



## PHYTOCHEMICAL AND ANTI-OXIDANT PROPERTIES OF *PANDANUS ODORIFER* OF ANDAMAN AND NICOBAR ISLANDS

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### ABSTRACT

In present study, the seed, seed oil, pulp and leaf of *P. odorifer* from three different geographical locations of Andaman and Nicobar Islands were analysed for its phytochemical and antioxidant properties which revealed that the pulp had excellent amount of carbohydrate ( $76.343 \pm 1.531$  %) as well as notable ascorbic acid ( $0.086 \pm 0.112$  %), added with good anti-nutritional contents like phenols ( $4.903 \pm 0.967$  %), flavonoids ( $17.223 \pm 1.113$  %), saponins ( $9.503 \pm 1.194$  %) and oxalate ( $0.593 \pm 0.695$  %) content. In addition, it also had good TSS ( $5.56 \pm 1.064^\circ$  Brix) content with exceptional antioxidant activity like DPPH ( $93.933 \pm 3.95$  % RSA) and ABTS ( $36.14 \pm 0.118$  % RSA). Notably, the seed had good protein ( $23.81 \pm 2.757$  %) content and the leaf had good tannin ( $10.98 \pm 1.005$  %), chlorophyll ( $5.278 \pm 1.048$   $\mu\text{g/g}$ ) and carotenoid ( $10.56 \pm 1$   $\mu\text{g/g}$ ) concentration added with notable hydroxyl radical scavenging activity ( $88.5 \pm 4.951$  %RSA). Exceptionally, the seed oil recorded good superoxide radical scavenging activity ( $89.14 \pm 6.836$  %RSA). Further correlation studies revealed strong correlation ascorbic acid with carbohydrates ( $r=1$ ,  $p \leq 0.05$ ), DPPH ( $r=0.99$ ,  $p \leq 0.05$ ) and carbohydrates itself with DPPH ( $r=0.97$ ,  $p \leq 0.05$ ), saponins ( $r=0.90$ ,  $p \leq 0.05$ ) and TSS ( $r=0.88$ ,  $p \leq 0.05$ ). Good correlation levels of ABTS with tannins ( $r=0.94$ ,  $p \leq 0.05$ ) and phenols ( $r=0.93$ ,  $p \leq 0.05$ ) as well as flavonoids ( $r=0.81$ ,  $p \leq 0.05$ ) were also observed. On analysis, these plant parts could have potent applications in the field of food, nutraceutical and pharmaceutical industries due to their exemplary antioxidant potential.

**Keywords:** *P. odorifer*, Phytochemicals, Antioxidants, Nutritional and Anti nutritional, Andaman and Nicobar Islands.

### INTRODUCTION

Andaman and Nicobar Islands serves as one of the hotspots of biological diversity holding flourishing biome of the members of Pandanaceae family especially the genera, *Pandanus* Parkinson as well as *Freycinetia* Gaudich (Nadaf *et al.*, 2012). *Pandanus odorifer* (Forssk.) Kuntze, commonly known as fragrant screw pine and umbrella tree, synonymously named as *P. odoratissimus* Linn.f., *P. fascicularis* Lam. (Nasim *et al.*, 2020) of Pandanaceae family is familiar for its aromatic essential oil, Kewda, produced from the male inflorescence which has a greater economic significance due to its diverse properties. Originated from south east Asia, *P. odorifer* has distributed

all around especially in Indian coastlines and Andaman Islands (Padhy *et al.*, 2016). *P.odorifer* is a perennial, evergreen dioecious, monocotyledonous aromatic shrubs or small trees which grow to a height of about 9 m with numerous prop roots and erect or decumbent stem branching either dichotomously or irregularly (Solomon Raju *et al.*, 2020). The plant has linear-ensiform leaves which are whole, deep green in colour, growing up to 2 m length and 4-7 m width with acute apex and margin along with three rows of yellow spiny midrib where the spines are close to each other at the middle and crowded towards the apex. Terminal and ephemeral inflorescence which gives a strong fragrance which decays rapidly at maturity.

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Flowering at July till October and fruiting throughout the year, the plant is highly polymorphous (Nadaf *et al.*, 2012). *P. odorifer* is predominantly cultivated for the extraction of essential oil from the male flower for its breath taking fragrance garlanding the pharmaceutical, flavour, fragrance and cosmetic industries (Nasim *et al.*, 2021). The oil is extracted by hydro-distillation method (Nasim *et al.*, 2018) and the phytochemical analysis of the oil has revealed that the characteristic smell was due to the presence of phenyl ethyl methyl ether (PEME) (Naqvi and Mandal, 1996). Apart from PEME, it also contains eugenol, capric acid, germacrene B, camphor, dodecane, geranial and linalool (Raina *et al.*, 2004). Further, *P. odorifer* also has excellent medicinal background that it has been used in Siddha and Ayurveda to treat various diseases in India, Sri Lanka and Taiwan. The flower is used to treat diseases like diabetes, asthma, urinary tract illness, syphilis and skin diseases. On the other hand, the leaves were used to treat leprosy, tumour, small pox, heart and brain diseases whereas the root was used to treat constipation, diabetes, fever, urinary tract illness as well as thyroid disorders (Sathasivampillai *et al.*, 2021). Although, phytochemical and antioxidant studies on fruit and its parts are meagre which could unravel bountiful information. In this line, phytochemical and antioxidant activities in seed, seed, seed oil, pulp and leaves of three accessions of *P. odorifer* collected from three geographical locations of Andaman and Nicobar Islands were analysed and possible benefits and applications were discussed in this study.

## MATERIALS AND METHODS

### Sample collection

Fresh Pandan (*Pandanus odorifer*) leaves and fruits were collected from three different locations of Andaman and Nicobar Islands as three accessions. Accession 1 was collected from Katchal, Accession 2 from Kamorta and Accession 3 from Car Nicobar. The collected samples of *P. odorifer* were washed in running water to get rid of the dust and foreign material adhered to the surface. Then the seed, seed oil, pulp and leaves of the plant were separated from the plant. Further, the samples were air dried under shade at room temperature and grounded into a coarse powder.

### Extraction and phytochemical screening

Dry samples and methanolic extracts (1:20) of leaves, fruit pulp and seed of the plants were prepared for analysis. Oil was extracted from seed using Soxhlet apparatus. About 50 g of sample powder was extracted with 1000 mL of methanol in the soxhlet apparatus till the extracted color in the siphon tube has becomes less intense and cooled the extract to room temperature and filtered through Whatman No.1 filter paper (Abubakar and Haque, 2020). Subsequently, the filtrates were used for analysis where the measurements were carried out in triplicate.

### Nutritional analysis

Total Carbohydrates was estimated by Phenol-Sulfuric Acid-UV method (Albalsmeh, 2013) where 1 g of fresh

sample was analysed and read against a blank at 315 nm and the results expressed in mg glucose equivalents/g of fresh weight. The total protein content was estimated by Bradford method (Bonjoch and Tamayo, 2001) where 0.2 g of fresh sample was analysed and read against 595 nm where the results were expressed as  $\mu\text{g BSA/g}$  fresh sample. The ascorbic acid content was determined by titration method (Al-Alimi *et al.*, 2017) where the samples were titrated against  $0.005 \text{ mol L}^{-1}$  Iodine solution. The appearance of permanent blue-black colour (starch-iodine complex) indicates the presence of ascorbic acid. The titration was carried out three times and a concordant value was obtained and mg/100 mL of ascorbic acid obtained in the sample were determined using the formula.

$$\text{Percentage of ascorbic acid in sample} = \frac{\text{Concentration of ascorbic acid}}{\text{Concentration of sample solution}} \times 100$$

### Anti-nutritional analysis

The total phenol, flavonoid and tannin content were estimated as described by Kavitha and Indira (2016) where the total phenolic content in 1 ml plant extract was determined by Folin-Ciocalteu method where the samples were treated with 1 ml of Folin-Ciocalteu phenol reagent and 7% sodium carbonate solution and the absorbance were read against 760 nm and the total phenolic content was expressed as mg of GAE per g of dried sample. Similarly, the total tannin content was also determined by Folin-Ciocalteu method where in 100  $\mu\text{l}$  of plant extract was treated with 500  $\mu\text{L}$  of Folin-Ciocalteu phenol reagent and 1mL of 35% sodium carbonate and the absorbance was read against 700 nm. The total tannin content was expressed as grams of tannic acid equivalents /100 g of dried sample. The total flavonoid content was determined by adding 5% sodium nitrite, 10% Aluminium chloride ( $\text{AlCl}_3$ ) and 1M sodium hydroxide to 1 ml of sample extract and the absorbance was read at 510 nm where Quercetin (1 mg/ mL) in methanol was used as a standard and the total flavonoid content was expressed in terms of Quercetin equivalents (mg QE per gram of dried sample). Total saponins content was determined using the Vanillin-Sulphuric acid colorimetric method as described by Makkar *et al.*, (2007) where vanillin reagent and 72% Sulphuric acid was added to the sample and the absorbance was read at 544nm. The total saponin content was calculated with the diosgenin calibration curve and expressed as mg diosgenin equivalent (DE) per 100 g of dried sample. The total oxalate content was determined by titration method (Mishra *et al.*, 2017) where 1 g of sample was boiled in 30 mL of 0.5 N dilute  $\text{H}_2\text{SO}_4$  for 15 minutes to extract the oxalate ions. Then the filtered boiled extract was treated with 0.5N  $\text{H}_2\text{SO}_4$ , heated to 60 °C and was titrated against standard  $\text{KMnO}_4$  solution till appearance of pale permanent pink colour.

### Antioxidant Activity

Methanolic extracts of dry samples of seed, leaves, pulp and seed oil of *P. odorifer* were subjected to antioxidant

analysis in which the DPPH, HRSA, SRSA was determined as the method described by Yu *et al.*, 2019 with Vitamin C as positive control. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by using 0.1 mM DPPH solution and the absorbance was read at 517 nm. The hydroxyl radical scavenging activity (HRSA) was determined using 1.8mM Salicylic acid, 1.8mM ferrous sulphate solution and 0.03% hydrogen peroxide solution and the absorbance was read at 510 nm. The superoxide radical scavenging activity (SRSA) was determined using Tris-Hydrochloride and pyrogallol acid and the absorbance was read at 320 nm. The ABTS radical cation decolorization assay and FRAP activity was done by the method described by Rajurkar and Hande, 2011. The ABTS assay was performed using ABTS solution (7 mmol/L ABTS solution mixed with 5 mmol/L Potassium persulfate prepared and incubated in the dark, at room temperature for 12-16 hours before use) was diluted with ethanol till the absorbance was 0.7 at 734nm. 1mL of diluted ABTS solution was added to sample and incubated for 5 minutes. The absorbance was measured at 734 nm using UV/Visible spectrophotometer (Eppendorf Bio spectrometer® basic). Ascorbic acid is used as a standard reference.

The above four antioxidant activities were expressed in percentage which was calculated using the formula

$$\text{Radical Scavenging Activity (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

FRAP assay was done using freshly prepared FRAP reagent where 3.995 mL FRAP reagent was mixed with 5 $\mu$ L of sample and mixed thoroughly and incubated at 37° for 30 minutes. The absorbance was measured at 593 nm using UV/Visible spectrophotometer (Eppendorf Bio spectrometer® basic). The analysis was carried out in triplicate. Ferrous Sulfate (FeSO<sub>4</sub>) was used as a standard and the FRAP activity was expressed in terms of mg of Trolox equivalent per gram dry sample.

### Physicochemical factors

Chlorophyll a, Chlorophyll b and Total Chlorophyll content was estimated by Arnon's method (Kavitha and Indira, 2016) where the absorbance was read at 663nm and 645nm for chlorophyll a whereas 645 nm and 663 nm for chlorophyll b respectively. The chlorophyll a, chlorophyll b and total chlorophyll were calculated using the formula

$$\text{Chl a (mg g}^{-1}\text{)} = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \text{ml acetone / mg leaf tissue}$$

$$\text{Chl b (mg g}^{-1}\text{)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \text{ml acetone / mg leaf tissue}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b}$$

The total carotenoid content was determined as described by Lichtenthaler and Buschmann, 2001 using acetone and the absorbance was read at 440 nm. The pH was determined using KCl buffer in pH meter (Microprocessor

based pH system, Model-1012). The total soluble sugars (TSS) were calculated using a digital refractometer (Optics Technology) as described by (Singh *et al.*, 2007) where the samples were read at 20°C.

### Statistical analysis

The statistical analysis was performed and the two-way ANOVA with replication was performed using MS-Excel 2010. Normally distributed data were expressed as Mean  $\pm$  SD and the criterion for statistical significance was  $p < 0.05$ . Post Hoc analysis was performed when the ANOVA test came out significant. The correlation analysis was performed using R – Software (R Studio, 2021).

## RESULTS AND DISCUSSION

The nutritional analysis of plant parts of three different accessions of *P.odorifer* revealed that the carbohydrates (76.343  $\pm$  1.531 %) , protein (23.81  $\pm$  2.757 %) and ascorbic acid (0.086  $\pm$  0.112 %) were comparatively high in pulp especially in Acc 2 and comparatively low in the leaf respectively. However, the statistical analysis revealed that the protein was significantly different for the plant parts as well as locations whereas carbohydrates and ascorbic acid were significantly same among the locations and different among the plant parts respectively. In detail, the carbohydrates of seed and leaf components are significantly same. Analyzing the anti-nutritional properties in the plant parts of *P.odorifer* revealed that the total phenols (4.903  $\pm$  0.967 %) and total flavonoids (17.223  $\pm$  1.113 %) were significantly high in the pulp of Acc 2 whereas the total saponins (9.503  $\pm$  1.194 %) and total oxalates (0.593  $\pm$  0.695 %) were significantly high in the pulp of Acc 3. Notably, the significantly high tannin (10.98  $\pm$  1.005 %) added with low saponin (0.047  $\pm$  0.047 %) and oxalate (0.053  $\pm$  0.01 %) concentration in the leaves adds essence towards the anti-nutritional properties of the plant. Although, all the analysed anti-nutritional properties except oxalates were significantly different among the plant parts as well as locations where oxalates were significantly same among the locations and different among the plant parts.

The pH was comparatively high in the leaf and seed whereas low in the pulp (Table 3) respectively. On the other hand, the TSS content was significantly high in the pulp and lower in the leaves. Especially, the TSS content was high in the pulp (5.56 $\pm$ 1.064 °Brix) of Acc 3 and lower in the leaf (0.127  $\pm$  0.021°Brix) of Acc 1 respectively. However, the physicochemical properties were significantly different among the plant parts whereas significantly same among the locations respectively (Table 4). Rich concentration of carotenoids (10.56  $\pm$  1  $\mu$ g/g) as well as chlorophyll (5.278  $\pm$  1.048  $\mu$ g/g) in the leaves were observed in Acc 2 and 1 respectively. A less chlorophyll a (1.86 $\pm$ 1.075  $\mu$ g/g) and chlorophyll b (3.07  $\pm$  1.015  $\mu$ g/g) concentration were observed in the leaves of Acc 3 and Acc 1 respectively. However, they were significantly same among the locations.

**Table 1.** Nutritional properties of *P.odorifer*.

Parameter	Location	Seed	Seed oil	Pulp	Leaf
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Carbohydrates (%)	Katchal	44.477±0.5 <sup>b</sup>	5.178±0.088 <sup>d,A</sup>	75.65±0.416 <sup>a,B</sup>	25.42±0.563 <sup>c</sup>
	Kamorta	35.47±0.501 <sup>c</sup>	3.806±1.146 <sup>d,B</sup>	76.343±1.531 <sup>a,A</sup>	37.349±0.574 <sup>b</sup>
	Car Nicobar	40.98±0.985 <sup>b</sup>	4.597±1.219 <sup>d,B</sup>	75.428±6.12 <sup>a,C</sup>	32.877±4.27 <sup>c</sup>
Protein (%)	Katchal	17.05±1.025 <sup>a,C</sup>	6.53±1.202 <sup>b,C</sup>	2.211±0.892 <sup>c,C</sup>	0.316±0.203 <sup>d,C</sup>
	Kamorta	23.81±2.757 <sup>a,A</sup>	11.06±1.015 <sup>b,A</sup>	2.313±0.958 <sup>c,B</sup>	0.419±0.512 <sup>d,A</sup>
	Car Nicobar	21±2.646 <sup>a,B</sup>	9.187±2.046 <sup>b,B</sup>	2.34±0.802 <sup>c,A</sup>	0.362±0.562 <sup>d,B</sup>
Ascorbic acid (%)	Katchal	0.031±0.051 <sup>c</sup>	0.003±0.003 <sup>d</sup>	0.084±0.015 <sup>a</sup>	0.034±0.014 <sup>b</sup>
	Kamorta	0.047±0.047 <sup>b</sup>	0.005±0.002 <sup>c</sup>	0.086±0.112 <sup>a</sup>	0.043±0.031 <sup>d</sup>
	Car Nicobar	0.069±0.042 <sup>b</sup>	0.008±0.002 <sup>d</sup>	0.074±0.015 <sup>a</sup>	0.024±0.011 <sup>c</sup>

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

**Table 2.** Anti-nutritional properties of *P.odorifer*.

Parameter	Location	Seed	Seed oil	Pulp	Leaf
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total Phenol (%)	Katchal	2.413±0.856 <sup>d,B</sup>	3.44±1.598 <sup>c,B</sup>	4.13±1.501 <sup>a,C</sup>	3.823±1.859 <sup>b,C</sup>
	Kamorta	3.347±1.344 <sup>d,A</sup>	3.703±1.003 <sup>c,A</sup>	4.903±0.967 <sup>a,A</sup>	4.1±0.915 <sup>b,B</sup>
	Car Nicobar	0.737±0.307 <sup>c,C</sup>	0.025±0.012 <sup>d,C</sup>	4.523±1.409 <sup>a,B</sup>	4.427±1.397 <sup>b,A</sup>
Total Flavonoids (%)	Katchal	12.05±0.97 <sup>b,C</sup>	11.25±1.002 <sup>c,A</sup>	1.997±1.822 <sup>d,C</sup>	14.28±1.412 <sup>a,C</sup>
	Kamorta	12.063±0.999 <sup>c,B</sup>	1.487±0.985 <sup>d,C</sup>	17.223±1.113 <sup>a,A</sup>	14.88±1.018 <sup>b,A</sup>
	Car Nicobar	12.15±1.895 <sup>c,A</sup>	1.5±0.964 <sup>d,B</sup>	16.513±1.504 <sup>a,B</sup>	14.577±1.221 <sup>b,B</sup>
Total Tannins (%)	Katchal	4.21±0.866 <sup>d,C</sup>	4.717±0.931 <sup>c,C</sup>	6.347±1.496 <sup>b,C</sup>	10.01±1 <sup>a,C</sup>
	Kamorta	7.447±1.064 <sup>c,B</sup>	5.128±1.036 <sup>d,A</sup>	8.249±0.836 <sup>b,B</sup>	10.657±1.12 <sup>a,B</sup>
	Car Nicobar	8.01±1.001 <sup>c,A</sup>	4.96±1.059 <sup>d,B</sup>	9.457±0.912 <sup>b,A</sup>	10.98±1.005 <sup>a,A</sup>
Total saponin (%)	Katchal	5.85±1.002 <sup>b,A</sup>	1.26±0.827 <sup>c,A</sup>	9.503±1.194 <sup>a,A</sup>	0.327±0.05 <sup>d,B</sup>
	Kamorta	3.558±1.12 <sup>b,B</sup>	0.637±0.494 <sup>d,C</sup>	8.38±1.133 <sup>a,C</sup>	0.757±0.69 <sup>c,A</sup>
	Car Nicobar	2.51±0.817 <sup>b,C</sup>	0.953±0.796 <sup>c,B</sup>	8.95±0.951 <sup>a,B</sup>	0.047±0.047 <sup>d,C</sup>
Total oxalate (%)	Katchal	0.447±0.582 <sup>b</sup>	0.287±0.095 <sup>c</sup>	0.513±0.588 <sup>a</sup>	0.079±0.011 <sup>d</sup>
	Kamorta	0.493±0.512 <sup>d</sup>	0.543±0.604 <sup>a</sup>	0.503±0.604 <sup>c</sup>	0.053±0.01 <sup>b</sup>
	Car Nicobar	0.556±0.082 <sup>c</sup>	0.7±0.702 <sup>a</sup>	0.593±0.695 <sup>b</sup>	0.07±0.032 <sup>d</sup>

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

Varied activity of antioxidants was observed between the plant parts as well as locations where the DPPH activity was high among all the plant parts especially in the pulp of Acc 3 (93.933±3.95 %) and Acc 2 (93.737 ± 1.896 %) respectively. Comparatively lower DPPH activity was observed in the seed oil and the leaf (Table 5). In contrast, high HRSA was observed in the leaves of Acc 2 (88.5 ± 4.951 %) and lowest activity was recorded in the seed (35.313 ± 9.361 %) of Acc 1 respectively. Notably, high SRSA was recorded in the seed oil than the plant parts especially in Acc 1 (89.14 ± 6.836 %) and lower activity was recorded in the seed (52.813 ± 5.501 %) of Acc 2 respectively. Unlike other antioxidant activity, the DPPH and SRSA were significantly different among the plant parts and same among the locations. However, varied ABTS activity was observed where higher activity was observed in the pulp of Acc 1 (36.14 ± 0.118 %) whereas lower activity was recorded in the Acc 2 seed oil (7.523 ± 2.021

%) respectively. Moreover, higher FRAP activity was recorded in Acc 2 leaves (1620.667 ± 977.166 μM (TE)/g DW) and lower activity was recorded in Acc 1 pulp (164.84 ± 12.31 μM (TE)/g DW) respectively. Correlation studies among the plant parts (Table 6) figured out strong correlation between ascorbic acid with carbohydrates (r=1, p ≤ 0.05), DPPH (r=0.99, p ≤ 0.05), TSS (r=0.91, p ≤ 0.05) and total saponins (r=0.90, p ≤ 0.05). Moreover, the carbohydrates also had strong correlations with DPPH (r=0.97, p ≤ 0.05), saponins (r=0.90, p ≤ 0.05) and TSS (r=0.88, p ≤ 0.05) respectively. On the other hand, the TSS was also correlated with saponins (r=0.97, p ≤ 0.05) and in addition, and the DPPH was also correlated with total saponins (r=0.84, p ≤ 0.05) and TSS (r=0.88, p ≤ 0.05) respectively. Notable correlations of tannins with phenols (r=0.77, p ≤ 0.05), flavonoids (r=0.87, p ≤ 0.05) as well as HRSA with phenols (r=0.70, p ≤ 0.05) were observed. Although strong correlations of ABTS with tannins (r

=0.94,  $p \leq 0.05$ ) and phenols ( $r = 0.93$ ,  $p \leq 0.05$ ) as well as flavonoids ( $r = 0.81$ ,  $p \leq 0.05$ ) were also observed. The nutritional properties assessed by the composition of carbohydrates, proteins and ascorbic acid in the plant parts of *P. odorifer* revealed that the pulp had significantly high amount of carbohydrates ( $76.343 \pm 1.531$  %) and ascorbic acid ( $0.086 \pm 0.112$  %) whereas the seed recorded significantly high amount of proteins especially in Acc 2 ( $23.81 \pm 2.757$  %). A similar phenomenon of high carbohydrate (62.63 %) and protein (32.40 %) in the pulp and seed respectively was observed in *Balanites aegyptiaca* (L.) Delile by Murthy *et al.*, 2020. Moreover, the nutritional concentration of *P. odorifer* of Andaman and

Nicobar Islands was substantially higher than the earlier reports in the pulp and seed of *Pandanus odoratissimus* (Kewda) where the carbohydrate, protein and Vitamin C concentration was 17 g/100 g, 1.3 mg/ 100 g, 5 mg /100 g respectively (Adkar and Bhaskar, 2014) who has reported that fresh Pandanus was an important source of Vitamin C. However, comparatively lower nutritional concentration was observed in the seed oil. A lesser carbohydrates, protein and ascorbic acid were observed in the leaf irrespective of the location. This may be due to the sugar nature of the plant parts as well as stress regulatory metabolism from the source to the sink (Akšić *et al.*, 2019).

**Table 3.** Physicochemical properties of *P.odorifer*.

Parameter	Location	Seed Mean $\pm$ SD	Seed oil Mean $\pm$ SD	Pulp Mean $\pm$ SD	Leaf Mean $\pm$ SD
pH	Katchal	6.1 $\pm$ 1.126 <sup>b</sup>	5.567 $\pm$ 1.05 <sup>c</sup>	4.7 $\pm$ 1.167 <sup>d</sup>	6.567 $\pm$ 1.253 <sup>a</sup>
	Kamorta	6.267 $\pm$ 1.206 <sup>b</sup>	5.867 $\pm$ 0.906 <sup>c</sup>	4.467 $\pm$ 1.277 <sup>d</sup>	6.567 $\pm$ 1.13 <sup>a</sup>
	Car Nicobar	6.267 $\pm$ 1.119 <sup>a</sup>	5.533 $\pm$ 1.081 <sup>c</sup>	4.6 $\pm$ 0.917 <sup>d</sup>	5.8 $\pm$ 1.054 <sup>b</sup>
TSS (°Brix)	Katchal	3.26 $\pm$ 1.054 <sup>b</sup>	0.463 $\pm$ 0.563 <sup>c</sup>	3.62 $\pm$ 131 <sup>a</sup>	0.127 $\pm$ 0.021 <sup>d</sup>
	Kamorta	2.72 $\pm$ 1.1 <sup>b</sup>	0.31 $\pm$ 0.115 <sup>d</sup>	5.26 $\pm$ 1.1 <sup>a</sup>	0.327 $\pm$ 0.05 <sup>c</sup>
	Car Nicobar	3.42 $\pm$ 1.34 <sup>b</sup>	0.61 $\pm$ 0.07 <sup>c</sup>	5.56 $\pm$ 1.064 <sup>a</sup>	0.407 $\pm$ 0.531 <sup>d</sup>

Note: Superscripts (a-c) in columns represent statistically significant at  $p < 0.05$ . No significant difference was observed among the plant parts (Rows).

**Table 4.** Pigmentation in *P.odorifer* leaves.

Parameter	Location	Chlorophyll a Mean $\pm$ SD	Chlorophyll b Mean $\pm$ SD	Total chlorophyll Mean $\pm$ SD	Carotenoids Mean $\pm$ SD
Pigmentation	Katchal	2.25 $\pm$ 1.051 <sup>B</sup>	3.07 $\pm$ 1.015 <sup>C</sup>	5.278 $\pm$ 1.048 <sup>A</sup>	9.14 $\pm$ 0.987 <sup>C</sup>
	Kamorta	2.43 $\pm$ 0.827 <sup>A</sup>	3.123 $\pm$ 1.016 <sup>bB</sup>	5.17 $\pm$ 1.055 <sup>C</sup>	10.56 $\pm$ 1 <sup>A</sup>
	Car Nicobar	1.86 $\pm$ 1.075 <sup>C</sup>	3.596 $\pm$ 1.848 <sup>A</sup>	5.27 $\pm$ 1.031 <sup>B</sup>	9.28 $\pm$ 0.975 <sup>B</sup>

Note: Superscripts (A-C) in rows represent statistically significant at  $p < 0.05$ .

**Table 5.** Antioxidant activities in *P.odorifer*.

Parameter	Location	Seed Mean $\pm$ SD	Seed oil Mean $\pm$ SD	Pulp Mean $\pm$ SD	Leaf Mean $\pm$ SD
DPPH (%)	Katchal	85.467 $\pm$ 5.896 <sup>b</sup>	70.1 $\pm$ 8.945 <sup>d</sup>	91.233 $\pm$ 4.446 <sup>a</sup>	79.14 $\pm$ 4.84 <sup>c</sup>
	Kamorta	84.477 $\pm$ 6.183 <sup>b</sup>	65.813 $\pm$ 10.575 <sup>d</sup>	93.737 $\pm$ 1.896 <sup>a</sup>	83.387 $\pm$ 5.1 <sup>c</sup>
	Car Nicobar	85.703 $\pm$ 6.333 <sup>b</sup>	63.613 $\pm$ 10.535 <sup>d</sup>	93.933 $\pm$ 3.95 <sup>a</sup>	74.18 $\pm$ 11.03 <sup>c</sup>
Hydroxyl RSA (%)	Katchal	35.313 $\pm$ 9.361 <sup>d,C</sup>	66.53 $\pm$ 5.049 <sup>c,C</sup>	83.93 $\pm$ 5.656 <sup>a,A</sup>	75.383 $\pm$ 7.59 <sup>b,C</sup>
	Kamorta	62.45 $\pm$ 10.546 <sup>d,B</sup>	83.3 $\pm$ 5.095 <sup>b,A</sup>	76.827 $\pm$ 7.591 <sup>c,B</sup>	88.5 $\pm$ 4.951 <sup>a,A</sup>
	Car Nicobar	76.527 $\pm$ 7.686 <sup>c,A</sup>	82.627 $\pm$ 3.056 <sup>b,B</sup>	75.133 $\pm$ 5.19 <sup>d,C</sup>	83.4 $\pm$ 5.505 <sup>a,B</sup>
Superoxide RSA (%)	Katchal	53.067 $\pm$ 6.102 <sup>d</sup>	89.14 $\pm$ 6.836 <sup>a</sup>	78.42 $\pm$ 5.551 <sup>b</sup>	60.513 $\pm$ 10.535 <sup>c</sup>
	Kamorta	52.813 $\pm$ 5.501 <sup>d</sup>	88.16 $\pm$ 9.98 <sup>a</sup>	78.643 $\pm$ 8.638 <sup>b</sup>	60.547 $\pm$ 10.546 <sup>c</sup>
	Car Nicobar	53.923 $\pm$ 5.522 <sup>d</sup>	86.637 $\pm$ 7.051 <sup>a</sup>	80.647 $\pm$ 5.131 <sup>b</sup>	66.617 $\pm$ 10.67 <sup>c</sup>
ABTS (%)	Katchal	21.333 $\pm$ 1.904 <sup>c,A</sup>	13.82 $\pm$ 0.958 <sup>d,B</sup>	36.14 $\pm$ 0.118 <sup>a,A</sup>	25.943 $\pm$ 8.614 <sup>b,C</sup>
	Kamorta	16.447 $\pm$ 1.463 <sup>c,B</sup>	7.523 $\pm$ 2.021 <sup>d,C</sup>	27.53 $\pm$ 1.721 <sup>b,B</sup>	32.917 $\pm$ 2.005 <sup>a,A</sup>
	Car Nicobar	14.633 $\pm$ 1.953 <sup>d,C</sup>	18.95 $\pm$ 1.666 <sup>c,A</sup>	19.843 $\pm$ 2.329 <sup>b,C</sup>	31.45 $\pm$ 2.361 <sup>a,B</sup>
FRAP ( $\mu$ M (TE)/g DW)	Katchal	1150.933 $\pm$ 21.1 <sup>b</sup>	1499 $\pm$ 353.275	164.84 $\pm$ 12.31	1508.667 $\pm$ 829.975 <sup>a</sup>
	Kamorta	1066.303 $\pm$ 50.76 <sup>b</sup>	369.34 $\pm$ 136.661	1173.667 $\pm$ 24.542	1620.667 $\pm$ 977.166 <sup>a</sup>
	Car Nicobar	1257.667 $\pm$ 122.98 <sup>a</sup>	507.12 $\pm$ 29.841	1309.667 $\pm$ 358.199	168.133 $\pm$ 12.584 <sup>b</sup>

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

**Table 6.** Correlation Analysis of *P.odorifier* among the plant parts (seed, seed oil, leaf, and pulp).

	CH	Pr	AA	TP	TF	TT	TS	TO	pH	TSS	DPPH	HRSA	SRSA	ABTS	FRAP
CH	1.00														
Pr	-0.21	1.00													
AA	1.00	-0.12	1.00												
TP	0.66	-0.87	0.59	1.00											
TF	0.60	-0.21	0.61	0.56	1.00										
TT	0.40	-0.62	0.37	0.77	0.87	1.00									
TS	0.90	-0.03	0.90	0.42	0.21	-0.02	1.00								
TO	0.19	0.50	0.22	-0.40	-0.58	-0.82	0.59	1.00							
pH	-0.62	0.37	-0.58	-0.49	0.20	0.16	-0.82	-0.58	1.00						
TSS	0.88	0.16	0.91	0.27	0.27	-0.06	0.97	0.61	-0.68	1.00					
DPPH	0.97	-0.04	0.99	0.53	0.69	0.41	0.84	0.15	-0.44	0.88	1.00				
HRSA	-0.03	-0.96	-0.13	0.70	-0.03	0.44	-0.14	-0.46	-0.32	-0.35	-0.22	1.00			
SRSA	-0.18	-0.44	-0.24	0.14	-0.74	-0.43	0.11	0.38	-0.65	-0.08	-0.39	0.60	1.00		
ABTS	0.63	-0.73	0.58	0.93	0.81	0.94	0.28	-0.61	-0.18	0.20	0.59	0.52	-0.23	1.00	
FRAP	0.15	0.36	0.21	-0.08	0.77	0.50	-0.17	-0.48	0.68	0.01	0.35	-0.52	-0.99	0.29	1.00

CH = Carbohydrates (%), P =Protein (%), AA = Ascorbic acid (%), TP = Total Phenol (%), TT = Total Tannin (%), TS = Total Saponins (%), TF = Total flavonoid (%), TO = Total Oxalate (%), TSS = Total Soluble Solids (°Brix), DPPH = 1, 1-Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity (%), HRSA = Hydrogen Peroxide (H<sub>2</sub> O<sub>2</sub> ) Radical Scavenging Activity (%), SRSA = *Superoxide Radical Scavenging Activity* (%), ABTS = (2, 2-Azinobiz-3-Ethylbenthiazoline-6-Sulfonic Acid) Radical Cation Scavenging Activity (%), FRAP = (Ferric Reducing Antioxidant Power) (µM (TE)/g DW).

The proposed anti-nutritional properties were high in the pulp except total tannins which was high in the leaves irrespective of location as depicted in Table 2. In detail, the leaf and pulp had comparatively higher concentration of phenol concentration whereas; predominantly lower phenol concentration was observed in the seed ( $0.737 \pm 0.307$  %) and seed oil ( $0.025 \pm 0.012$  %) especially in Acc 3. However, varied flavonoid concentration was observed where the pulp of Acc 2 ( $17.223 \pm 1.113$  %) and 3 ( $16.513 \pm 1.504$  %) recorded a significantly higher concentration. This was followed by the leaves and seed which had almost similar concentration among the accessions. However, lower concentration was observed in the seed oil. Notably, extremely lower concentration of flavonoids was observed in the pulp of Acc 1 ( $1.997 \pm 1.822$  %) respectively. However, it was higher than the phenol ( $80.27 \pm 3.21$  mg GAE/g) and flavonoid ( $260.03 \pm 11.13$  mg RE/g) contents in the methanolic extract of *P. conoideus* fruit (Rohman *et al.*, 2010). Hassanpour *et al.*, 2011 stated that the astringent taste in some leaves and fruits was due to the presence of tannins and tannin biosynthesis was regulated by various factors in the plant system where the biosynthesis in leaves was regulated by light availability during photosynthesis. In this study varied tannin content was observed irrespective of the plant parts as well as the accessions except the leaf which recorded almost same as well as higher concentration among the accessions (Table 2) especially in the leaves of Acc 3 ( $10.98 \pm 1.005$  %) stressing the significance of variation in the light availability during photosynthesis. Comparatively lower tannin content was reported by Amadi *et al.*, 2018 in the leaves ( $0.07 \pm 0.01$  %) of *Artocarpus heterophyllus* which was followed by its seed ( $0.06 \pm 0.01$  %) and pulp ( $0.03 \pm 0.01$  %) respectively. Although, Acc 1 recorded comparatively lower concentration of tannins respectively. Meanwhile, a sequential concentration of saponins were observed among the plant parts which follows the order pulp > seed > seed oil > leaf.

A study conducted by Ezeabara *et al.*, 2014 over the saponin concentration in the different plant parts of a range of citrus varieties observed varied saponin concentration over the plant parts and varieties and documented higher concentration in the leaves of *Citrus sinensis* ( $0.98 \pm 0.003$  %) followed by the peels of *Citrus aurantifolia* ( $0.96 \pm 0.01$  %) who stated that the saponin concentration may vary widely within the plant varieties and suggested the use of citrus plant parts as an ethnomedicine. Indeed, plant parts of *P. odorifer* with relatively high saponin concentration could also be used as a promising ethnomedicine. Furthermore, the overall oxalate concentration was high in the pulp and leaves and low in the leaf and seed oil. Indeed, the seed oil ( $0.7 \pm 0.702$  %), pulp ( $0.593 \pm 0.695$  %) and seed ( $0.556 \pm 0.082$  %) of Acc 3 recorded comparatively higher concentration than the other accessions. Notably the oxalate concentration was significantly different only among the plant parts whereas significantly same among the locations which was well

depicted in the Table 2. In line, comparatively higher oxalate concentration was observed in the seeds of green ( $116.9 \pm 2.8$  mg/100g) and golden ( $97.3 \pm 1.4$  mg/100g) cultivars of kiwifruit to that of its pulp [green ( $19.3 \pm 0.3$  mg/100g); golden ( $15.7 \pm 0.1$  mg/100g)] (Nguyen and Savage, 2013). The higher concentration of oxalates in the seed and pulp might be due to the crystallization of oxalates in the outer layers of the inner pericarp and the seed (Rassam and Laing, 2005).

The analyzed physicochemical properties was significantly different among the plant parts whereas significantly same among the locations. The pH was almost same in the leaf of Acc 1 ( $6.567 \pm 1.253$ ) and 2 ( $6.567 \pm 1.13$ ) as well as seed of Acc 2 ( $6.267 \pm 1.206$ ) and 3 ( $6.267 \pm 1.119$ ) respectively. However, it was high in the leaf ( $5.8 \pm 1.054$  to  $6.567 \pm 1.253$ ) and substantially low in the pulp ( $4.467 \pm 1.277$  to  $4.7 \pm 1.167$ ) than the other plant parts. Meanwhile, the TSS content was high in the pulp especially in Acc 3 ( $5.56 \pm 1.064^\circ$  Brix) and 2 ( $5.26 \pm 1.1^\circ$  Brix) followed by the seed and substantially low in the leaf. Relatively higher pH as well as TSS was observed in the pulp [pH ( $5.60 \pm 0.20$ ); TSS ( $15 \pm 0.1^\circ$  Brix)], seed [pH ( $8.15 \pm 0.10$ ); TSS ( $1.5 \pm 0.4^\circ$  Brix)] and seed oil [pH ( $6.12 \pm 0.50$ ); TSS (not determined)] of *Adansonia digitata* L. suggests that the lower pH and higher sugar content of the pulp could be used indeed for wine production (Oyeleke, 2002) claiming similar applications in *P. odorifer*. The pigmentation in the leaves varied significantly among the accessions where the chlorophyll a, b as well as carotenoid concentrations showed strong variations among the accessions (Table 4). However, the total chlorophyll concentration was almost same among the accessions 1 ( $5.278 \pm 1.048$  µg/g) and 3 ( $5.27 \pm 1.031$  µg/g). However, the total chlorophyll and carotenoid content was lower than that of *P. amaryllifolius* which ranged 77.75 to 125.39 mg/100 g FW and 1.04 mg/ g DW to 1.50 mg/g DW respectively (Suryani *et al.*, 2020). Moreover, the chlorophyll concentration was attributed to the plant type, climate, soil and environmental conditions (Li *et al.*, 2018) coupled with light availability as well as the size of mesophyll in the leaves whereas the chlorophyll a and b and carotenoid concentration was attributed to the leaf maturity (Suryani *et al.*, 2020).

The DPPH activity was high in the pulp and almost same in the pulp of Acc 2 ( $93.737 \pm 1.896$  %) and 3 ( $93.933 \pm 3.95$  %). Contrarily, a lower and almost same concentration was recorded in the seed of Acc 1 ( $85.467 \pm 5.896$  %) and Acc 3 ( $85.703 \pm 6.333$  %) respectively. This was higher than the DPPH activity in the pulp ( $87.07 \pm 0.7$  %) and seed ( $85.8 \pm 3.2$  %) of *Canarium odontophyllum* Miq. Fruit as reported by Prasad *et al.*, in 2010 where he documented that the high DPPH activity may be attributed to the high phenolic concentration and hence reveals that the pulp acts a good hydrogen donor. Moreover, varied hydroxyl RSA was observed among the plant parts as well as locations where, the highest activity

was observed in the leaf of Acc 2 (88.5±4.951 %) followed by the pulp of Acc 1 (83.93±5.656 %) and Acc 2 seed oil (83.3±5.095 %) and the lowest activity was observed in the Acc 1 seed (35.313±9.361 %) respectively. Interestingly, the superoxide RSA was high in the seed oil especially in Acc 2 (89.14±6.836 %) and 3 (88.16±9.98 %) and low activity was recorded in the seed. Besides, antioxidant study conducted by Londonkar and Kamble, (2009) obtained comparatively lower DPPH (91.55 %), HRSA (78.17 %), and SRSA (74.12 %) in the methanolic pulp extracts of *P. odoratissimus*. Besides, the ABTS activity was high in the pulp and leaf especially in Acc 1 pulp (36.14±0.118 %). However, the plant parts of Acc 3 recorded comparatively lower activity. The FRAP activity was high in the leaves of Acc 2 (1620.667±977.166 µM (TE)/g DW) and 1 (1508.667±829.975 µM (TE)/g DW). Reports reveal that the ABTS [pulp (205.28 ± 0.03); seed (641.57 ± 6.20)] and FRAP [pulp (54.72± 0.88 mg TE/g); seed (11.42 ± 0.09 mg TE/g)] activity recorded in the methanolic extracts of *Randia monantha* Benth was suggested due to the presence of phenolic compounds (Naida Juárez-Trujillo *et al.*, 2018) whereas the high FRAP value may be attributed to its reducing power and phenolic concentration in the leaves (Kubola & Siriamornpun, 2011). Moreover, an experimental study conducted by Bhumi Parikh and Patel, 2016 on an underutilized Indian fruit, *Manilkara hexandra* (Roxb.) documented high antioxidant activity and reported that the high reducing power suggests strong antioxidant potential. In line, plant parts of *P. odorifer* might have uncompromising antioxidant potential. Notable correlation was observed among the components of the plant parts where strong correlation between ascorbic acid with carbohydrates ( $r=1$ ,  $p \leq 0.05$ ), DPPH ( $r=0.99$ ,  $p \leq 0.05$ ), TSS ( $r=0.91$ ,  $p \leq 0.05$ ) and total saponins ( $r=0.90$ ,  $p \leq 0.05$ ) were observed. Moreover, the carbohydrates also had strong correlations with DPPH ( $r=0.97$ ,  $p \leq 0.05$ ), saponins ( $r=0.90$ ,  $p \leq 0.05$ ) and TSS ( $r=0.88$ ,  $p \leq 0.05$ ) respectively. On the other hand, the TSS was also correlated with saponins ( $r=0.97$ ,  $p \leq 0.05$ ) and in addition, and the DPPH was also correlated with total saponins ( $r=0.84$ ,  $p \leq 0.05$ ) and TSS ( $r=0.88$ ,  $p \leq 0.05$ ) respectively. Notable correlations of tannins with phenols ( $r=0.77$ ,  $p \leq 0.05$ ), flavonoids ( $r=0.87$ ,  $p \leq 0.05$ ) as well as HRSA with phenols ( $r=0.70$ ,  $p \leq 0.05$ ) were observed. In line positive correlation ( $r=0.76$ ,  $p \leq 0.05$ ) between TSS and sugars was observed in strawberry wherein it has been stated that the TSS levels rise with maturity of the fruit because during ripening stages, the fruits' starch degrades and was converted into TSS components by the ripening enzymes like  $\alpha$ -amylase and  $\beta$ -amylase (Basak *et al.*, 2022). Furthermore, strong correlations of ABTS with tannins ( $r=0.94$ ,  $p \leq 0.05$ ) and phenols ( $r=0.93$ ,  $p \leq 0.05$ ) as well as flavonoids ( $r=0.81$ ,  $p \leq 0.05$ ) were also observed. Similar correlation was also reported in BARI Mango variety where positive correlations of total phenols, total flavonoid, DPPH, carotenoids and FRAP documenting a strong correlation

between phytochemicals and antioxidant activities (Sabuz *et al.*, 2024). Further, correlation among DPPH, ABTS, phenols and tannins have been reported in citrus fruits suggesting their relation for a better antioxidant activity (Fang *et al.*, 2022).

## CONCLUSION

The high TSS and carbohydrate in the pulp added with high protein in the seed provokes the fruit of *P.odorifer* towards nutraceutical application. Significantly, the high carbohydrates coupled with low pH and high TSS of Acc 3 has feasible wine production properties. The high phenol and flavonoid content enhance the flavoring property of the pulp. The leaves with high tannin content and hydroxyl RSA may be studied further in line of bio protection. The seed oil with high superoxide anion RSA could be studied further for its medicinal property. The plant parts of *P.odorifer* therefore has strong phytochemical and antioxidant potential which could be better utilized in pharmaceutical and nutraceutical industries.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

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