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Proximate composition, Phytochemical analysis and Antioxidant potential of a Rare Anticancer Plant: Unveiling the Secrets of *Pyrenacantha volubilis* Wight Fruit Peel and Pulp.

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ABSTRACT: *Pyrenacantha volubilis* wight is a medicinally important plant recognized for its therapeutic potential and promising anticancer properties. This woody liana is restricted to Eastern coast of South India having potent antitumor compound Camptothecin. Assessment of proximate composition and phytochemical constituent provides insights of their medicinal efficiency. In this study proximate composition, phytochemicals and antioxidant potential of fruit peel and pulp were evaluated. Proximate analysis showed significantly different ($p < 0.05$) components across various parameters between samples. Peel and pulp showed different mineral composition, with pulp enriched with trace elements like Zn, Fe and Mg, while peel with metals like Na and K. Quantitative estimation of alkaloids, phenolics, condensed tannins, saponins and flavonoids across methanol and aqueous extracts, clearly shows that methanolic peel extracts were richer in bioactive compounds than pulp. ABTS and DPPH radical scavenging activities revealed methanolic pulp extract with highest radical scavenging activity with IC_{50} value at 120.35 $\mu\text{g/mL}$ and 372.06 $\mu\text{g/mL}$ respectively followed by aqueous peel extract (131.17 $\mu\text{g/mL}$ and 468.08 $\mu\text{g/mL}$), aqueous pulp extract (150.11 $\mu\text{g/mL}$ and 557.19 $\mu\text{g/mL}$), and methanolic peel extract (204.14 $\mu\text{g/mL}$ and 613.8 $\mu\text{g/mL}$). CUPRAC and Phosphomolybdenum assay were also conducted to determine reducing capacity and total antioxidant potential. Overall, the methanolic peel extract showed approximately 5 times higher total antioxidant activity than aqueous pulp extract suggesting that peel is phytochemically richer than pulp. These findings indicate that fruit peels may serve as important reservoirs of bioactive metabolites and cost effective source for nutraceuticals and pharmaceuticals.

INTRODUCTION:

India is a land of rich biodiversity and home to rare medicinal plants whose nutritional and phytochemical profiles remain underexplored (Sharma et al., 2020; Ved & Goraya, 2008). We rely on these botanicals not only for therapeutic properties but also for nutritional benefits and disease prevention. These benefits stem directly from the metabolites inherently present in them. A significant global surge in demand for medicinal flora is prompting the pharmaceutical sector to exploit these valuable resources (Pandey et al., 2018). One such rare and underexplored plant for its pharmacological potential is *Pyrenacantha volubilis* Wight, belonging to the family Icacinaceae; it is a woody twiner (Reddy and Parthasarthy., 2003) exhibiting vertical growth from low-lying stems to tall climbers along the eastern coast of South India, that bear flowers and fruits in late monsoon and winter, respectively (Ramesha et al., 2013 and Suma et al., 2017). The fruits are small, ovoid, and laterally compressed drupes with a smooth, fleshy outer skin that changes from green to a bright orange-red upon ripening. Inside, a single seed is enclosed within a thin, hard endocarp featuring a punctate outer surface and a papillose inner layer that extends into the ruminant endosperm and a pulpy mesocarp (Indian Biodiversity Portal). Fruits are rich sources of essential nutrients and are also abundant in a wide

variety of bioactive phytochemicals, including flavonoids, alkaloids, tannins, phenolic acids, saponins, and terpenoids, which play a crucial role in promoting human health by exhibiting antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Kaur & Kapoor, 2001; Akinmoladun et al., 2007).

P. volubilis is a potential source of antitumor compounds, notably Camptothecin, present at 1.35% in cotyledons and 0.60% in ripened whole fruits (dry weight), along with related derivatives and biosynthetic precursors such as 10-hydroxycamptothecin, 9-methoxycamptothecin, 20-deoxycamptothecin, strictosidine, deoxypumiloside, and strictosamide (Suma et al., 2014). It has been reported to exhibit notable pharmacological effects, including anticancer activity (Suma et al., 2017), as well as anti-inflammatory and antiarthritic properties (Anjali et al., 2020). Although these studies highlight *P. volubilis* with rich phytochemical content, a clear gap remains in understanding its phytochemical and nutritional profile. A detailed proximate analysis is essential to assess its overall nutritional potential. This becomes even more relevant when considering the promising nutritional attributes observed in other members of the Icacinaceae family—such as *Icacina trichantha* (Alawode 2024), *I. oliviformis* (Fay. 1991), and *I. senegalensis* (Ekwealor et al., 2023), which have been recognized for their high nutrient content and potential role in supporting food and nutritional security.

The present investigation aims to uncover nutritional value through proximate analysis and quantify its bioactives through phytochemical evaluation. Phytochemical screening serves as a preliminary step in identifying these compounds, guiding further isolation and pharmacological evaluation. Moreover, phytochemical content can vary widely by species, cultivar, maturity stage, and environmental factors, underscoring the necessity of such analysis in fruit-based research and development (Kalt et al., 1999). As global interest in functional foods, nutraceuticals and new drug molecules continues to grow, the need for comprehensive phytochemical profiling of *Pyrenacantha volubilis* fruits has become increasingly important.

MATERIALS AND METHODS:

Plant material:

Freshly ripened fruits were collected from *Pyrenacantha volubilis* located along National Highway 132B, Palur, Chengalpattu district of Tamil Nadu (12°45'09.0''N 79°55'50.1''E) and stored in a clean Ziplock cover. In the lab, they were thoroughly washed with tap water to eliminate surface contaminants, followed by rinsing with sterile distilled water. Thereafter, it was surface-sterilized using 70% ethanol and again rinsed with sterile distilled water. Wet fruits were then blot-dried using blotting sheets. Fruit peels were manually separated using a sterile knife. Seeds were separated by peeling off the pulp residue. Both peel and pulp were homogenized using a pestle and mortar and stored separately at 4°C until further processing (**Figure 1**).

Chemicals: Atropine, gallic acid, quercetin, catechin, diosgenin, and bromocresol green were purchased from Sigma-Aldrich. All the other chemicals used in the study were of analytical grade purity and were procured from Himedia, SD-fine.

Extract Preparation:

Ultrasonic Bath Extraction was carried out using the method described by Pelayo et al. (2019) with slight modifications. Briefly, fifteen grams of homogenized fruit peel and pulp were weighed and transferred into a 250 mL sterile conical flask. Extraction was carried out using 45 mL of sterile distilled water under dark conditions in an ultrasonic bath (Ti-643, 40 KHz $\pm$ 3 KHz, 230V) for 30 minutes with temperature being regularly monitored to be stable at 30 ($\pm$ 1) °C. The extracts were centrifuged at 4000 rpm for 5 minutes. The supernatant was collected, and the pellet was re-extracted twice under identical conditions. All supernatants were pooled and filtered through Whatman No. 1 filter paper. The final volume was adjusted to 150 mL with sterile distilled water. Similarly, methanol extraction was done and the extracts were stored in sterile glass flasks at 4 °C for subsequent use (**Figure 1**).

The moisture, ash content, crude fibre, and total solids were assessed from fresh homogenized fruit peel and pulp, while pH, protein content, phytochemical screening, quantification and antioxidant activities were carried out using extracts obtained through ultrasonic bath treatment.



Figure: 1. A- *Pyrenacantha volubilis* fruits, B- Peel, C- Pulp, D- Methanol and Aqueous extract of peel, E- Methanol and Aqueous extract of pulp.

1. Proximate analysis: The homogenized peel and pulp were used to determine the proximate composition. Ash content was determined by incinerating the sample in muffle furnace at 550°C for 3 h to constant weight. The moisture content was determined by oven drying method by calculating the loss of weight. Fat content was estimated using Mojonnier ether extraction method. Crude fiber was determined by sequential acid-base digestion followed by filtration and drying the residue in oven to get constant weight. Total solids were determined by drying the sample in oven to obtain constant weight of the residue. Titratable acidity was estimated by titrating 0.1N NaOH using phenolphthalein as indicator. The total carbohydrate content was determined by arithmetic difference method. All the above-mentioned composition was estimated using standard AOAC methods (2016) with slight modifications. pH was measured using digital pH meter. All measurements were performed in triplicates, and the results were recorded as mean percentage values $\pm$ standard deviation.

2. Total Proteins: The protein content in the fruit was quantified using the Lowry method, as described by Waterborg et al. (1984), with slight modifications. To estimate the protein content in the fruit peel and pulp extracts, a standard BSA (Bovine Serum Albumin) curve was prepared using concentrations of 10, 20, 30, 40, and 50 μg in a final volume of 500 μL with sterile distilled water. To this, 100 μL of 2 N NaOH was added, followed by heating in a boiling water bath at 100°C for 10 minutes to ensure proper hydrolysis. After allowing the mixture to cool to room temperature, 1 mL of freshly prepared complex-forming reagent [2% sodium carbonate: 1% copper sulfate pentahydrate: 2% sodium potassium tartrate in the ratio 100:1:1 (v/v)] was added to each tube. After mixing, the reaction mixture was allowed to stand at room temperature for 10 minutes. Subsequently, 100 μL of 1 N Folin–Ciocalteu reagent was added with the aid of a vortex mixer, and the solution was kept at room temperature for 30 minutes for color development. A blank sample was prepared following the same steps but without adding BSA. Absorbance was measured at 750 nm using a Visible Spectrophotometer 106 (Systronics). The protein content in the fruit peel and pulp extracts was quantified by comparing the absorbance values with the standard BSA curve and expressed as a percentage.

3. Mineral composition: To assess the mineral content in the fruit peel and pulp, a slightly modified version of the AOAC (2016) method was followed. About 5 grams of thoroughly homogenized fruit peel and pulp were carefully weighed into pre-cleaned, dry silica crucibles. These crucibles were placed in a hot air oven set at 100–105°C to remove any residual moisture. After drying, the samples were incinerated in a muffle furnace at 550 $\pm$ 5°C for approximately 4 to 6 hours until a consistent light grey or white ash was produced. Once incineration was complete, the crucibles were cooled in a desiccator and reweighed. Repeated weighing was done until a constant mass was obtained. The ash residue was then dissolved in 10 mL of 10% nitric acid and filtered using Whatman’s No.41 filter paper to remove any insoluble particles. The clear filtrate was used for quantitative mineral analysis. An Atomic Absorption Spectrophotometer (AAS) was used to determine the concentrations of

minerals such as Manganese (Mn), Iron (Fe), Zinc (Zn), Lead (Pb), Nickel (Ni), Copper (Cu) and Cadmium (Cd). Sodium (Na) and Potassium (K) levels were assessed using a flame photometer.

4. Qualitative phytochemical screening: Qualitative phytochemical screening was carried out following standard protocols described by Annapurna et al. (2021), Mathew et al. (2012) and Balamurugan et al. (2019). Extracts from fruit peel and pulp were tested for the presence of phytochemicals such as alkaloids, saponins, flavonoids, phenols, glycosides, tannins, carotenoids, carbohydrates, proteins, and essential oils by observing specific color changes or precipitate formation.

5. Quantitative phytochemical estimation:

a. Total phenolics Content (TPC):

The total phenolic content was estimated using the Folin–Ciocalteu colorimetric method, as described by Singleton et al. (1999), with slight modifications. Briefly, varying concentrations of standard gallic acid (5–50 µg) were pipetted from 0.1mg/mL stock into separate test tubes and the volume was made up to 2 mL with distilled water. To each tube, 200 µL of Folin–Ciocalteu reagent was added and incubated at room temperature for 5 minutes followed by adding 2 mL of 7% (w/v) sodium carbonate solution, and the volume was brought up to 5 mL using distilled water. The mixture was thoroughly mixed and kept in the dark for 1.5 h. Following incubation, the absorbance was recorded at 725 nm using a visible spectrophotometer against the blank that was prepared similarly, without the addition of standard. A standard curve was plotted, and the phenolic content from 10 µL of fruit peel and pulp extracts was determined from the calibration curve. All analyses were performed in triplicates, and results were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GE/g FW).

b. Total flavonoid content (TFC):

Total flavonoid content was estimated using the aluminium chloride (AlCl₃) colorimetric method with slight modifications, based on the procedure by Chang et al. (2002). Varying concentrations of quercetin (10–100 µg) were prepared from a 0.1 mg/mL stock solution and diluted to 3 mL with 95% (v/v) ethanol in separate test tubes. To each tube, 200 µL of 10% aluminium chloride and 200 µL of 1 M potassium acetate were added. The volume was then brought up to 9 mL with distilled water. The mixtures were incubated at room temperature for 30 minutes, after which absorbance was measured at 415 nm using a visible spectrophotometer against the blank prepared in the same manner but without quercetin. A standard calibration curve was constructed using the quercetin absorbance values. The total flavonoid content in 50 µL of the fruit peel and pulp extracts was determined from the curve and expressed as milligrams of quercetin equivalent per gram of fresh weight (mg QE/g FW).

c. Total condensed tannins (TCT):

Total Condensed Tannins was estimated using the method described by Sun et al. (1998), with slight modifications. Different concentrations of catechin (10–100 µg) were prepared from a 2 mg/mL stock solution and diluted to 50 µL with methanol. To each tube, 3 mL of 4% vanillin solution was added, followed by 1.5 mL of concentrated hydrochloric acid (HCl). The mixture was allowed to stand at room temperature for 15 minutes. Absorbance was then measured at 500 nm using a visible spectrophotometer, with a blank prepared in the same manner but without catechin. A standard curve was plotted using the absorbance values of the catechin solutions. The total condensed tannin content of the fruit peel and pulp extracts (50 µL) was calculated from the curve and expressed as milligrams of catechin equivalents per gram of fresh weight (mg CE/g FW).

d. Total Alkaloid Content (TAC):

The total alkaloid content was estimated using the bromocresol green (BCG) complexation method, as outlined by Shamsa et al. (2008), with slight modifications. A series of atropine standard solutions (40 –120 µg) were prepared from a 0.1 mg/mL stock and adjusted to a volume of 1.2 mL using distilled water. To each standard tube, 5 mL of phosphate buffer (pH 4.7) and 5 mL of BCG reagent were added. The mixture was then extracted with 1 mL of chloroform using a separating funnel, and the extraction was repeated three times. The pooled chloroform layer was made up to 5 mL. The absorbance of each solution was measured at 470 nm using a visible spectrophotometer against the blank (except standard). A standard calibration curve was constructed using the atropine standards. Alkaloid content in 100 µL of fruit peel and pulp extract was determined by comparing its absorbance to the standard curve. Results were expressed as milligrams of atropine equivalents per gram of fresh weight (mg AE/g FW).

e. Total Saponin Content (TSC):

The total saponin content was estimated using the vanillin-sulfuric acid colorimetric method, based on the procedure described by Hiai et al. (1976), with slight modifications. A series of diosgenin standard solutions (10–100 µg) were prepared from 1 mg/mL stock solution and adjusted to 100 µL with methanol. To each tube, 100 µL of 8% (w/v) vanillin solution and 1 mL of 75% (v/v) sulfuric acid were added. The mixtures were incubated in a water bath at 60°C for 10 minutes. After incubation, the absorbance was recorded at 540 nm using a visible spectrophotometer against the black (without standard). A standard calibration curve was plotted using the absorbance values of the diosgenin standards. Saponin content in 5 µL and 100 µL of fruit peel and pulp extracts respectively was determined by comparing their absorbance with the standard curve. The results were expressed as milligrams of diosgenin equivalents per gram of fresh weight (mg DE/g FW).

6. Antioxidant Activity:

a. Radical scavenging activities: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity were performed following the methods of Sharma and Bhat (2009) and Re et al., (1999) respectively, for methanol and aqueous extracts of peel and pulp. Briefly, 50 µM of DPPH was dissolved in methanol and mixed with 50 µL of peel and pulp extracts. The reaction mixture was incubated in dark for 30 min and absorbance was measured at 517 nm. Ascorbic acid was used as the standard. In ABTS, free radicals were generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate. 50 µL of peel and pulp extracts were incubated with 1 mL of ABTS free radicals in dark for 30 min and absorbance was measured at 734 nm. Trolox was used as the standard. IC₅₀ values were calculated and percent inhibition was calculated using the formula

$$\text{Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

b. Reducing capacity and total antioxidant activity: CUPRAC (Cupric reducing antioxidant capacity) and Phosphomolybdenum assay were carried out for methanol and aqueous extracts of peel and pulp following the methods described by Apak et al. (2004) and Prieto et al. (1999) respectively. In CUPRAC, Briefly, 1 mL each of 10 mM Cu (II) chloride solution, 7.5 mM neocuproine in ethanol, and 1 M ammonium acetate buffer (pH 7.0) were added to the different concentration of peel and pulp extracts followed by 30 min incubation in dark and measuring the absorbance at 450 nm. The cupric reduction potential was expressed as standard Trolox equivalents (TE). In Phosphomolybdenum assay, peel and pulp extracts at different concentrations were mixed with the Phosphomolybdenum reagent having 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated for 90 min at 95°C and after cooling to room temperature, the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as standard ascorbic acid equivalents (AAE).

Statistical analysis: The experimental data were analyzed using one-way analysis of variance (ANOVA) in GraphPad Prism version 5. Results are presented as mean ± standard deviation (SD) of triplicate determinations. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION:

1. Proximate analysis: Table 1 shows the proximate composition of *P. volubilis* fruit peel and pulp. Most parameters differed significantly ($p < 0.05$) between the two parts. The pulp had significantly higher moisture content ($80.63 \pm 0.20\%$) than the peel ($56.42 \pm 1.49\%$). In contrast, the peel showed significantly higher essential nutrients such as ash content ($10.25 \pm 0.44\%$), crude fat ($0.48 \pm 0.06\%$), crude fiber ($2.68 \pm 0.06\%$), protein ($3.77 \pm 0.03\%$), carbohydrates ($26.39 \pm 1.06\%$) and total solids ($43.58 \pm 1.49\%$). Interestingly, both peel and pulp showed identical titratable acidity (0.34%), with no significant difference, indicating similar acid-base characteristics. The nutritional assessment in our study suggests that peel, with lowest moisture content has higher levels of macronutrients like protein, carbohydrates, fiber, and total solids compared to pulp. Several fruit peels retain less moisture content with higher amounts of proteins, fiber, carbohydrates, and fat than its pulp (Ajila et al., 2007). Izidoro et al. (2023) also noted similar nutritional richness in mango peel flour compared with pulp flour across other parameters. Even in *Citrus maxima* and *Citrus reticulata*, peels generally have higher concentrations of carbohydrates, fibre, fat, and ash compared to their inner pulp (Goyal & Kaushal., 2018; Batool et al., 2020). Alawode. (2024) reported parallel observations from the same family member, *Icacina tricantha*, where the tubers share a similar profile to the peel of *P. volubilis*, with moisture content at 55.8% and carbohydrates around 27.6%. These observations show that nutrient accumulation is higher

in outer tissues compared to inner parts across various species and peel, often discarded as waste, is a nutrient-dense component with a promising application in food formulation and nutritional enhancement.

Parameter	Fruit peel	Fruit Pulp	P value	Statistically significant
pH	6.28 ± 0	6.73 ± 0	0	-
Ash Content (%)	10.25 ± 0.44	4.77 ± 0.36	0.00007	Yes
Moisture content (%)	56.42 ± 1.49	80.63 ± 0.20	0.000009	Yes
Fat content (%)	0.48 ± 0.06	0.29 ± 0.09	0.0365	Yes
Crude fibre (%)	2.68 ± 0.06	1.04 ± 0.08	0.000008	Yes
Protein content (%)	3.77 ± 0.03	2.02 ± 0.06	0.000001	Yes
Carbohydrate content (%)	26.39 ± 1.06	19.28 ± 0.40	0.00040	Yes
Total solids (%)	43.58 ± 1.49	11.26 ± 0.58	0.000003	Yes
Titrateable acidity (%)	0.34 ± 0	0.34 ± 0	-	No

Table 1. Proximate composition of fruit peel and pulp. Values are expressed as mean percentage ± standard deviation along with p value. p<0.05 is considered significant.

2. Mineral composition: Table 2 illustrates the mineral content of *P. volubilis* fruit peel and pulp, which varies significantly between the two parts. The fruit pulp had significantly higher Zinc content at 69.0 ± 0.7 mg/g, about 3.7 times higher than that of the peel at 19.10 ± 0.35 mg/g. Likewise, pulp showed higher Iron (11.502 ± 0.18 mg/g) and Manganese (1.03 ± 0.05 mg/g) than peel (8.4725 ± 0.12 mg/g Iron and 0.5525 ± 0.03 mg/g), suggesting pulp is a better source of micronutrients. In contrast, alkaline metals like Sodium and Potassium were found to be substantially higher in peel (50.10 ± 0.45 mg/g and 55.4 ± 0.50 mg/g respectively) than in pulp (30.8 ± 0.35 mg/g and 38.5 ± 0.42 mg/g respectively). Both peel and pulp also contained small amounts of heavy metals like Lead and Cadmium, with peel (0.171 ± 0.012 mg/g and 0.020 ± 0.003 mg/g, respectively) showing slightly more than pulp (0.0945 ± 0.0008 mg/g and 0.01 ± 0.002 mg/g, respectively). These findings show that peel and pulp offer different nutritional benefits; pulp is enriched with trace elements, while peel has higher electrolyte levels. The mineral profile of fruit peel and pulp revealed significant differences in the distribution of micronutrients and alkali metals. Notably, the pulp showed higher levels of trace elements, which are involved in immune support, enzymatic activity, and antioxidant defense mechanisms. Radhika et al. (2024) found that Zinc and Iron levels were higher in the pulp of *Eriobotrya japonica* than in the peel. Singh et al. (2015) recorded similar observations in grapefruit that pulp had higher concentrations of Zinc, Copper, and Iron than peel. These elements act as electrolytes that play a vital role in fluid balance, osmoregulation, and nerve transmission (Dharmarajan. 2021). Higher concentrations of these elements in the peel suggest its potential role in maintaining homeostasis. Similar to our findings, Emaga et al. (2007) reported higher Potassium and Sodium retention in banana peel than in pulp.

Mineral element	Copper (mg/g)	Zinc (mg/g)	Nickel (mg/g)	Manganese (mg/g)	Iron (mg/g)	Sodium (mg/g)	Potassium (mg/g)	Cadmium (mg/g)	Lead (mg/g)
Fruit Peel	0.48 ± 0.02	19.10 ± 0.35	0.16 ± 0.015	0.55 ± 0.03	8.47 ± 0.12	50.10 ± 0.45	55.40 ± 0.50	0.020 ± 0.003	0.17 ± 0.012
Fruit Pulp	0.47 ± 0.018	69.00 ± 0.70	0.21 ± 0.018	1.03 ± 0.05	11.50 ± 0.18	30.80 ± 0.35	38.50 ± 0.42	0.010 ± 0.002	0.09 ± 0.008
P value	0.554813	4.03164 × 10 ⁻⁸	0.0209066	0.000140538	0.000017128	5.06529 × 10 ⁻⁷	1.48101 × 10 ⁻⁶	0.00862393	0.000656
Statistically significant	No	yes	yes	yes	Yes	yes	yes	No	yes

Table 2. Mineral composition of fruit peel and pulp. Values are expressed as mean ± standard deviation (mg/g FW) along with p value. p<0.05 is considered significant.

The accumulation of heavy metals in the fruit peel, with Lead at 0.171 ± 0.012 mg/g and Cadmium at 0.02 ± 0.003 mg/g, which substantially exceeds the maximum permissible limits suggested by international food safety standards, might be due to the accumulation of these metals at the place of collection. This indicates that external sources, likely from environmental exposure such as air or soil pollution, may contribute to the accumulation of these toxic elements in the peel. At the same time these metals were present at much lower concentrations in the pulp. Detection of heavy metals in the peel suggest its potential health risks if consumed without adequate processing or treatment. Similar observations were made regarding wild plants in South Africa that accumulated Lead and Cadmium despite safe levels in edible parts (Bvenura & Afolayan. 2015).

3. Qualitative Phytochemical screening: The phytochemical screening of *P. volubilis* showed that both fruit peel and pulp have beneficial plant compounds (**Table 3**). The peel showed a broader range of these compounds than the pulp. Both peel and pulp contained carbohydrates, proteins, alkaloids, glycosides, saponins, phenols, flavonoids, carotenoids and mucilage. Interestingly, terpenoids and quinones were present only in peel. Tannins, steroids, and anthraquinones were absent in both peel and pulp. Although both peel and pulp contain bioactive substances, the peel appears to possess higher nutritional and therapeutic potential than the pulp due to its richer phytochemical profile. This abundance of phytochemicals in peel suggests higher bioactive potential, consistent with the other studies on mango and citrus, where peels often contain higher concentrations of protective phytochemicals (Ajila et al., 2007; Budiarto et al., 2024). These bioactive compounds are known for their strong antioxidant, antimicrobial, and therapeutic properties, supporting our view that the fruit peel of *P. volubilis* may have nutraceutical and pharmaceutical applications. The presence of alkaloids and saponins in both peel and pulp further emphasizes their significance in anticancer, anti-inflammatory, and hypoglycemic effects (Venkatesan et al., 2019).

Phytochemical	Fruit Peel	Fruit Pulp
Carbohydrates	+	+
Starch	–	–
Proteins	+	+
Glycosides	+	+
Saponins	+	+
Terpenoids	+	–

Steroids	–	–
Alkaloids	+	+
Phenols	+	+
Tannins	–	–
Flavonoids	+	+
Anthraquinones	–	–
Quinones	+	–
Gums and Mucilage	+	+
Carotenoids	+	+

Table: 3. Qualitative phytochemical constituents of fruit peel and pulp extracts. “+” indicates presence; “–” indicates absence of the compound in the respective fruit part.

4. Quantitative Phytochemical estimation: Alkaloids, flavonoids, condensed tannins, and saponins from both peel and pulp were better extracted in methanol, whereas water was a better solvent for extracting phenolics. The methanolic peel extract had 5.01 ± 0.10 mg AE/g FW alkaloids, compared to the aqueous peel extract with 1.51 mg AE/g FW. The flavonoids and condensed tannins were 16.38 mg QE/g FW and 0.6311 ± 0.04 CE/g FW, respectively in methanolic peel extract. The saponin content in methanolic peel extract was 69.77 ± 4.42 mg DE/g FW and 59.86 ± 5.18 mg DE/g FW in aqueous peel extract. In contrast, aqueous peel extract showed higher phenolic content of 4.77 ± 0.17 mg GE/g FW than methanol extract 2.83 ± 0.1 mg GE/g FW (Table 4). The overall findings indicate that methanol served as a superior solvent for the extraction of the majority of phytochemicals, although water was more effective in recovering phenolics. Azzahra et al. (2025) reported that solvent polarity and chemical affinity of compounds influence recovery; therefore, different solvents extract different groups selectively. The pulp showed significantly lower amounts of phytochemicals than the peel, with saponins highest, followed by flavonoids, phenolics, alkaloids, and condensed tannins, (Table 4). In aqueous extracts, the peel showed 5.9-fold more saponins than pulp, suggesting that peel is a rich source of saponins. The peel also showed higher flavonoid accumulation than pulp, with peel extracts showing nearly 5 times more than the pulp extracts. Flavonoids and phenolics are particularly involved in defense mechanisms, suggesting their accumulation is higher in protective parts like peel than in pulp. These findings suggest that the peel, often discarded as waste, is a rich source of bioactive phytochemicals with potential antioxidant, and therapeutic properties, supporting its potential use in the development of functional foods, nutraceuticals, and pharmaceutical products. Consistent with our findings, Lasota et al. (2024) made similar observations across citrus species, reporting significantly higher levels of polyphenols and flavonoids in peel than in pulp. Sir Elkhatim et al. (2018) reported peels with 2.8–3.6-fold higher phenolics than pulp in grapefruit and tangerine, and lime peels also showed richer flavonoid contents (Budiarto et al., 2024). Peels of fruit species like *Annona*, *Cydonia*, and *Fortunella* yielded higher diversity and higher concentrations of antioxidant compounds (Lasota et al., 2024) indicating peels are more functionally significant than pulp in concentrating bioactive compounds. In our study, saponins were the most abundant compound, similar to earlier findings by Johnson et al. (2012) and Neglo et al. (2021), who reported significantly higher amounts of saponins in the pulp, seed, and rind of watermelon. In the case of *P. volubilis*, the exceptionally high saponin content in the aqueous peel extract, which is six times higher than the aqueous pulp extract, suggests its potential as a valuable raw material. The strong alignment between previous literature and our findings, highlights the potential of fruit peels as cost-effective sources for nutraceuticals and pharmaceuticals.

Phytochemical estimation	Peel Aqueous	Peel Methanol	Pulp Aqueous	Pulp Methanol
Total Alkaloids mg of AE/ g F W	1.51± 0.26	5.01 ± 0.10	1.30± 0.10	1.63 ± 0
Total flavonoids mg of QE/ g FW	9.76± 0.16	16.38± 0.72	2.75 ± 0	3.90 ± 0.28
Total phenolics mg of GE/ g FW	4.77± 0.17	2.83 ± 0.10	2.00 ± 0.11	1.45 ± 0.02
Total condensed tannins mg of CE/ g FW	0.54 ± 0.01	0.63 ± 0.04	0.31 ± 0.02	0.26 ± 0.01
Total saponins mg of DE/ g FW	59.86 ± 5.18	69.77 ± 4.42	10.08 ± 0.28	23.54 ± 0.14

Table: 4. Quantitative estimation of Phytochemicals of fruit peel and pulp. Values are expressed as mean ± SD (mg/g FW) in triplicate. AE: Atropine Equivalent, QE: Quercetin Equivalent, GE: Gallic Acid Equivalent, CE: Catechin Equivalent, and DE: Diosgenin Equivalent.

5. Antioxidant activities

a. Radical scavenging activities: ABTS assay showed that the methanolic pulp extract exhibited the highest radical scavenging activity, with an IC_{50} value of 120.35 $\mu\text{g/mL}$, followed by aqueous peel extract (131.97 $\mu\text{g/mL}$), aqueous pulp extract (150.11 $\mu\text{g/mL}$), and methanolic peel extract (204.14 $\mu\text{g/mL}$). Thus, methanolic peel extract showed the lowest concentration to achieve 50% inhibition, suggesting that compounds in the pulp were more efficient against ABTS radical cations. Interestingly, unlike the pulp extracts, the aqueous peel extract showed a lower IC_{50} than the methanolic peel extract. A similar trend was also observed in DPPH scavenging activity, although the IC_{50} values were much higher than those obtained in the ABTS Assay. Methanolic pulp extracts showed the highest activity with the lowest IC_{50} value at 372.06 $\mu\text{g/mL}$, followed by aqueous pulp extract (468.08 $\mu\text{g/mL}$), aqueous peel extract (557.19 $\mu\text{g/mL}$), and methanolic peel extract (613.8 $\mu\text{g/mL}$). Overall, radical scavenging activity followed the order: Pulp methanol > Pulp aqueous > Peel aqueous > Peel methanol (**Figure 2**). Our results suggest that *P. volubilis* fruit extracts have considerable radical scavenging activity, but the extent of activity varied by the part used, the extraction solvent, and the assay system. Methanolic pulp showed the lowest IC_{50} value in both assays, suggesting a higher ability to neutralize the ABTS and DPPH radicals. This relatively strong antioxidant activity in the methanolic pulp extract is consistent with observations by Nandhakumar and Indumathi. (2013), who studied methanol and aqueous extracts of *Annona squamosa* fruit pulp with higher antioxidant activity in methanolic extracts, correlating it with having higher phenolics and flavonoids.

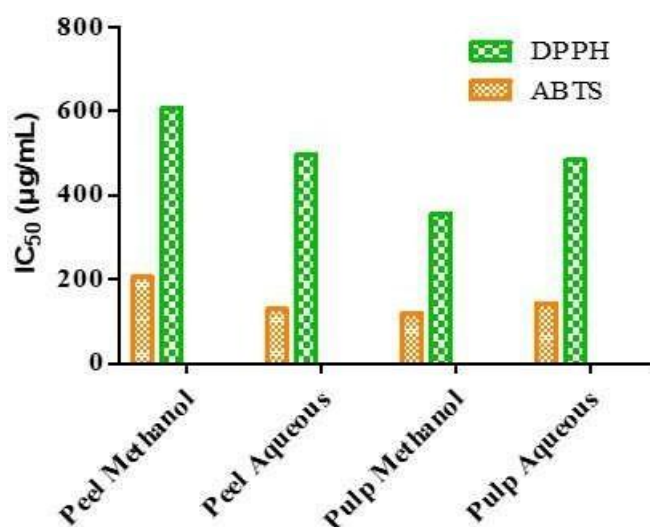


Figure 2. DPPH and ABTS radical scavenging activity of methanolic and aqueous fruit peel and pulp extracts expressed in IC₅₀ (µg/mL) values

The ABTS results were particularly notable because they showed lower IC₅₀ values from 120.35 to 204.14 µg/mL. These values fall within the range reported for several medicinal plants (Singh et al. 2016), which ranged from 24.08 to 513.4 µg/mL. They also demonstrated that lower IC₅₀ values correspond to higher radical-scavenging activity. The IC₅₀ values obtained in our study show appreciable scavenging potential when compared to the variations among other medicinal plants.

It's very interesting to note that aqueous peel extract showed stronger ABTS activity than methanolic peel extract, whereas the methanolic pulp extract showed stronger activity than the corresponding aqueous extract. This contrasting solvent response between peel and pulp suggests that antioxidant compounds with different polarities may be distributed differently within the fruit. Therefore, the antioxidant activity of *P. volubilis* should not be attributed to a single class of compounds or to one extraction solvent alone because antioxidant activity can result from synergistic interactions among phenolics, flavonoids, and other constituents.

b. Reducing capacity and total antioxidant activity: In CUPRAC assay, methanolic peel extract showed the highest cupric-ion-reducing capacity at 26.68 ± 0.18 mg TE/g FW. This was followed by aqueous peel extract (15.08 ± 0.13 mg TE/g FW), methanol pulp extract (10.83 ± 0.13 mg TE/g FW), and aqueous pulp extract (5.57 ± 0.06 mg TE/g FW). Similarly, methanolic peel extract (39.10 ± 0.05 mg AAE/g FW) showed the highest total antioxidant capacity, followed by aqueous peel extract at 24.99 ± 0.12 mg AAE/g FW, methanolic pulp extract at 17.31 ± 0.08 mg AAE/g FW and aqueous pulp extract at 7.82 ± 0.04 mg AAE/g FW. Thus, methanolic peel extract showed the highest total antioxidant capacity and reducing capacity, whereas aqueous pulp extract showed the lowest in both. Therefore, both the assays followed the same order of Peel methanol > Peel aqueous > Pulp methanol > Pulp aqueous (**Figure 3**). Our findings suggest that *P. volubilis* peel and pulp extracts exhibit good total antioxidant and reducing capacities that depend on the fruit tissue and extraction solvent. In the present study, methanolic peel extract showed the highest Phosphomolybdenum and CUPRAC values, indicating higher electron-donating and reducing capacity than the other extracts. Yerlikaya et al. (2017) also noted a comparable solvent-dependent response, reporting differences in Phosphomolybdenum activity among methanol, ethyl acetate, and aqueous extracts of *Ononis natrix* subsp. *hispanica*, in which the methanolic extract showed higher activity (1.53 mmol TE/g extract) than the aqueous extract (1.13 mmol TE/g extract). CUPRAC also yielded 170.51 mg TE/g extract for the methanolic extract and 94.16 mg TE/g extract for the aqueous extract. This supports the possibility that methanol extracted a greater proportion of redox constituents from *P. volubilis* peel, and the high CUPRAC value suggests that methanolic peel was abundant in compounds capable of reducing Cu²⁺ due to the presence of multiple macromolecules and phytochemicals, as evident from our study. The higher activity in the peel may be attributed to higher levels of phytochemicals and provide the chemical basis for the stronger antioxidant activity observed in peel extracts. This interpretation is also supported by Dilkalal et al. (2024), who reported strong antioxidant activity in

phenol-enriched fractions. Therefore, our findings indicate that the peel, particularly the methanolic peel extract, is a major source of polyphenolic compounds with electron-donating/reducing antioxidant capacity.

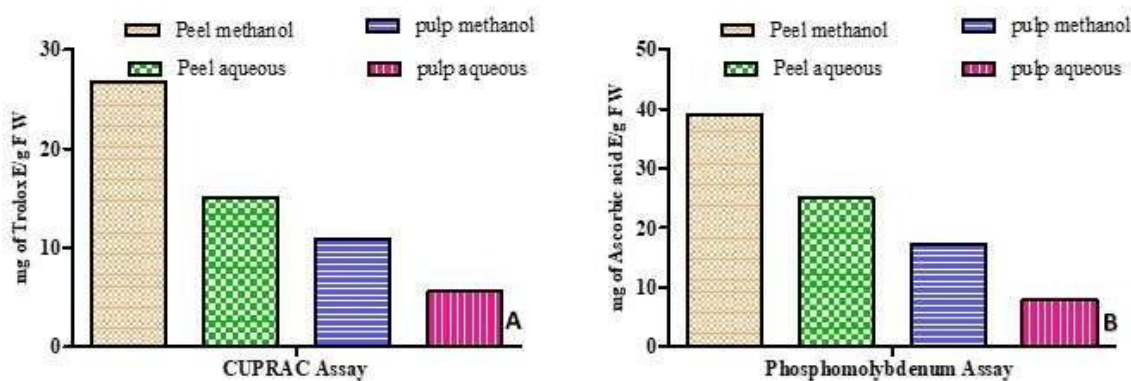


Figure 3. A- CUPRAC Assay of methanolic and aqueous Fruit peel and pulp extracts expressed as mg of Trolox Equivalent per g FW and B- Phosphomolybdenum Assay of methanolic and aqueous fruit peel and pulp extracts expressed as mg of Ascorbic acid Equivalent per g FW.

CONCLUSIONS:

Overall study shows substantial variation in the proximate, mineral, phytochemical, and antioxidant profiles of the investigated fruit tissues, highlighting their distinct nutritional and functional attributes. The peel showed comparatively higher levels of ash, proteins, carbohydrates, fats, crude fiber, and total solids than the pulp, whereas the pulp had higher moisture content. The mineral analysis further revealed tissue-specific accumulation patterns, with the peel showing greater concentrations of several trace elements. Meanwhile the pulp was comparatively enriched in the major electrolytes Sodium and Potassium. Notably, detecting heavy metals in the peel underscores the importance of appropriate safety assessment and processing before any potential dietary or therapeutic applications.

Phytochemical screening and quantitative analyses showed greater recovery of bioactive constituents from methanolic than from aqueous extracts, highlighting the influence of solvent polarity on phytochemical extraction efficiency. The pronounced antioxidant activity of the extracts, reflected by their free-radical-scavenging and reducing capacities, further supports the presence of redox-active phytoconstituents. Collectively, the findings suggest that the peel, in particular, represents a promising source of nutritionally and biologically relevant constituents compared with the pulp. However, the observed antioxidant activity alone does not establish therapeutic efficacy. Comprehensive chemical characterization, identification and quantification of individual bioactive compounds, assessment of toxicological and heavy-metal-related risks, and well-designed *in vitro* and *in vivo* studies are necessary to establish the biological relevance, safety, and therapeutic potential of these plant-derived constituents. Such investigations may provide a stronger scientific foundation for assessing the potential of these underexplored plant resources as sources of bioactive compounds for nutraceutical and pharmaceutical applications.

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