fat contents (7.73±0.05 and 7.48±0.146 g/100g, respectively) in their pulp.The inherent variations of pulp of *P. leram* accessions was observed for their energy contents as it varied from 318.35±1.042 to 359.74±4.352 K cal/100g suggesting innate diversity in the fruit pulp of various accessions.

Biochemical features of *P. leram* accessions

Additionally, various other biochemical components, including total polyphenol content (TPC), total tannin, total saponins, total flavonoid content (TFC), and total oxalate, exhibited significant differences among the *P. leram* accessions (Table 3). Accession 3 (IC 0646205) recorded the highest values for TPC (35.61±0.413 mg GAE/g), total tannin (141.92± 1.507 mg TA/g), and total saponins (66.47 mg DSG/g). Accession I (IC 0646202) followed closely with TPC (34.61±0.103 mg GAE/g), while Accession 2 had the highest values for total tannin (140.27±1.179mg TA/g) and total saponins (4.57±0.05%). Accession 2 also significantly recorded the highest ascorbic acid content (5.28 ± 0.036mg/g) total oxalate (1.88±0.047mg/g), and TFC (66.04±0.092 mg QE/g).

Table 3. Biochemical features of *Pandanus leram* pulp accessions collected from Andaman and Nicobar Islands.

Parameters	IC 0646202	IC 0646206	IC 0646205
Ascorbic acid (mg/g)	3.70 ± 0.088^{c}	5.28 ± 0.036^{a}	4.40±0.017^{b}
Total phenols (mg GAE/g)	34.61 ± 0.103^{a}	34.26 ± 0.061^{a}	35.61 ± 0.413^{a}
Total tannins (mg TA/g)	132.46±2.186^{c}	140.27±1.179^{a}	141.92±1.507^{a}
Total saponin (mg DSG/g)	35.03±1.102^{c}	45.67±0.530^{b}	66.47±0.506^{a}
Total flavonoids (mg QE/g)	50.35±0.332^{b}	66.04±0.092^{a}	43.69±0.420^{c}
Total oxalates (mg/g)	1.32±0.034^{c}	1.88±0.047^{a}	1.64±0.040^{b}

Three independent experiments were performed and data are presented as mean ± SD CD- Critical Difference at 5% level of significance; Different letters in each column after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT).

Anti-oxidant potential of *P. leram* fruit pulp

The anti-oxidant potential of *P. leram* pulp extracts are depicted in Table 4. The DPPH

Table 4. Anti-oxidant potential of *P. leram* pulp accessions collected from Andaman and Nicobar Islands.

Parameter	IC 0646202	IC 0646206	IC 0646205
DPPH (%)	70.81 ± 1.62^{a}	65.69 ± 0.62^{c}	69.21 ± 0.41^{b}
Hydroxyl RSA (%)	70.62 ± 0.46^{b}	72.31 ± 0.29^{a}	52.94 ± 0.95^{c}
Superoxide RSA (%)	70.31 ± 0.40^{a}	71.29 ± 0.37^{a}	69.21 ± 0.85^{a}
ABTS (%)	33.24 ± 0.3^{a}	17.42 ± 0.46^{b}	16.64 ± 0.47^{b}
FRAP (μM (TE)/g DW)	903.85 ± 37.35^{c}	951.02 ± 42.00^{b}	1071.08 ± 65.44^{a}

Three independent experiments were performed and data are presented as mean ± SD CD- Critical Difference at 5% level of significance; Different letters in each column after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT).

activity followed the sequence IC 0646202>IC 0646205> IC 0646206. However, hydroxyl RSA of the pulp extracts showed that the accession IC0646206 exhibited highest activity compared to other two accessions. A similar trend was observed for superoxide RSA of *P. leram* pulp extracts although there were no significant differences among the accessions. The observed anti-oxidant activity could be attributed to the phenolic and flavonoids in the pulp of *P. leram*fruits. The ABTS radical assay measures the scavenging of free radicals as the discoloration of the ABTS blue reactant. A significantly high ABTS antioxidant activity of 33.24 ± 0.3% was observed for IC 0646202 whereas accessions IC 0646206 and IC 0646205 showed comparable ABTS activities of 17.42 ± 0.46 % and 16.64 ± 0.47 %, respectively. However, the values obtained for ABTS assay were significantly lower than that obtained in DPPH eventhough ABTS offers a number of advantages over the DPPH assay, as the former can be used in a wide pH range, and solubility range (both in aqueous and organic solvents) thereby estimating the antioxidant activity of both the hydrophilic and lipophiliccomponents. However measurement of reducing power of the extract of *P. leram* pulp in FRAP suggests that IC 0646205 showed a significantly high activity of 1071.08 ± 65.44 µM (TE)/g DWas against the values of 903.85 ± 37.35 and 951.02 ± 42.00 obtained for accessions IC 0646202and IC 0646206, respectively. The presence of polyphenolic compounds contributes to the total antioxidant potential of *P.leram* pulp.

Biochemical finger printing by GC-MS

GC-MS analysis of methanolic extracts of *P. leram* pulp of three different accessions namely IC 0646202, IC 0646206, IC 0646205 revealed a comprehensive overview of biochemical compounds in these accessions (Fig. S1; Fig.S2: Fig. S3).The details of accessions and the associated compounds along with their molecular formula, molecular weight, peak area

Chromatograms obtained from GC-MS analysis of methanolic extracts of *P. leram* pulp of three different accessions namely IC 0646202, IC 0646206, and IC 0646205.

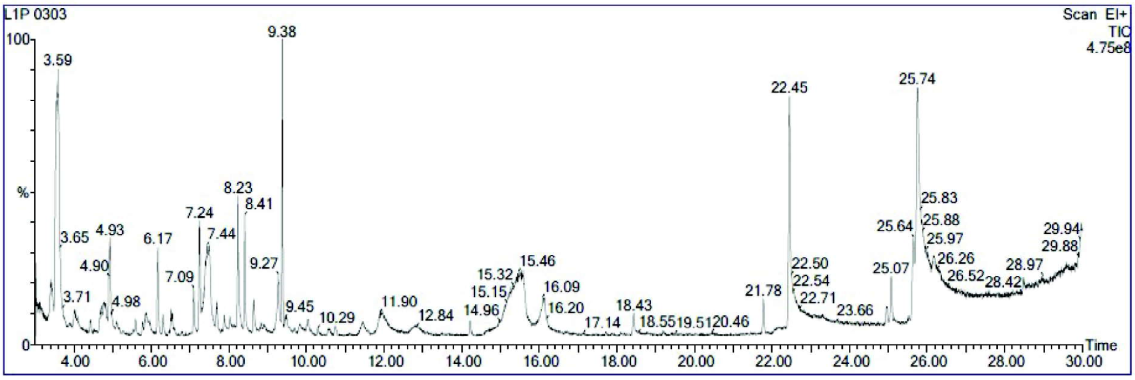


Fig S1. Chromatogram of GC-MS profile of IC 0646202 pulp samples

Fig S2. Chromatogram of GC-MS profile of IC 0646206 pulp samples

%, are presented in Table 5, Table 6 and Table 7, respectively. The chromatogram revealed 34 compounds in *P. leram* accession IC 0646202 whereas each 32 distinct compounds are found in accessions IC 0646206 and IC 0646205, respectively. The GC-MS chromatogram pertaining to the pulp of accession IC 0646202 showed oleic acid (14.218%), glyceraldehyde dimer (10.14%), 3-deoxy-d-mannoic lactone (9.38%), n-Hexadecanoic acid (8.945%),9S-Hydroxy-10E,12Z,15Z-octadecatrienoic acid (2.356%) as predominant biocompounds (Table 5).Similarly, in the fruit pulp of accession IC 0646206 among the 34 compounds identified in GC-MS profiling, the major metabolites found are 9-Octadecenoic acid,(E)(41.489%),n-Hexadecanoic acid (18.172%), Hexadecanoic acid, ethyl ester(1.676%), 9,12-Octadecadienoic acid (Z,Z)- (1.207%), 3-Deoxy-d-mannoic lactone (1.157%),Glycidyl palmitate (1.138%) (Table 6).In the accession, IC 0646205, GC-MS profiling has identified 33 compounds and the major metabolites found are DL-Arabinose (16.9%), trans-13-Octadecenoic acid (12.1%), 1,2-Cyclopentanedione (10.473%), n-Hexadecanoic acid (7.598%), 3-Deoxy-d-mannoic lactone (4.745%) (Table 7).The pie charts of compounds identified in the three *Pandanus lerum* accessions using the GC–MS peak area data are presented in Fig 3**.**

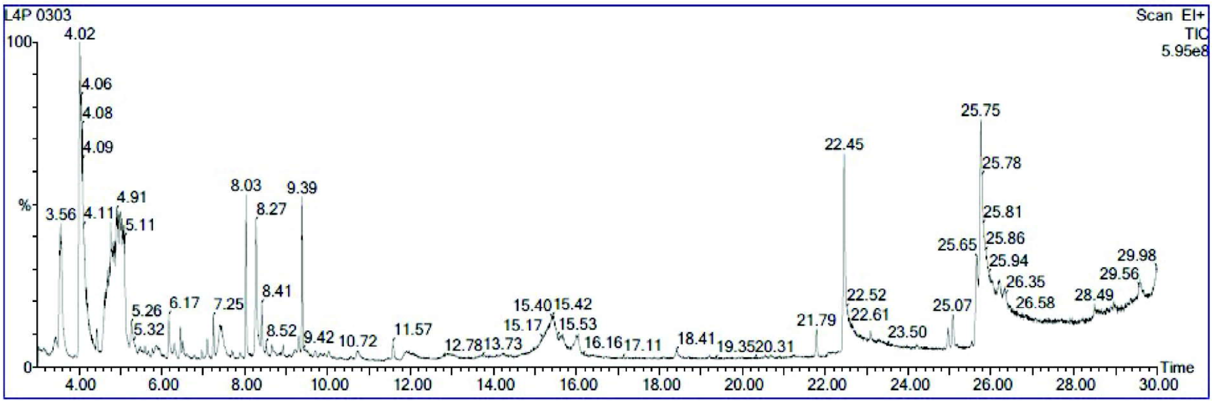


Fig S3. Chromatogram of GC-MS profile of IC 0646205 pulp samples

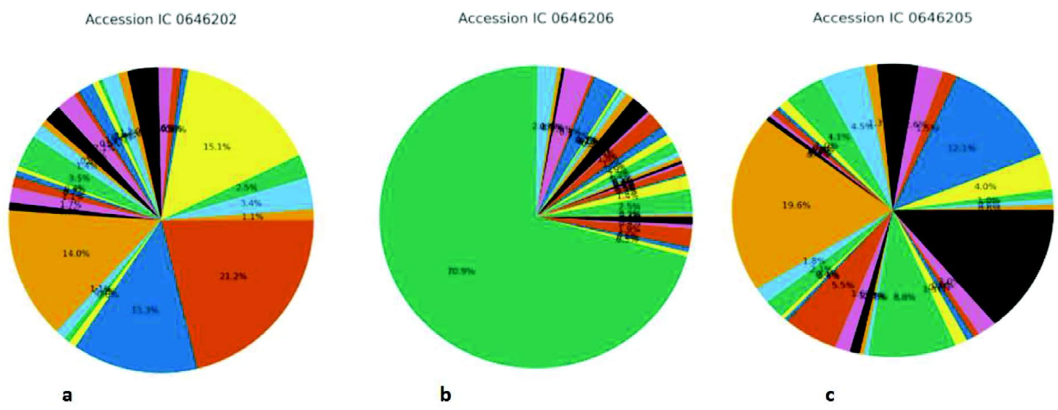


Fig 3. Pie charts of compounds identified in the three *Pandanus lerum* accessions using the GC–MS peak area data.

a) IC 0646202is dominated by Oleic acid (14.2%), dl-Glyceraldehyde dimer (10.1%), n-Hexadecanoic acid (8.9%), and 3-Deoxy-d-mannoic lactone (9.4%)
b) IC 0646206: overwhelmingly dominated by 9-Octadecenoic acid (E)- (41.5%), with smaller contributions from others
c) IC 0646205: largest fractions from DL-Arabinose (16.9%), trans-13-Octadecenoic acid (12.1%), and 1,2-Cyclopentanedione (10.5%)

Table 5. The chromatogram area and height of the phytocompounds of *Pandanus lerum* accession IC 0646202 from GC–MS analysis report.

Peak	Compounds	RT	Peak Area (%)	Molecular weight (g/mol)	Molecular Formula	Compound class/ activity	Reference
1	Glyceraldehyde	3.023	0.768	90.08	$C_3H_6O_3$	Triose monosaccharide, used in nutrition; in the preparation of polyesters and adhesives	Flefel*et al.*, 2012.
2	2-Furanmethanol	3.128	2.257	98.09	$C_5H_6O_2$	Heteroaromatic compound enhance cellulose enzymatic hydrolysis	Gallasch, B A. *et al.*,2000.
3	Propanoic acid, 3-nitro-, methyl ester	3.409	1.683	*118.13*	$C_4H_7NO_4$	Anti-Microbial properties	Gandhi, V.V.*et al.*, 2022.
4	dl-Glyceraldehyde dimer	3.594	10.141	*180.16*	$C_6H_{12}O_6$	Sugar moiety and Preservative	Ganesan, T. *et al.*,2024.
5	1,2-Cyclopentanedione	4.019	0.474	98.09	$C_5H_6O_6$	Lactones,methyl derivative has moderate antioxidant activity	Gangaiah, B.,*et al.*, 2017.
6	Urea	4.714	0.617	60.06	CH_4N_2 O	Carbamide, evaluate renal function	Gesteiro, E.,*et al* 2019.
7	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	4.779	1.056	144.13	$C_6H_8O_4$	Furan (Aroma compound/ antioxidant/ anticancer)	Ghosh, G., *et al.,*2015.
8	2-Propen-1-ol	4.929	2.172	58.08	$C_{11}H_{140}$	Allyl alcohol; used as pesticides, perfume, high catalytic activity	Goya, L.*et al.,*2023.
9	2(3H)-Furanone, 5-acetyldihydro-	5.870	0.692	128.13	$C_6H_8O_3$	Antimicrobial, antioxidant	Grosso, G., *et al.,* 2018.
10	Thymine	6.170	1.152	126.11	$C_5H_6N_2O$	DNA require Thymine for the synthesis of Thymidylate	Hamedi, A., *et al.,* 2019.
11	3-Pyridinol	6.535	0.299	95.09	C_5H_5NO	Hydroxypyridines, cytotoxic effect, phenolic properties	Hameed, R.H.,*et al.,*2018.
12	Ethanamine, N-ethyl-N- nitroso-	7.090	0.453	102.14	$C_4H_{10}N_{20}$	proliferation	Hasan, M.M., *et al.,*2017.

13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.240	1.138	144.13	$C_6H_8O_4$	Strong anti-oxidant, antimicrobial and anti-inflammatory	Helrich, K., *et al.* 1990. Henningsen, B.M., . *et al.,*2015.
14	Procyclidine	7.685	0.345	287.44	$C_{19}H_{29}NO$	anticholinergic	Igile, G.O., *et al.,*2018.
15	1,11-Undecanedi carboxylic acid	8.230	1.439	244.33	$C_{13}H_{24}O_4$	high-resolution mass spectrometry	Jeeva, S.*et al*.,2018.
16	5-Hydroxymethylfurfural	8.406	1.198	126.11	$C_6H_6O_3$	Antioxidant and Antiproliferative Activity	Kamble, M.Y., *et al.,*2008.
17	L-Sorbose	8.636	0.508	180.16	$C_6H_{12}O_6$	Natural sweetener	Katiyar, S.K.,*et al.* 1989.
18	1-(Methylthio)-3-pentanone	9.271	0.964	132.22	$C_6H_{12}OS$	Flavouring agent	Kaushik, S., *et al.,*2020.
19	9S-Hydroxy-10E,12Z, 15Z-octadecatrienoic acid	9.376	2.356	*294.43*	$C_{18}H_{30}O_3$	atherogenic risk factors	Kumari, P.,*et al.,*2014.
20	Melezitose	9.821	0.297	504.44	$C_{18}H_{32}O_{16}$	metabolic marker	Lenski, M., *et al.*,2022.
21	Glutaric acid, 2-methylpent-3-yl 8-chlorooctyl ester	10.031	0.444	362.93	$C_{11}H_{19}ClO_3$	Dermatology study	Lim, T.K.*et al*.,2012.
22	Sucrose	11.917	0.755	342.29	$C_{12}H_{22}O_{11}$	Non-toxic	Liu, G.*et al.,*2019.
23	à-D-Mannofuranoside, 1-O-decyl-	11.947	1.121	*320.42*	$C_{16}H_{32}O_6$	Antiviral agent	Looby, N., *et al.,*2021.
24	1-Nitro-á-d-arabino furanose, tetraacetate	12.812	0.538	*363.27*	$C_{13}H_{17}NO_{11}$	Cytotoxic activity against cancer cell lines	Makkar, H.P.S., *et al.*, 2007.
25	3-Deoxy-d-mannoic lactone	15.538	9.385	*162.14*	$C_6H_{10}O_5$	Antibacterial activity	Malinowski, J.,*et al.*, 2021.
26	3-Deoxy-d-mannonic acid	16.094	0.766	-	$C_6H_{10}O_5$	Antifungal activity	Mazumder, K. e*t al.,*2020.
27	Tetradecanoic acid	18.430	0.421	228.37	$C_{14}H_{28}O_2$	antielastase and antioxidant	Mishra, D.P. *et al.,*2017.

28	Hexadecanoic acid, methyl ester	21.776	0.513	270.45	$C_{12}H_{34}O_{7}$	Antibacterial activity	Muthulakshmi, *et al*., 2012.
29	n-Hexadecanoic acid	22.446	8.945	256.42	$C_{16}H_{32}O_{2}$	Antioxidant, Hypocholesterolemic Nematicide, Antiandrogenic, Flavor, Hemolytic	Nalawade T. M..*et al*.,2015.
30	4H-Cyclopropa5',6' benz1',2':7,8azuleno5, 6-boxiren-4-one, 8-(acetyloxy)-1,1a,1b, 1c,2a,3,3a,6a,6b,7,8, 8a-dodecahydro-3a,6b, 8a-trihydroxy-2a-(hydroxymethyl)-1, 1,5,7-tetramethyl-, 1ar-(1aà,1bá,1cà,2aà, 3aá,6aà,6bà,7à,8á,8aà)-	23.291	0.970	522.5	-	Antibacterial, hepato-protective, hypocholesterolemic, antiarthritic, nematicide properties	Nashalian, O.*et al*., 2017. Ogawa,S.*et al*.,2000.
31	12,15-Octadecadienoic acid, methyl ester	24.952	0.287	*294.5*	$C_{19}H_{32}O_{2}$	nutritional benefits	Pelley J.W.,*et al*., 2012
32	9,12-Octadecadienoyl chloride, (Z,Z)-	25.067	0.764	*280.46*	$C_{18}H_{31}ClO_{2}$	antiviral.	Placidi, L., *et al*.,2000.
33	Linoelaidic acid	25.642	1.829	280.446	$C_{18}H_{32}O_{2}$	Essential fatty acid in mammalian nutrition and is used in the biosynthesis of prostaglandins and cell membranes.	Rajurkar, N.S.*et al*.,2011.
34	Oleic Acid	25.742	14.218	*282.46*	$C_{18}H_{34}O_{2}$	Antibacterial activity	Rose, D.R., *et al*.,2012.

Table 6. The chromatogram area and height of the phytocompounds of *Pandanus lerum* accession IC 0646206from GC–MS analysis report.

Peak	Compounds	RT	Area (%)	Molecular weight	Molecular Formula	Compound class/ activity	Reference
1	Acetic acid	3.093	0.172	60.05	CH_3COOH	Chronic Wound Healing	Rudrapal, M.,*et al.,*2022.
2	Propanoic acid, 3-nitro-, methyl ester	3.399	0.122	*118.13*	$C_4H_7NO_4$	Anti-Microbial properties	Gandhi, V.V.,*et al.,* 2022.
3	dl-Glyceraldehyde dimer	3.554	1.444	*180.16*	$C_6H_{12}O_6$	Sugar moiety and Preservative	Ganesan, T.,*et al*.,2024.
4	1,2-Cyclopentanedione	3.979	0.833	*98.09*	$C_6H_{12}O_6$	Lactones,methyl derivative has moderate antioxid ant activity	Gangaiah, B.,*et al.,* 2017.
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.719	0.131	144.13	$C_6H_8O_4$	Strong anti-oxidant, antimicrobial and anti-inflammatory	Helrich, K., *et al.,* 1990. Henningsen, B.M.,*et al.,* 2015.
6	Formic acid, 2-propenyl ester	4.889	0.503	86.09	$C_4H_6O_2$	Antifungal	Sales-Campos, H., *et al.,* 2013.
7	2(3H)-Furanone, 5-heptyldihydro-	5.825	0.186	184.28	$C_{11}H_{20}O_2$	antifungal	Saravana Kumar, P., *et al.*,2014.
8	Thymine	6.140	0.150	126.11	$C_5H_6N_2O$	DNA require Thymine for the synthesis of Thymidylate	Hamedi, A., *et al.,*2019.
9	2(3H)-Furanone, dihydro -4-hydroxy-	7.305	0.260	102.09	$C_4H_6O_3$	antiviral activity	Saravanan, P.,*et al.,* 2014.
10	Thiophene, 2,3-dihydro-	7.980	0.301	86.16	C_6H_6 S	high reactivity and excellent enantios electivity	Schulien, I.*et al.,* 2021.

11	2(3H)-Furanone, 5-ethoxydihydro-	8.230	0.718	130.14	$C_6H_{10}O_3$	antiviral activity	Saravanan, P.,*et al.,* 2014.
12	1-Deoxy-d-arabitol	8.295	0.530	*136.15*	$C_5H_{12}O$	Glucose precursors and hence energy suppliers	Seong, S.H.,*et al.,*2022.
13	5-Hydroxymethylfurfural	8.376	0.611	126.11	$C_6H_6O_3$	Antioxidant and Antiproliferative Activity	Kamble, M.Y.,*et al.,*2008.
14	Coumestrol	9.341	1.030	268.22	$C_{15}H_{10}O_6$	competitive MAO-A inhibitor	Shaaban, M.T.,*et al.,*2021.
15	Sucrose	11.857	0.224	342.29	$C_{12}H_{22}O_{11}$	Non-toxic	Saravanan, P.,*et al.,* 2014.
16	3-Deoxy-d-mannoic lactone	15.363	1.157	*162.14*	$C_6H_{10}O_5$	Antibacterial activity	Malinowski J.,*et al.,* 2021.
17	á-D-Glucopyranoside, methyl	15.613	0.503	*194.18*	$C_7H_{14}O_6$	evaluation of intestinal lactase	Sharma, M.D.,*et al.,* 2015.
18	3-Deoxy-d-mannonic acid	15.999	0.349	-	$C_6H_{10}O_5$	Antifungal activity	Mazumder, K.,*et al.,* 2020.
19	Tetradecanoic acid	18.410	0.107	228.37	$C_{14}H_{28}O_2$	antielastase and antioxidant	Nalawade, T.M.*, et al.,* 2015.
20	Hexadecanoic acid, methyl ester	21.741	0.155	270.45	$C_{12}H_{34}O_7$	Antibacterial activity	Nashalian, O.*et al.,* 2017.
21	n-Hexadecanoic acid	21.891	1.567	256.42	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic Nematicide, Antiandrogenic, Flavor, Hemolytic	Ogawa, S.*et al.*,2000.
22	Ethyl iso-allocholate	22.071	0.187	436.63	$C_{13}H_{14}N_2O_3$	Antimicrobial, anti-inflammatory, antiasthma	Sheela, D.*et al.,* 2013.

23	Hexadecanoic acid, ethyl ester	23.041	1.676	284.48	$C_{18}H_{36}O$	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Antiandrogenic	Shobana, S.,*et al.,*2009.
24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	24.927	0.151	294.47	$C_{19}H_{32}O_3$	Production of fatty acid	Shreenivasan, Y.S.,*et al.*,2022.
25	10-Octadecenoic acid, methyl ester	25.037	0.262	296.49	$C_{19}H_{32}O_2$	Production of fatty acid	Shreenivasan, Y.S.,*et al.*,2022.
26	9,12-Octadecadienoic acid (Z,Z)-	25.507	1.207	280.45	$C_{18}H_{32}O_2$	Antioxidant, antiarthritic, hepatoprotective	Shubharani, R.,*et al.,* 2014.
27	9-Octadecenoic acid, (E)-	25.812	41.489	282.46	$C_{18}H_{36}O_2$	fatty acid metabolite	Singh, D., *et al.,*2012.
28	Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester	27.683	0.291	*568.90*	$C_{19}H_{38}O_4$	Antibacterial activity	Muthulakshmi,A.R.J M *et al.,* 2012.
29	Fluroxypyr 1-methy lheptyl ester	27.768	0.319	367.24	$C_{15}H_{21}Cl_2FN_2O_3$	Herbicide	Sokmen, B.B.,*et al.,* 2014.
30	Glycidyl palmitate	28.393	1.138	312.50	$C_{19}H_{36}O_3$	food ingredient	Springer, D.,*et al*., 2003.
31	Linoleic acid ethyl ester	29.274	0.259	308.49	$C_{20}H_{36}O_2$	Protects lipids from oxidative damage.	Stagner, W.C.,*et al*.,2019.
32	9-Octadecenamide, (Z)-	29.329	0.464	281.47	$C_{18}H_{35}NO$	hypolipidemic effect	Teoh, Y.P., *et al.,*2012.

Table 7.The chromatogram area and height of the phytocompounds of *Pandanus leram*accessionIC 0646205from GC–MS analysis report.

Peak	Compounds	RT	Area (%)	Molecular weight	Molecular Formula	Compound class/ activity	Reference
1	Methyl-4-azido-4-desoxyá -arabinopyranoside	3.033	0.559	*189.17*	$C_6H_{11}N_3$	Not reported	-
2	2-Furanmethanol	3.163	0.533	98.09	$C_5H_6O_2$	enhance cellulose enzymatic hydrolysis	Gallasch, B.A.*et al.,* 2000.
3	Propanoic acid, 3-nitro-, methyl ester	3.434	0.876	*118.13*	$C_4H_7NO_4$	Anti-Microbial properties	Gandhi, V.V.,*et al.,* 2022.
4	dl-Glyceraldehyde dimer	3.564	3.466	180.16	$C_6H_{12}O_6$	Sugar moiety and Preservative	Ganesan, T., *et al.,* 2024.
5	1,2-Cyclopentanedione	4.024	10.473	98.09	$C_6H_{12}O_6$	Lactones, methyl derivative has moderate antioxidant activity	Gangaiah, B.,*et al.*, 2017.
6	3-Methyl-a-pyrrolidino propiophenone	4.189	1.277	253.80	$C_{14}H_{19}NO$	stimulant	Tyagi, T.*et al.*, 2017.
7	Glycerin	4.684	2.214	92.09	$C_3H_8O_3$	Bactericidal	Tyson, B., *et al.*,2022.
8	Acetic acid	4.769	3.522	60.05	CH_3COOH	Chronic Wound Healing	Rudrapal, M.,*et al.,* 2022.
9	Aldicarb	4.839	1.122	190.26	$C_7H_{14}N_2O_2S$	carbamate pesticide	van der Krieken, S.E.,*et al.*, 2018.
10	2-Propen-1-ol	4.914	3.893	58.07	$C_{11}H_{14}O$	Allyl alcohol; used as pesticides, perfume, high catalytic activity	Goya, L.*et al.,* 2023.
11	2H-Pyran-2,6(3H)-dione	5.004	3.521	112.08	$C_5H_4O_3$	three-dimensional network.	Wakama tsu, J.,*et al.,* 2019.

12	1,2,3,4-Cyclopentane tetrol, (1à,2á,3á,4à)-	5.274	0.905	-	$C_5H_{10}O_4$	α-mannosidase inhibitor hence a potential anti-cancer or anti-infection compounds	Wang, H.,*et al.,*2014. Wang, J., *et al.,*2015.
13	2(3H)-Furanone, 5-acetyldihydro-	5.880	0.328	128.13	$C_6H_8O_3$	Antimicrobial, antioxidant	Grosso, G.,. *et al.,* 2018
14	Thymine	6.170	0.589	126.11	$C_5H_6N_2O$	DNA require Thymine for the synthesis of Thymidylate	Hamedi, A.,*et al.,*2019.
15	1,2,3-Propanetriol, 1-acetate	6.445	0.355	134.13	$C_5H_{10}O_4$	primary inhibitor	Yu, X.,*et al.,*2013.
16.	4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl-	7.245	0.384	144.13	$C_6H_8O_4$	Strong anti-oxidant, antimicrobial and anti-inflammatory	Helrich, K *et al.*, 1990. Henningsen, B.M.,*et al.*, 2015.
17	DL-Arabinose	7.395	16.900	*150.13*	$C_5H_{10}O_5$	Crystallization behavior	Zhao, L.,*et al.,*2013.
18	Thiophene, 2,3-dihydro-	8.030	1.530	86.16	C_4H_6S	high reactivity and excellent enantio selectivity.	Schulien, I.*et al.,* 2021.
19	(S)-(+)-2',3'-Dideoxyri	8.270	1.806	116.12	-	β-l-nucleoside analogs, antiviral activity bonolactone	[90]
20	5-Hydroxymethylfurfural	8.411	0.360	126.11	$C_6H_6O_3$	Antioxidant and Antiproliferative Activity	Kamble, M.Y.,*et al.,*2008.
21	1,2,3-Butanetriol	9.301	0.343	106.12	$C_4H_{10}O_3$	Antioxidative	[91]
22	3-Deoxy-d-mannoic lactone	15.418	4.745	162.14	$C_6H_{10}O_5$	Antibacterial activity	Malinowski, J.,*et al.,* 2021.
23	à-D-Glucopyranoside, methyl	15.663	1.369	*194.18*	$C_7H_{14}O_6$	evaluation of intestinal lactase	Sharma, M.D.,*et al*.,2015.
24	3-Deoxy-d-mannonic acid	15.999	0.831		$C_6H_{10}O_5$	Antifungal activity	Mazumder, K.,*et al.,* 2020.
25	L-Glucose	16.039	0.385	180.15	$C_6H_{12}O_6$	source of energy	[92]

26	Hexadecanoic acid, methyl ester	21.786	0.394	270.45	$C_{12}H_{34}O_7$	Antibacterial activity *et al.,*2012.	Muthulakshmi, A.R.J.M.
27	n-Hexadecanoic acid	22.451	7.598	256.42	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic Nematicide, Antian drogenic, Flavor, Hemolytic	Nalawade, T.M.,*et al.*,2015.
28	Hexadecanoic acid, ethyl ester	23.086	1.086	284.47	$C_{18}H_{36}O_2$	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Antiandrogenic	Shobana, S.,*et al.,* 2009.
29	Butylaldehyde, 4-benzy loxy-4-2, 2,-dimethyl-4-dioxolanyl-	23.301	0.643	-	$C_{16}H_{22}O_4$	anti-inflammatory	[93]
30	6-Octadecenoic acid, methyl ester	25.067	0.540	296.49	$C_{19}H_{32}O_2$	Production of fatty acid	Shreenivasan, Y.S.,*et al.*,2022.
31	9,12-Octadecadienoic acid (Z,Z)-	25.647	1.713	280.45	$C_{18}H_{32}O_2$	Antioxidant, antiarthritic, hepatoprotective	Shubharani, R.,*et al.,* 2014.
32	trans-13-Octadecenoic acid	25.747	12.100	282.46	$C_{18}H_{36}O_2$	fatty acid metabolite	Singh, D., *et al.*,2012.

Discussion

As per the findings of the International Plant Genetic Resources Institute (IPGRI) in Rome, Italy, there exists the potential to cultivate around 7,000 plant species for food purposes. However, a mere 150 crops are commercially grown. Currently, a significant portion of global calorie consumption, approximately half, is derived from just three crops—namely rice, wheat, and maize. The remaining (6,850 plant species) either grow in the wild or are cultivated in small areas for sustenance. These plants are often referred to as underutilized, underdeveloped, neglected, minor, promising, orphan, or relict crops, as well as life support species. They embody a rich source of agro-biodiversity capable of contributing to enhanced incomes, food security, and nutrition (Singh, *et al*., 2012).This diverse range of species plays a crucial role in addressing 'hidden hunger' caused by micronutrient deficiencies. They also enhance diversity, thereby ensuring stability in agro ecosystems, particularly in fragile environments like island ecosystem as in Andaman and Nicobar Islands 6.

In addition, theindigenous tribes of Andaman and Nicobar Islands have relied on medicinal plants to address a diverse range of ailments. These traditional medicinal practices have been integral to the healthcare systems of these aboriginal communities over the course of history. The rich biodiversity of medicinal flora (536 species spanning across 382 genera and 138 families of plants) reflects the longstanding and profound connection between these indigenous communities and the healing properties of their natural environment (Kamble, *et al*.,2008). *P. leram* is considered a staple food in Nicobar Islands as the fruit, which is either yellow or red colour, is consumed after boiling and processing it into a cake (Jaisankar, *et al*.,2022). In addition, the traditional dietary pattern of the tribes in the region utilizes *P. leram* and coconut to prepare weaning food for the infants suggesting its inherent nutritional potential (Jaisankar, *et al*.,2022).

Meanwhile, the present study focuses primarily on the fruits of *P. leram* for extraction and characterization of phytochemicals of nutritional and therapeutic importance. The qualitative preliminary phytochemical analysis revealed the presence of various phytoconstituents in the pulp of *P. leram* fruits.Although carbohydrates predominate the pulp of fruit (71.36±0.904 to 84.76±0.501 g/100g), a relatively high protein content of 4.4±0.122 g/100g, was found in accession IC 0646205.Similarly, variations are observed for the crude fibre content of the pulp of the accessions and a relatively high crude fibre content of 21.63±0.14 g/100g was reported in the pulp of accession IC 0646206. Hence, this carbohydrate rich fruit pulp was combined with fat and protein rich endosperm of coconut (*Cocos nucifera* L.) by the tribal population of Nicobar Islands to prepare a wholesome traditional weaning food for infants (Jaisankar, *et al*., 2022).

The fruit pulp of *P. leram* also showed a relatively high content of phenolics (34.26 ± 0.061 to 35.61 ± 0.413 mg GAE/g) and flavonoids (43.69±0.420 to 66.04±0.092 mg QE/g) with a commensurating antioxidant potential (Table 3 and Table 4). Scientific reports reveal that the dietary intake of polyphenols and polyphenol-rich foods has a positive effect on human health due to their effect toward the prevention and treatment of non-communicable diseases (Grosso 2018; Goya and de Pascual-Teresa 2023). Similarly, dietary uptake of vegetables and foods rich in polyphenols have demonstrated exemplary protective qualities against free radicals, and is associated with the reduced risk of cancer, cardiovascular diseases and stroke (Rudrapal *et al*., 2022).

To the best of our knowledge there are no reports of GC-MS based biochemical characterization of *P. leram* fruit pulp. Methanol extract of the fruit pulp of three different accessions revealed 34, 32 and 32 compounds in IC 0646202, IC 0646206, IC 646205, respectively. Several of the constituents revealed by GC–MS are biologically active, as reported elsewhere. It is known that the majority of these compounds possess multiple pharmacological activities. The predominant compound in IC 0646202, oleic acid, 3-deoxy-d-mannoic lactone are known to possess antibacterial activity and heart health promoting activities (Sales-Campos*et al*.,2013),whereas n-Hexadecanoic acid is shown to have better antioxidant, and hypocholesterolemic properties (Ganesan,*et al*.,2022)and 9S-Hydroxy-10E,12Z,15Z-octadecatrienoic acid are hydroxyl-oxylipins with multiple physiological and ecological significance (Kumari,*et al*., 2022). Similarly, in the fruit pulp of accession IC 0646206, besides fatty acids such as oleic acid (9-Octadecenoic acid), 9,12-Octadecadienoic acid (Z,Z) (omega 6 unsaturated fatty acid), cyclic ester like 3-Deoxy-d-mannoic lactone were predominant. The cardioprotective properties of fatty acids such as oleic acid and omega 6 unsaturated fatty acids are well known along with that anti-bacterial property of 3-Deoxy-d-mannoic lactone present in the pulp are a desirable addition (Ghosh, *et al*., 2015). Pulp of accession IC 0646205 revealed presence of sugars and fatty acids in abundance however a notable exception is presence of 1,2-Cyclopentanedione which is proven to be a phytocompound that downregulates transcription factor, nuclear factor-kappa B (NF-κB)- a pivotal factor involved in inflammation and aging process (Chung,*et al*., 2007).

Conclusion

Thus, this investigation sheds light on the biochemical properties and bioactive ingredients of three different accessions of *P. leram* fruit pulp endemic to Nicobar Islands. These plant-derived compounds have the potential to be utilized as prospective remedies for human diseases triggered by oxidative stress and as anti-inflammatory agents. Hence, it is crucial to conduct additional investigations into isolating pure compounds from the most potent extract. Moreover, further exploration of the biological activities, including the cardio protective effects and cytotoxic effects of the pure compounds from the plant extract, is essential to confirm their diverse bioactivity profiles. Also, this study warrants further investigation delving into exploring the clinical effects of these bioactive compounds and development of nutraceutical products from these fruits. It would go long way in harnessing the bioactive potential of underutilized fruits into mainstream food habits.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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