Phytochemical properties and antioxidant activities of *Pandanus amaryllifolius*

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ABSTRACT: *This study was performed to spread more public awareness as well as to know the medicinal and nutritional values of this plant in favour of humanity. Samples of Pandanus amaryllifolius were collected from three different locations in Andaman and Nicobar Islands and investigated for their nutritional, anti-nutritional and physicochemical characteristics, as well as antioxidant activity and the potent component was identified using Heat Map and PCA. The results revealed that a good concentration of carbohydrate (608.7 mg glucose/g), phenol (46.7 mg GAE/g), flavonoid (20.85 mg QE/g) in the plant parts added with notable TSS (4.80 ° Brix), chlorophyll (7.28 µg/g) and carotenoid (9.67µg/g) content in the leaves were ecorded. The antioxidant activities (80.21 % RSA) in plant parts propound potent applications in nutraceutical industries. The heat map and PCA illustrated that the leaf was the most potent plant parts than other plant parts and hence, P.amaryllifolius of Andaman and Nicobar Islands has potent phytochemical and antioxidant properties.*

Research Article

ARTICLE INFO

Received: 05.04.2023

Accepted: 25.11.2023

Keywords:
Pandanus amaryllifolius, Nutritional, Anti-nutritional, Physicochemicals, Antioxidants

1. INTRODUCTION

P. amaryllifolius Roxb. of the Pandanaceae family commonly known as Pandan Wangi is a native of the Moluccas Islands, Indonesia. It is a short shrub of about 1.2-1.5 m in height and 60 – 90 cm in width with a stout stem, often branched low down and has uniquely fragranced leaves of about 80 cm long and 5 cm wide (Wongpornchai, 2006). Flowering is rare and mostly male whereas, female flowers are unknown. The plant has aerial roots that are distinct not only in growth forms but also have root caps at the apex[2]. Owing to the aromatic properties of leaves, the plant is cultivated as a spice in tropical peninsular countries and in certain coastal regions of India (Wakte *et al.*, 2009; Stone, 1978). The fragrance and aroma of leaves are due to the presence of 2-acetyl-pyrroline (2AP) which is the principal aromatic compound in basmati and other scented rice varieties. The epidermal papillae are bag-like structures present under the leaf surface that serve as a sink for 2AP (Buttery *et al.*, 1983; Wakte *et al.*, 2007).

Besides, the leaves are widely used as a flavouring agent in Southeast Asian foods such as bakery products, sweets, biryani and home cooking and natural food colour due to their increased chlorophyll content (Gurmeet and Amrita, 2016). The Pandan protein in the leaves is known to have magnificent potential for a healthy body also the Pandan leaf consists of several alkaloids like Norpandamarilactonine-A, -B, Pandamarilactone-1, Pandamarilactonine-A, -B, -C, Pandamarine, and Pandanamine (Bhuyan and Sonowal, 2021). The juice extracted from the leaves is used for colouring cakes (Setyowati and Siemonsma, 1999). The dried leaf powder is used for preparing traditional pandan-flavoured rice (Yahya *et al.*, 2011) and Pandan-flavoured coconut jam called 'Kaya' in Malaysia (Khong *et al.*, 2007). The leaves have excellent health benefits when used in food and hence are exploited well in the flavour industries (Wakte *et al.*, 2007).

Besides its fragrant usage, it is also used medicinally as a diuretic, cardio-tonic and anti-diabetic (Wakte *et al.*, 2010). Pandan contains phytochemicals *viz.*, phenols, steroids, alkaloids, saponins, flavonoids, terpenoids, isoflavones, coumetarol, lignans, glycosides, carbohydrates, amino acids, and vitamins. It is also used as a potential natural anti-oxidant (Nor *et al.*, 2008). This aspect brings stimulus for further research in this plant. The research gap identified from the previous studies indicated that there are no reports on other plant parts except the leaves and roots of *P. amaryllifolius*. Considering unravels and the health benefits of this plant for society, our present research was designed to study the nutritional, anti-nutritional, physicochemical factors and anti-oxidant activities of *P. amaryllifolius* collected from three geographical locations of Andaman and Nicobar Islands, India.

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2. MATERIALS AND METHODS

Sample collection

Fresh Pandan plants were collected from three different locations in the Andaman and Nicobar Islands (Table 1).

The study area map depicting the collected sample locations is shown in Fig. 1. The study area map was prepared using ArcGIS 10.8 and QGIS (v3.10) platform open-source GIS Software (ESRI, 2021).

Extraction and phytochemical screening

The collected plants of *P. amaryllifolius* were washed in running water to get rid of the dust and foreign material adhered to the surface. Then the roots, stem and leaves were detached, cut into small pieces of about 5 cm, air dried under shade at room temperature, ground into coarse powder and methanolic extracts of fresh and dry samples of leaf, stem, and root were prepared for analysis using soxhlet apparatus in which about 50 g of sample powder was extracted with 1000 mL of methanol till the extracted colour in the siphon tube has become less intense and the extract collected in the flask was cooled to room temperature and filtered through Whatman No.1 filter paper and the filtrate was used for analysis (Abubakar and Haque, 2020).

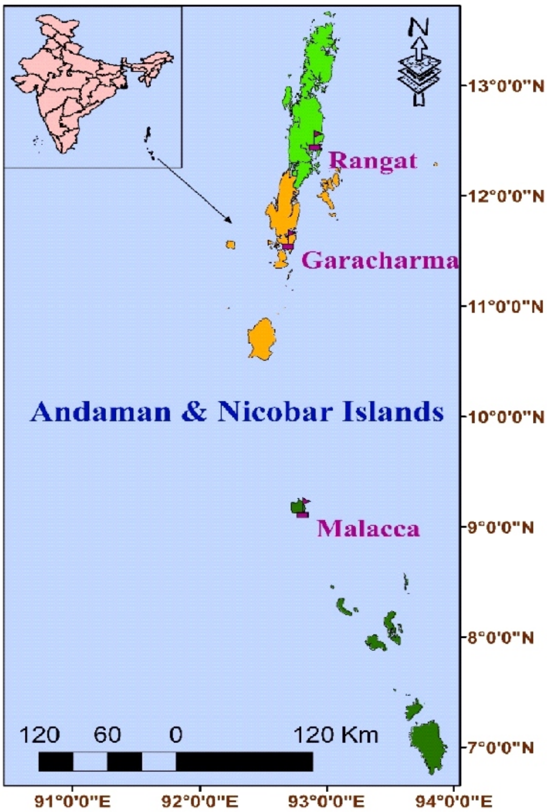

Fig. 1. Area map depicting the location of *P. amaryllifolius* collection

Nutritional Factors

The nutritional content in *P. amaryllifolius* was analyzed using the methanolic extracts of fresh samples where the Total Carbohydrate Content (Albalasmeh, 2013), Protein Content (Bonjoch, N.P. Tamayo, 2001) and Ascorbic acid Content (Al-Alimi *et al.*, 2017) were determined by Sulfuric Acid – UV method, Bradford method and titration method respectively.

Anti-nutritional Factors

Anti-nutritional factors such as total phenol content, total flavonoid content, total tannin content (Kavitha and Indira, 2016), total saponin content (Makkar *et al.*,2007), total anthocyanin content (Ayu *et al.*, 2018) were analysed in methanolic extracts of dry samples of root, stem and leaves of *P. amaryllifolius.*

Antioxidant activity

Methanolic extracts of dry samples of root, stem and leaves of *P. amaryllifolius* were subjected to antioxidant analysis *viz.*, α, α-diphenyl-β-picrylhdrazyl (DPPH) Radical Scavenging activity, Hydroxyl Radical Scavenging Activity (HRSA), Superoxide Radical Scavenging activity (SRSA) (Yu *et al.*, 2019) 2, 2' -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Free Radical Scavenging Activity and Ferric Reducing Antioxidant Power (FRAP) (Rajurkar *et al.*, 2011).

Physicochemical factors

Physicochemical factors such as Total Chlorophylls (Kavitha and Indira, 2016), Total Carotenoid content (Lichtenthaler and Buschmann, 2001), pH and Total Soluble Solids (TSS) (Singh *et al.*, 2001) were analysed in methanolic extracts of fresh samples of root, stem and leaves of *P. amaryllifolius*.

Statistical Analysis

Statistical analysis of phytochemical and antioxidant activities was performed where the data were analysed using R Studio version 4.1.1. Normally distributed data were expressed as Mean ±SD. The comparison

Table 1: Details of Pandan Accessions collected from Andaman and Nicobar Islands

Sl. No.	Accession number	Place of sample collection	Island group	Geographical location		
				Latitude (N)	Longitude (E)	Altitude (mMSL)
1.	Acc. 1	Rangat	Middle Andaman	12º35´17.01´´	92º57´32.06´´	7
2.	Acc. 2	Garacharma	South Andaman	11º37´51.36´´	92 º 44´02.82´´	10
3.	Acc. 3	Malacca	Nicobar	9º10´11.14´´	92º49´45.46´´	6

was performed using two-way ANOVA without repetition. The criterion for statistical significance was $p < 0.05$. Post Hoc analysis was performed when the ANOVA test came out significant (R Studio, 2021).

Heat Map and Principal Component Analysis (PCA)

The heat map is a visual representation of a two-dimensional plot, in which data is represented by colors and distinguished using various *dendrograms*. The colour bar in heat map indicates the amount in number from the highest (blue) to the lowest (red), intuitive to see the characteristics of different factors. The PCA is one of the most used multivariate analysis methods to reduce the number of variables in multivariate data sets, whilst retaining as much as possible of variation in the present data set. This reduction was achieved by taking *n* variables X_1, X_2..., X_n and finding the combinations of these to produce principal components/factors PC_1, PC_2..., PC_n, which were uncorrelated. These principal components are also termed eigenvectors. The lack of correlation was a useful property as it indicated that factors were measuring different dimensions in the data. Nevertheless, factors were ordered so that the PC_1 exhibits greatest amount of variation, PC_2 exhibits the second greatest amount of variation and so on. The variance (PC_1) The eigenvalues reflect the quality of the projection from the N-dimensional initial to a lower number of dimensions. The method for assessing an adequate number of factors/PCs is the eigenvalue - one criterion, also known as the Kaiser criterion, in which only the factors where eigenvalue $\geq$1 is retained (Kaiser, 1958). On the other hand, any factor with an eigenvalue of less than 1 contained less information than one of the original variables and so was not worth retaining. Both the heat map and PCA are performed using 'pheatmap' and 'FactoMineR & 'factoextra' packages, respectively in R Studio 4.1.1 (R Studio, 2021).

3. RESULTS AND DISCUSSION

Nutritional properties of Pandan

Among the plant parts analyzed, the root had the highest carbohydrate (496.67 ± 97.78 mg glucose/g) content, the lowest ascorbic acid (4.40 ± 0.72 mg/g) content and leaves had the highest protein (23.94 ± 1.52 mg BSA/g), ascorbic acid (9.14 ± 0.78 mg/g) content respectively. Among the locations, highest carbohydrate (461.73 ± 168.98 mg glucose/g) and protein (24.47 ± 1.22 mg BSA/g) content was recorded in Acc. 2 whereas the ascorbic acid (6.65 ± 3.05 mg/g) content was high in Acc. 1. Significantly highest carbohydrate content of 608.7 mg glucose/g was recorded in Acc. 2 root and lowest carbohydrate content in Acc. 2 leaves (277.1 mg glucose/g). Protein content was high in Acc. 1 root (25.65 mg BSA/g) and low in Acc. 3 root (21.95 mg BSA/g). The highest ascorbic acid content of about 9.99 mg/g was recorded in Acc. 1 leaf and lowest in Acc. 3 root (3.98 mg/g) (Table 2). However, this was lower than the carbohydrate (21.89%), protein (1.45%), and ascorbic acid (12.04%) content in Pandan leaf powder observed by Prathiba.[27] Besides, the root (608.7 mg glucose/g) and root (9.99 mg/g) of Acc. 3 and 1 respectively possessed comparatively high carbohydrates and ascorbic acid concentration respectively. On the other hand, Acc. 2 possessed high levels of protein which was higher than the varied protein (0.0026-0.773g/100g) and carbohydrate (0.198-5.212 g/100g) concentrations observed by Deb and Khruomo (2021) in leafy vegetables of Nagaland. The varied carbohydrate, protein and ascorbic acid concentrations could have a significant role in plant immunity therefore could be used as foliar sprays (Trouvelot *et al.*, 2014) suggesting novel information regarding the application of *P.amaryllifolius* in crop protection studies.

Table 2. Nutritional properties of *P.amaryllifolius*

Parameter	Location	Root	Stem	Leaf	Mean ± SD
Carbohydrates (mg glucose/g)	Acc. 1	452.80	287.90	363.30	368.00 ± 82.55
	Acc. 2	608.70	499.40	277.10	461.73 ± 168.98
	Acc. 3	428.50	356.40	510.20	431.70 ± 76.95
	Mean ± SD	496.67 ± 97.78	381.23 ± 107.91	383.53 ± 117.86	
Protein (mg BSA/g)	Acc. 1	25.65A,a	22.65C,b	22.49C,c	23.60 ± 1.78
	Acc. 2	23.14B,c	24.75A,b	25.53A,a	24.47 ± 1.22
	Acc. 3	21.95C,c	23.42B,b	23.80B,a	23.06 ± 0.98
	Mean ± SD	23.58 ± 1.89	23.61 ± 1.06	23.94 ± 1.52	
Ascorbic acid (mg/g)	Acc. 1	4.00	5.96	9.99	6.65 ± 3.05
	Acc. 2	5.23	6.02	8.45	6.57 ± 1.68
	Acc. 3	3.98	6.98	8.97	6.64 ± 2.51
	Mean ± SD	4.40 ± 0.72	6.32 ± 0.57	9.14 ± 0.78	

Note: Superscripts (a-c) in columns and (A-C) in rows represent statistical significance at $p<0.05$.

Anti-nutritional properties in Pandan

The anti-nutritional factors such as phenols, flavonoids, tannins, saponins and anthocyanin content in Pandan revealed that the total tannin content significantly varied among the plant parts as well as locations whereas the total saponin content varied significantly among the locations. Howbeit, the total phenol, total flavonoid and total anthocyanin showed no significant difference either among the plant parts or the locations (Table 3). Among the plant parts, the leaves recorded high phenol (33.17 ± 11.74 mg GAE/g), tannins (2.75 ± 1.08 mg/g) and saponin (1.28 ± 0.38 mg DE/g) content whereas high flavonoid (17.35 ± 3.65 mg QE/g) and anthocyanin (3.43 ± 1.12 mg CYE/100g) content was recorded in root and stem respectively. While comparing locations, Acc. 1 recorded high saponin (1.27 ± 0.23 mg DE/g) and anthocyanin (3.43 ± 1.12 mg CYE/100g) content whereas Acc. 2 recorded high phenols (32.93 ± 13.1 mg GAE/g), flavonoids (18.24 ± 2.78 mg QE/g) and Acc. 3 had high tannin (2.37 ± 1.08 mg/g) content respectively. Collectively, the phenol was high in Acc. 2 leaves (46.7 mg GAE/g) and low in Acc. 2 stem (20.73 mg GAE/g). The flavonoid content was high in Acc. 2 root (20.85 mg QE/g) and low in Acc. 3 root respectively. The tannin content was significantly high in Acc. 3 leaves (3.58 mg/g) and low in Acc. 1 root (0.05 mg/g). The saponins were high in Acc. 1 leaves (1.52 mg DE/g) and low in Acc. 3 root (0.66 mg DE/g) whereas the anthocyanin content was high in Acc. 1 stem (4.34 mg CYE/ 100 g) and low in Acc. 3 root (0.56 mg CYE/ 100 g) respectively.

Similar studies conducted by Quyen *et al*., (2020) in *P. amaryllifolius* methanolic extracts obtained comparatively lower total phenol (38.12±1.49 mg GAE/g) and flavonoid (11.79±0.44mg QE/g) content and reported to have antioxidant activity in IC_{50} value of about 129.32 µg/ml DPPH and 104.31 µg/ml ABTS activity respectively. Generally, young leaves are rich in phenolic compounds whereas the root and stem are attributed to specific phenolic compounds like proanthocyanidins and quinic acid respectively (Liu *et al*., 2020; Jiang *et al*., 2013). This ensures the potency of plant parts of *P.amaryllifolius* for desirable applications. However, the expression pattern of the related genes in phenol biosynthesis was often specific towards the tissue and developmental phase of the plant (Fathi *et al*., 2019). Therefore, understanding the phenolic hologram of *P.amaryllifolius* could give novel information on the effect and regulation of phenol biosynthetic pathways. Moreover, the highest flavonoid content was observed in the root of Acc. 2 (20.85 mg QE/g). Furthermore, Pandan collected from different locations in Malaysia had comparatively lower total phenol (6.72mg/g) and total flavonoid (1.87mg/g DW) content and possessed antioxidants as well as anticancer activities (Ghasemzadeh and Jaafar, 2013).

Table 3. Anti-nutritional factors of *P.amaryllifolius*

Parameter	Location	Root	Stem	Leaf	Mean ± SD
Total Phenols (mg GAE/g)	Acc. 1	24.31	23.06	27.19	24.85 ± 2.12
	Acc. 2	31.36	20.73	46.7	32.93 ± 13.1
	Acc. 3	25.51	22.09	25.63	24.41 ± 2.01
	Mean ± SD	27.06 ± 3.77	21.96 ± 1.17	33.17 ± 11.74	
Total Flavonoids (mg QE/g)	Acc. 1	17.64	13.86	14.63	15.38 ± 2.00
	Acc. 2	20.85	15.32	18.54	18.24 ± 2.78
	Acc. 3	13.56	15.42	17.59	15.52 ± 2.02
	Mean ± SD	17.35 ± 3.65	14.87 ± 0.87	16.92 ± 2.04	-
Total Tannins (mg/g)	Acc. 1	$0.05^{C,c}$	$1.24^{C,b}$	$1.53^{C,a}$	0.94 ± 0.78
	Acc. 2	$0.41^{B,c}$	$2.08^{A,b}$	$3.13^{B,a}$	1.87 ± 1.37
	Acc. 3	$1.5^{A,c}$	$2.04^{B,b}$	$3.58^{A,a}$	2.37 ± 1.08
	Mean ± SD	0.65 ± 0.76	1.79 ± 0.47	2.75 ± 1.08	-
Total Saponins (mg DE/g)	Acc. 1	1.08^{A}	1.20^{A}	1.52^{A}	1.27 ± 0.23
	Acc. 2	0.90^{B}	0.77^{B}	1.48^{B}	1.13 ± 0.74
	Acc. 3	0.66^{C}	0.69^{C}	0.84^{C}	0.73 ± 0.10
	Mean ± SD	0.87 ± 0.54	0.89 ± 0.27	1.28 ± 0.38	-
Total Anthocyanins (mg CYE/100g)	Acc. 1	1.51	4.34	4.05	3.30 ± 1.56
	Acc. 2	0.91	2.18	0.94	1.34 ± 0.72
	Acc. 3	0.56	3.78	3.79	2.71 ± 1.86
	Mean ± SD	0.99 ± 0.48	3.43 ± 1.12	2.93 ± 1.73	-

Note: Superscripts (a-c) in columns and (A-C) in rows represent statistical significance at $p<0.05$.

Although, on comparing the plant parts of this study, strikingly, the leaves had high levels of total phenol, total tannin, and total saponin concentration whereas the root and stem recorded high levels of flavonoid and anthocyanin content suggesting to enhance the utilization of these leaves for value addition in food. Similarly, the high flavonoid content in the root can exert antimicrobial activity through different mechanisms such as inhibition of nucleic acid synthesis, disturbing the functions of the cytoplasmic membrane and energy metabolism (Tim *et al*., 2005). This claims further research on the quorum sensing studies of the plant and its microbiome. Further, the saponin levels were lesser in Acc. 3 and higher in Acc. 1 and were straight opposite to the tannin levels concerning geography which opens novel studies rooting towards the plant's adaption to the environmental, genetic, climate and edaphic conditions. Similar variation in secondary metabolites were observed in *Marrubium vulgare* L. collected from two different localities of Northeast Morocco were reported that the variation was due to edaphic factors (Hayat *et al*., 2020). Moreover, the antinutritional content of this study was on par with the reports of Popova and Mihayloya (2019). This adds essence to the use *P.amaryllifolius* as a substitute food for legumes, tubers and nightshades stressing its significance in nutraceutical research and application.

Physicochemical factors of Pandan

The physicochemical factors varied significantly among plant parts as well as locations (Table 4). The pH was high in Acc. 3 root (6.94) and low in Acc. 1 stem (6.22). In terms of plant parts, the pH was high in the root and low in the stem whereas on comparing locations, the pH was high in Acc. 3 (Malacca) and low in Acc. 1 (Rangat). Moreover, it was reported that there was an inverse association between the chlorophyll concentration and leaf apoplastic pH (Mengel *et al*., 1994). The leaves of Acc. 1 recorded significantly high TSS (4.80˚Brix), total chlorophyll (7.28 µg/g) and carotenoid (9.67 µg/g) content. On the other hand, the TSS was low in the root (0.30˚Brix) of Acc. 1 and 3 whereas the total chlorophyll content was nil in the root of the three accessions respectively. Whilst, the carotenoid content was high in Acc. 1 leaves and comparatively low in Acc. 3 roots (5.60 µg/g).

Moreover, the chlorophyll content was higher in the leaf than in the stem and root, and unlike chlorophyll, the carotenoid levels had significant variation among the three Accessions (Table 4) in terms of plant parts as well as locations. However, this variation might be due to environmental factors (Li *et al*., 2018). Howbeit, the chlorophyll levels reflect on the photosynthetic efficiency of the plant (Liu *et al*., 2017). Therefore, this similarity in chlorophyll levels claims the involvement of similar plant hormones in the photosynthesis of *P.amaryllifolius*. Although, the mean TSS was high in the leaf (4.67 ± 0.15º Brix) and low in the root (0.33±0.06º Brix). TSS often accumulates in plant parts at water stress thereby reducing osmotic potential and has a pivotal role by serving an indicator for drought stress (Levy *et al*., 2006). Consequently, the reduced TSS levels in the root of *P.amaryllifolius* may be a strong indicator that the three accessions grown in water sufficient area. This was in line with Singh *et al*., (2001) who reported

Table 4. Physicochemical factors of *P.amaryllifolius*

Parameter	Location	Root	Stem	Leaf	Mean ± SD
pH	Acc. 1	6.70a,C	6.22c,C	6.68b,C	6.53 ± 0.27
	Acc. 2	6.88a,B	6.26c,B	6.80b,B	6.65 ± 0.34
	Acc. 3	6.94a,A	6.50c,A	6.85b,A	6.76 ± 0.23
	Mean ± SD	6.84 ± 0.12	6.33 ± 0.15	6.78 ± 0.09	-
TSS (Brix)	Acc. 1	0.30c	1.50b	4.80a	2.20 ± 2.33
	Acc. 2	0.40c	1.20b	4.50a	2.03 ± 2.17
	Acc. 3	0.30c	1.40b	4.70a	2.13 ± 2.29
	Mean ± SD	0.33 ± 0.06	1.37 ± 0.15	4.67 ± 0.15	-
Total Chlorophyll (µg/g)	Acc. 1	0.00^{c}	5.21^{b}	7.28^{a}	4.16 ± 3.75
	Acc. 2	0.00^{c}	5.17^{b}	6.27^{a}	3.81 ± 3.35
	Acc. 3	0.00^{c}	5.25^{b}	7.15^{a}	4.13 ± 3.70
	Mean ± SD	0.00 ± 0.00	5.21 ± 0.04	6.90 ± 0.55	-
Carotenoids (µg/g)	Acc. 1	$7.65^{c,A}$	$8.67^{b,A}$	$9.67^{a,A}$	8.66 ± 1.01
	Acc. 2	$6.70^{c,B}$	$7.72^{b,B}$	$8.72^{a,B}$	7.71 ± 1.01
	Acc. 3	$5.60^{c,C}$	$6.89^{b,C}$	$7.62^{a,C}$	6.70 ± 1.02
	Mean ± SD	6.65 ± 1.03	7.76 ± 0.89	8.67 ± 1.03	-

Note: Superscripts (a-c) in columns and (A-C) in rows represent statistical significance at $p<0.05$.

that higher TSS of the common wild edible fruits were recorded in Andaman and Nicobar Islands. In addition, the presence of a significant quantity of TSS correlates with its sugar content which is linked to the development of non-structural carbohydrates and further phenolic compounds synthesis which leads to antioxidant properties (Resendez *et al.*, 2016).

Antioxidant activity of Pandan

Analysing the antioxidant activities like DPPH, Hydroxyl, Superoxide Anion, ABTS and FRAP activity in plant parts of *P.amaryllifolius* revealed that they don't show any significant difference among the plant parts as well as the locations. The DPPH activity was high in Acc. 3 leaves (80.21% RSA) and low in Acc. 2 root (45.74% RSA). This was higher than the DPPH (64.27%) activity of Pandan from three different locations in Malaysia which possessed anti-cancer promoting activity against the MCF-7 (Michigan Cancer Foundation-7) cell line which is the human breast cancer cell line, and the maximum cell line inhibition was achieved at 66.3% antioxidant activity (Ghasemzadeh and Jaafar, 2013). The HRSA was high in Acc. 3 root (60.00% RSA) and low in Acc. 1 leaves (18.00% RSA) whereas the SRSA was high in Acc. 1 leaves (79.11% RSA) and low in Acc. 1 root (59.43% RSA). The ABTS activity was high in Acc. 2 leaves (13.20% RSA) and low in Acc. 1 stem (5.09% RSA) whereas the highest FRAP activity of 4712.48 µM (TE)/g DW was recorded in Acc. 1 leaves and lowest of 1112.16 µM (TE)/g DW in Acc. 1 stem (Table 5).

Higher FRAP (3257.8±53.8 mmol Fe (II)/g extract), ABTS (25435.2±1798.1 µmol Trolox/ g extract) and hydroxyl radical scavenging activity (84.4%) added with strong correlation between DPPH (r = 0.972), FRAP (r = 0.928), ABTS (r = 0.995) and hydroxyl (r = 0.998) radical with total flavonoid content as well as respective correlation coefficient of 0.900, 0.828, 0.981, 0.970 with total phenolic content was reported in the methanolic extracts of leaves of *Bauhinia vahlii* (Sowndhararajan and Kang, 2013). Present study, the FRAP activity was higher than the observations by Thatsanasuwan *et al.*, (2015) in *P. amaryllifolius* leaves (23.16±0.23 mg TE/g DW). Further, the highest superoxide radical scavenging activity of 93.91±1.37% in the methanolic extracts of the leaves of *Blumea balsamifera* antioxidant activity was regulated by the presence of flavonoids and polyphenolic compounds (Fazilatun *et al.*, 2004). As the plant parts of *P. amaryllifolius* of Andaman and Nicobar Islands were rich in anti-nutritional compounds like phenols and flavonoids, it again emphasizes the significance of its application in relevant fields.

Heat map visualization

A dendrogram-based heat map was applied to visualize the differences in the nutritional, anti-nutritional, physicochemical and antioxidant factors

Table 5. Antioxidant factors of *P.amaryllifolius*

Parameter	Location	Root	Stem	Leaf	Mean ± SD
DPPH (%RSA)	Acc. 1	59.79	51.70	62.87	58.12 ± 5.77
	Acc. 2	45.74	51.81	74.36	57.30 ± 15.08
	Acc. 3	64.26	54.26	80.21	66.24 ± 13.09
	Mean ± SD	56.60 ± 9.66	52.59 ± 1.44	72.48 ± 8.82	-
Hydroxyl Radical Scavenging activity (%RSA)	Acc. 1	23.30	38.20	18.00	26.50 ± 10.47
	Acc. 2	34.40	35.10	50.40	39.97 ± 9.04
	Acc. 3	60.00	53.30	49.10	54.13 ± 5.50
	Mean ± SD	39.23 ± 18.82	42.20 ± 9.74	39.17 ± 18.34	-
Superoxide Radical Scavenging activity (%RSA)	Acc. 1	59.43	67.83	79.11	68.79 ± 9.88
	Acc. 2	64.36	66.85	68.84	66.68 ± 2.24
	Acc. 3	64.62	63.47	69.02	65.70 ± 2.93
	Mean ± SD	62.80 ± 2.92	66.05 ± 2.29	72.32 ± 5.88	
ABTS (%RSA)	Acc. 1	5.86^{C}	5.09^{C}	8.57^{C}	6.51 ± 1.83
	Acc. 2	11.38^{A}	12.05^{A}	13.20^{A}	12.21 ± 0.92
	Acc. 3	6.76^{B}	8.42^{B}	13.00^{B}	9.39 ± 3.23
	Mean ± SD	8.00 ± 2.96	8.52 ± 3.48	11.59 ± 2.62	-
FRAP[µM (TE)/g DW]	Acc. 1	1758.29^{c}	1112.16^{b}	4712.48^{a}	2527.64 ± 1919.51
	Acc. 2	2710.90^{a}	1036.30^{c}	3118.48^{c}	2288.56 ± 1103.47
	Acc. 3	2240.13^{b}	2684.04^{a}	4701.42^{B}	3208.53 ± 1311.79
	Mean ± SD	2236.44±476.32	1610.83±930.20	4177.46±917.12	-

Note: Superscripts (a-c) in columns and (A-C) in rows represent statistical significance at $p<0.05$.

for root, leaf, and stem of Pandan at Acc. 1, Acc. 2, and Acc. 3 (Fig. 2).

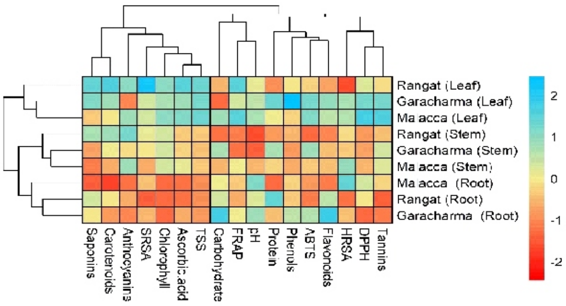

Fig. 2. Heat map visualization in *Pandanus amaryllifolius*

The blue colour indicates high values while the red colour indicates low values for different factors of Pandan. The heat map indicated that the leaf component of Pandan at Acc. 1, Acc. 2, and Acc. 3 is different from the root and stem components as it exhibited high values in most of the factors. Similarly, the root component at Acc. 1, Acc. 2, and Acc. 3 resulted in low values. The dendrogram connections revealed that HRSA, Phenols, Carbohydrates, SRSA, Chlorophyll, and Anthocyanins are exhibiting unique factors and are not correlated directly with other factors. Similarly, leaf and stem components at Acc. 1 and root components at the Acc. 3 are exhibiting unique values in terms of their factors. Thus, the optimum component for further use and analysis is the leaf.

Principal Component Analysis (PCA)

PCA was applied to 17 different nutritional, anti-nutritional, physicochemical, and antioxidant factors of the root, leaf, and stem of Pandan at Acc. 1, Acc. 2, and Acc. 3 locations. The factors analyzed were carbohydrates, protein, ascorbic acid, DPPH, HRSA, SRSA, ABTS, FRAP, phenols, flavonoids, tannins, saponins, anthocyanins, pH, TSS, chlorophyll, carotenoid (Table 6). PCA reduced the original 17 variables into five principal components, in which PC1 to PC5 had eigenvalues more than one. The first two eigenvalues represented 60.43% of the total variability in the data. Similarly, the first five eigenvalues represented 92.86% of the total variability in the data. This means, that representing the data on the first two axes can ensure good-quality projection of the initial multi-dimensional table.

The specific pattern and contribution of variables (factors) to the principal components can be further visualized with the help of a two-dimensional map of PCA (Fig. 3 a and b). All factors were clustered into 3 classes: Cluster 1: TSS, ascorbic acid, saponins, SRSA, chlorophyll, carotenoid, anthocyanin; Cluster 2: PH, HRSA, FRAP, tannin, DPPH, phenol; and Cluster 3: ABTS, protein, flavonoid, carbohydrate. Here, Cluster 1 factors are very important for representing Pandan as they are away from the centre (Fig. 3).

The arrows in the biplot and the angle between the arrows explain the relationship/correlation between the variables. All the variables are positively correlated as they are away from the centre except for HRSA and protein (Fig. 3b). The greater the length of the arrow, the greater the influence of the variable. Numbers 1, 2, and 3 represent the root component at 3 locations while 4, 5 and 6 represent the stem component at 3 locations, and 7, 8 and 9 represent the leaf component at 3 locations (Fig. 3b). Numbers 4, 5 and 6 are placed on the lower left side quadrant indicating lower negative values.

Table 6. Eigen value analysis of the significant principal components

Parameter	Dimension							
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Eigen value	6.60	3.67	2.36	1.67	1.48	0.69	0.41	0.11
Variance (%)	38.83	21.60	13.89	9.84	8.70	4.09	2.42	0.64
Cumulative variance (%)	38.83	60.43	74.32	84.16	92.86	96.95	99.36	100.00

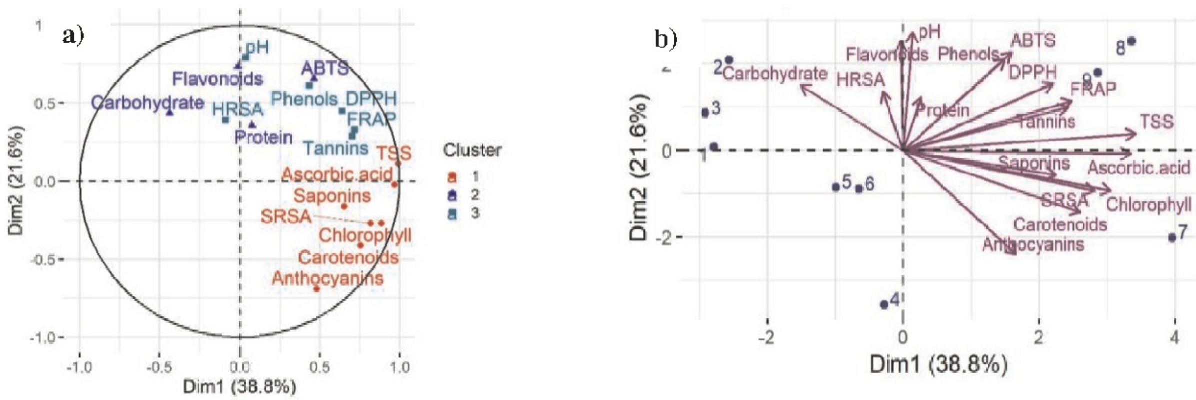

Fig. 3. Biplot graph of factors of *P. amaryllifolius* with PCA

PCA of phytochemical properties in *P. amaryllifolius* of Andaman and Nicobar Islands revealed that the pigments as well as the superoxide radical scavenging activity and saponins have been clustered distinctly and significantly. An analytical study conducted by PCA and Heat Map analysis on phytochemical and antioxidant activity of liverworts by Mukhia *et al.*, (2017) reported a strong correlation between polyphenolic compound and antioxidant activity and added that DPPH and ABTS were strongly correlated with steroid and tannin content of the plant which reveals the significance effects of antinutritional compounds over antioxidant activity. Notably, the ascorbic acid of *P. amaryllifolius* has been clustered distinctly which shows that it has much more capabilities to be revealed. Strikingly, all the above-mentioned factors were found higher in leaves, other than the stem and root, which emphasizes the significance of *P. amaryllifolius* of Andaman and Nicobar Islands. This was further confirmed in the heat map that the leaves have more strength than that of stem and root. Further, the dendrogram reveals that the leaves of three locations are related either directly or indirectly. This insists that the leaves of *P. amaryllifolius* may have some promising applications and claims for further research in this area.

The presence of both nutritional factors and anti-nutritional factors highlighted that *P. amaryllifolius* has both nutritional and anti-nutritional importance. Also, these anti-nutritional properties can be suggested to include the leaves as value addition in the food industry and also as nutraceutical due to the similar anti-nutritional composition of different foods. Concentration-oriented studies on the relation between anti-nutritional compounds and anti-oxidant properties would give extraneous information. The presence of chlorophyll and carotenoid as natural pigments in the leaves demonstrated to have antioxidant properties. The presence of a significant quantity of TSS in the leaves correlates with its sugar content, formation of non-structural carbohydrates, synthesis of phenolic compounds, and antioxidant properties. Statistical analysis by heat map and PCA revealed that the leaves have better potential than the stem and root. Further research on the pigments and antioxidant properties of the leaf may unravel more health benefits. The roots were rich in flavonoids which insisted the prevalence of better microbial activity emphasizing Quorum sensing studies. In terms of geographical location, the Rangat accession was potent compared to Garacharma and Malacca accessions. Further, our results concluded that *P. amaryllifolius* of Andaman and Nicobar Islands have exemplary nutritional value with antioxidant activity which can be further investigated for its various pharmacological benefits.

Declarations

Ethical approval: This article contains no studies on human or animal objects

Competing Interests: The authors declare that they have no competing interests.

Funding: This study is a part of the project entitled "Bio-prospecting of *Pandanus* sp. (Kewda) of Andaman and Nicobar Islands for its medicinal properties" funded by the National Medicinal Plants Board, Ministry of AYUSH, Government of India [Project Number R&D/AN-01/2020-21].

REFERENCES

Abubakar, A.R. and Haque, M. 2020. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bio allied Sci.*, 12: 1-10.

Al-Alimi, K.R., Abdul Razak, A.A. and Alabsi, A.M. 2017. Tannins acid, ascorbic acid and fluoride from khat chewing plant. *Int J Dent Oral Health*., 3: 01-05. http://dx.doi.org/10.16966/2378-7090.240.

Albalasmeh, A.A.;,Berhe, A.A. and Ghezzehei, T.A. 2013. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydr Polym*., 97: 253-261.

Ayu, A.C., Ida, M., Moelyono, M. and Fakhriati, S.G. 2018. Total anthocyanin content and identification of anthocyanidin from *Plectranthus scutellarioides* (L.) R. Br leaves. *Res J Chem Environ*., 22:11-17.

Bhuyan, B. and Sonowal, R. 2021.An overview of *Pandanus amaryllifoliu s*Roxb. ex Lindl. and its potential impact on health. *Curr Trends Phar Res*., 8:138-157.

Bonjoch, N.P. and Tamayo PR. 2001. Protein content quantification by Bradford method. In: Reigosa Roger, M J (ed) Handbook of plant ecophysiology techniques. Dordrecht: Springer, Netherlands, pp. 283-295. https://doi.org/10.1007/0-306-48057-3_19.

Buttery, R.G., Ling, L.C., Juliano, B.O. and Turnbaugh, J.G. 1983. Cooked rice aroma and 2-acetyl-1-pyrroline. *J Agric Food Chem*., 31:823-826. https://doi.org/10.1021/jf00118a036.

Deb, C. R. and Khruomo, N. 2021. Assessment of Nutrient composition and antioxidant activity of some popular underutilized edible crops of Nagaland, India. *Natural Resources*, 12(02): 44.

ESRI, R. 2021. ArcGIS Desktop: Release 10.8. Redlands, CA: Environmental Systems Research Institute.

Fathi, E., Majdi, M., Dastan, D. and Maroufi, A. 2019. The spatio-temporal expression of some genes involved in the biosynthetic pathways of terpenes/phenylpropanoids in yarrow (*Achillea millefolium*). *Plant Physiol. Biochem.*, 142: 43-52.

Fazilatun, N., Nornisah, M. and Zhari, I. 2004. Superoxide radical scavenging properties of extracts and flavonoids isolated from the leaves of *Blumea balsamifera*. *Pharm. Biol.*, 42(6): 404-408.

Ghasemzadeh, A.and Jaafar, H.Z. 2013. Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (*Pandanus amaryllifolius* Roxb.) extracts from different locations of Malaysia. *BMC Complement Altern Med.*, 13:1-9, https://doi.org/10.1186/1472-6882-13-34

Gurmeet, S. and Amrita, P. 2016. Unique pandanus-flavour, food and medicine. *J Pharmacogn Phyto chem.*, 5: 08-14.

Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S.and Belmalha, S. 2020. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon,* 6(11): e05609.

Jiang, X., Liu, Y., Li, W., Zhao, L., Meng, F., Wang, Y., Tan, H., Yang, H., Wei, C., Wan, X., Gao, L. and Xia, T. 2013. Tissue-specific, development-dependent phenolic compounds accumulation profile and gene expression pattern in tea plant [*Camellia sinensis*]. *PloS one*, 8(4): e62315.

Kaiser, H.F. 1958. The varimax criterion for analytic rotation in factor analysis. *Psychometrika*., 23:187-200.

Kavitha Chandran, C.I. and Indira, G. 2016. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (Neelakurinji). *J Med Plants Stud.*, 4:282-286.

Khong, M.K., Chin, N.Y., Ibrahim, M.N. and Taip, F.S. 2007. Processing of kaya using jacketed mixer with microcontroller. *Int J Eng Technol.*, 4: 36-43.

Levy, D., Fogelman, E., Itzhak, Y., Ma, Q., Turner, D. W. and Cowling, W. 2006. Osmotic adjustment in leaves of Brassica oilseeds in response to water deficit. *Canadian journal of plant science*. , 86(2): 389-397.

Li, Y., Nianpeng, H., Hou, J., Xu, L., Liu, C. Zhang, J. Wang, Q. Zhang, X. and Wu, X. 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front Ecol Evol.*, 6: 64. https://doi.org/10.3389/fevo.2018.00064.

Lichtenthaler, H.K. and Buschmann, C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr Protoc Food Anal Chem.*, 1: 4-3.

Liu, X., Li, Y. and Zhong, S. 2017. Interplay between light and plant hormones in the control of Arabidopsis seedling chlorophyll biosynthesis. *Front. Plant. Sci.*, 8: 1433.

Liu, Z., Bruins, M. E., de Bruijn, W. J. and Vincken, J. P. 2020. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. *J. Food Compos*., 86:103-385.

Makkar, H.P., Siddhuraju, P., Becker, K., Makkar, H.P., Siddhuraju, P. and Becker, K. 2007. Saponins. In: Clifton N J (ed) Plant secondary metabolites: Methods Mol Biol. Humana Press, Germany, pp 93-100. https://doi.org/10.1007/978-1-59745-425-4_16.

Mengel, K., Planker, R. and Hoffmann, B. 1994. Relationship between leaf apoplast pH and iron chlorosis of sunflower (*Helianthus annuus* L.). *J Plant Nutr*., 17: 1053-1065. https://doi.org/10.1104/pp.121.4.1069.

Mukhia, S., Mandal, P., Singh, D. K. and Singh, D. 2017. Study of bioactive phytoconstituents and in-vitro pharmacological properties of Thallose Liverworts of Darjeeling Himalaya. *J Pharm Res*, 11(5): 490-501.

Nor, F.M., Mohamed, S., Idris, N.A. and Ismail, R. 2008. Antioxidative properties of *Pandanus amaryllifolius* leaf extracts in accelerated oxidation and deep-frying studies. *Food Chem.*, 110:319-327. https://doi.org/10.1016/j.foodchem.2008.02.004.

Popova, A. and Mihaylova, D. 2019. Antinutrients in plant-based foods: A review. *Open Biotechnol. J.*, 13(1): 68-76.

Prabitha, M. 2023. Phytonutrient analysis and sensory evaluation study on *Pandanus amaryllifolius*. *Int J Create Res Thoughts*., 11:636-653.

Quyen, N.T.C., Quyen, N.T.N., Nhan, L.T.H. and Toan, T.Q. 2020. Antioxidant activity, total phenolics and flavonoids contents of *Pandanus amaryllifolius* (Roxb.). In Proceedings of IOP Conference Series*: Mater Sci Eng.*, 991:12-19.

R Studio Team. *R Studio.* 2021. *Integrated Development Environment for R. Boston, MA*; Retrieved from https://www.rstudio.com/

Rajurkar, N.S.and Hande, S.M. 2011. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian J Pharm Sci.*, 73:146-151.

Resendez, A.M., Solano, R.P., Carrillo, J.L.R., Perez, L.S., Lujan, M.R.M., Aragon, M.G.R. and Dimas, N.R. 2016. Organic manures improved the phenolic content, antioxidant capacity and soluble solids in Pepper. *Food Nutr Sci.*, 7:1401-1413.

Setyowati, F.M. and Siemonsma, J.S. 1999. *Pandanus amaryllifolius* Roxb. In de Guzman C, Siemonsma JS (ed), Plant Resour South-East Asia, Spices, 13th Edn. Backhuys, Leiden, pp. 164-166.

Singh, D.B., Attri, B.L., Sharma, T.V.R.S. and Sreekumar, P.V. 2001. Nutrient composition of some wild edible fruits of Andaman and Nicobar Islands*. J Appl Hortic.*, 3: 60-62.

Sowndhararajan, K.and Kang, S.C. 2013. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. *Saudi J. Biol. Sci.*, 20 (4): 319-325.

Stone, B.C. 1978. Studies in Malesian Pandanaceae XVII on the taxonomy of 'PandanWangi'A Pandanus cultivar with scented leaves. *Econ Bot.*, 32: 285-293. https://doi.org/10.1007/BF02864702.

Thatsanasuwan, N., Srichamnong, W., Chupeerach, C., Kriengsinyos, W. and Suttisansanee, U. 2015. Antioxidant activities of *Pandanus amaryllifolius* leaves extracted under four designed extraction conditions. *Food Appl Bio Sci*., 3:130-136.

Tim Cushnie, T.P.and Andrew, J. Lamb. 2005. Antimicrobial activity of flavonoids. *IntJ Anti microb Agents*., 26:343-356.

Trouvelot, S., Héloir, M.C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Trdá, L., Daire, X. and Adrian, M. 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Front. Plant Sci.*, pp.592.

Wakte, K.V., Nadaf, A.B., Krishnan, S.and Thengane, R.J. 2007. Studies on lower epidermal papillae, the site of storage of basmati rice aroma compounds in *Pandanus amaryllifolius* Roxb. *Curr. Sci.*, 93:238-242. http://www.jstor.org/stable/24099312.

Wakte, K.V., Nadaf, A.B., Thengane, R.J. and Jawali, N. 2009. *Pandanus amaryllifolius* Roxb. cultivated as a spice in coastal regions of India. *Genet Resour Crop Evol.*, 56:735-740. https://doi.org/10.1007/s10722-009-9431-5.

Wakte, K.V., Thengane, R.J., Jawali, N. and Nadaf, A.B. 2010. Optimization of HS-SPME conditions for quantification of 2-acetyl-1-pyrroline and study of other volatiles in *Pandanus amaryllifolius* Roxb. *Food Chem.*, 121: 595-600.

Wongpornchai, S. 2006. Pandan wangi. In: Peter K (ed) Handbook of herbs and spices. Woodhead, England, 3: 453-459. http://dx.doi.org/10.1533/9781845691717.3.453.

Yahya, F., Fryer, P.J.and Bakalis, S. 2011. The absorption of 2-acetyl-1-pyrroline during cooking of rice (*Oryza sativa* L.) with Pandan (*Pandanus amaryllifolius* Roxb.) leaves. *Procedia Food Sci*., 1:722-728, https://doi.org/10.1016/j.profoo.2011.09.109.

Yu, M., Chen, M., Gui, J., Huang, S., Liu, Y., Shentu, H., He, J., Fang, Z.,Wang, W. and Zhang, Y. 2019. Preparation of *Chlorella vulgaris* polysaccharides and their antioxidant activity *in vitro* and *in vivo*. *Int J Biol Macromol.*, 137: 139-150. https://doi.org/10.1016/j.ijbiomac. 2019.06.222