

# Total phenolic and saponin content and $\alpha$ -amylase inhibition of marketed *Pandanus tectorius* fruits and leaves

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## ABSTRACT:

*Pandanus tectorius* is widely marketed in Vietnam as crude herbal material and tea, yet comparative data on its phytochemical quality and  $\alpha$ -amylase inhibition remain limited. In this study, thirteen fruit and nine leaf samples were purchased from local markets and e-commerce sources and extracted with 90% ethanol. Total phenolic content (TPC) and total saponin content (TSC) were determined by Folin–Ciocalteu and vanillin–sulfuric acid assays, while  $\alpha$ -amylase inhibition was evaluated using a modified DNSA method. Results were expressed on both a sample basis (mg/g dry material) and an extract basis (mg/g extract). Extraction yields were higher in leaves (11.51–14.51%) than in fruits (9.59–11.94%). On a sample basis, fruits and leaves contained comparable levels of TPC (12.17 vs 12.99 mg GAE/g;  $p = 0.187$ ) and TSC (5.63 vs 5.31 mg AE/g;  $p = 0.214$ ). On an extract basis, fruit extracts were significantly more enriched, with TPC of 115.43 vs 99.38 mg GAE/g extract ( $p = 0.002$ ) and TSC of 53.42 vs 40.73 mg AE/g extract ( $p < 0.0001$ ). The  $\alpha$ -amylase inhibitory activity was moderate, with  $IC_{50}$  values ranging from 130.48 to 200.45  $\mu$ g/mL (fruits 163.77  $\mu$ g/mL, leaves 171.49  $\mu$ g/mL), compared with 88.96  $\mu$ g/mL for acarbose. Correlation analysis revealed a strong negative relationship between TPC and  $IC_{50}$  ( $r = -0.842$ ,  $R^2 = 0.709$ ), while TSC correlated only weakly ( $r = -0.319$ ,  $R^2 = 0.102$ ). These results indicate that phenolic compounds are the main contributors to the inhibitory activity and that fruit extracts represent a more concentrated source of bioactive metabolites suitable for functional food or nutraceutical applications.

**Keywords:** *Pandanus tectorius*, phenolic, saponin,  $\alpha$ -amylase

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## I. INTRODUCTION

The genus *Pandanus* (family Pandanaceae) comprises approximately 700–750 species predominantly distributed across tropical and subtropical regions of the Paleotropics, ranging from West Africa to the Pacific Islands [1, 2]. Members of this genus are morphologically distinctive, with spiral phyllotaxy, long, narrow leaves, and aerial prop roots that facilitate adaptation to sandy or coastal habitats. Ecologically, *Pandanus* species play an important role in shoreline stabilization and tropical forest ecosystems, while culturally they possess considerable economic and ethnobotanical value. Their leaves are widely used for weaving mats, baskets, and roofing materials, and in some regions as aromatic culinary ingredients [1, 2]. *Pandanus tectorius* Parkinson ex Du Roi is a widely distributed species occurring in tropical and subtropical coastal regions from South Asia to the Pacific Islands, and in Vietnam, it is commonly found from the northern midland areas to the coastal regions of Khanh Hoa [3]. Traditionally, this plant has been employed in folk medicine for the treatment of various ailments. Its roots have been used for edema, dysuria, and kidney stones, and externally for bone fractures and hemorrhoids, while its leaves, characterized by a sweet taste and cooling properties, are applied to reduce fever and treat colds. Young shoots have also been reported to support the treatment of kidney stones and pediatric convulsions [4-6]. Beyond medicinal uses, *P. tectorius* holds ecological significance in mangrove and coastal ecosystems.

Phytochemical studies have demonstrated that *P. tectorius* is a rich source of structurally diverse secondary metabolites. International and regional investigations have identified multiple compound classes, including phenolic acids and aldehydes (e.g., p-coumaric acid, ferulic acid, vanillin, syringaldehyde), flavonoids (e.g., vitexin, tricetin, chrysin, sakuranetin, naringenin), and lignans such as pinorexinol, syringaresinol, medioresinol, lyoniorexinol, and balanophonin [7-12]. Coumarins, benzofuran derivatives, and volatile

constituents such as geranyl acetate and ethyl cinnamate have also been reported [8, 11, 12]. In addition, other miscellaneous compounds, including long-chain fatty alcohol esters, methylsuccinic acid, sugar derivatives, and 5-hydroxymethylfurfural, have been documented [10, 13-16]. These phytoconstituents underpin a broad spectrum of biological activities. Extracts and isolated compounds from *P. tectorius* have demonstrated strong antioxidant, anti-inflammatory, antimicrobial, cytotoxic, and antidiabetic properties. In particular, phenolic-rich extracts exhibit potent radical scavenging capacity in DPPH, ABTS, and hydroxyl radical assays [11-14, 17], protective effects against oxidative stress in Schwann cells through the Nrf2/Keap1 pathway [18], lipid-lowering effects via PPAR $\alpha$  and AMPK signaling [19], and  $\alpha$ -glucosidase inhibitory activity contributing to hypoglycemic potential [10, 13, 14]. Cytotoxicity against several cancer cell lines, including A549, MCF-7, and HeLa, has also been documented [12, 15, 20]. Moreover, antimicrobial effects against gram-positive and gram-negative bacteria have been reported [11]. Recent studies have further highlighted the potential of *P. tectorius* fruit extracts in nanoparticle formulations for therapeutic applications in metabolic disorders [15].

In Vietnam, the fruits and leaves of *P. tectorius* are widely available on the market, either as raw materials or in packaged tea bags. Beyond their use in traditional medicine, they are also consumed as a common food product. The present study investigates the levels of major classes of secondary metabolites, including phenolics and saponins, and evaluates the  $\alpha$ -amylase inhibitory activity of marketed fruits and leaves of *P. tectorius* in Vietnam, thereby contributing to quality assessment and further exploration of the biomedical potential of this species.

## II. MATERIAL AND METHODS

### 2.1. Materials

Nine dried leaf samples and thirteen dried fruit samples of *P. tectorius* were purchased from herbal markets in Hanoi, Vietnam, or through e-commerce platforms. Common solvents such as ethanol, DMSO, and inorganic salts were obtained from Daihan Scientific (South Korea), while reference standards and other chemicals were supplied by Merck (Germany).

### 2.2. Extracts preparation

To prepare the extract for chemical analysis and bioassays, 50 g of each sample was powdered, then extracted in triplicate with 1 L of ethanol 90% in a sonication bath at 70°C. The solution was filtered and evaporated to yield the total extract.

### 2.3. Total phenolic assay

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the standard [21]. Absorbance was measured at 760 nm, and results were expressed as mg gallic acid equivalents per gram of dry sample (mg GAE/g).

### 2.4. Total saponin assay

The total saponin content (TSC) of the samples was assessed using the vanillin–sulfuric acid method [22]. For this procedure, 100  $\mu$ L of each extract was mixed with 100  $\mu$ L of 8% (w/v) vanillin in ethanol and 2800  $\mu$ L of 80% (v/v) sulfuric acid. The mixture was incubated at 70°C for 15 minutes. Solutions of the reference compound (aescin) and reagent blanks (with solvent) were also prepared. After incubation, the mixtures were allowed to cool at room temperature for 5 minutes, and absorbance was recorded at 560 nm against the blank.

### 2.5. $\alpha$ -amylase inhibition assay

$\alpha$ -amylase inhibition was evaluated using a modified DNSA method [23]. Reaction mixtures containing sample extracts and  $\alpha$ -amylase were incubated with starch substrate, and the reaction was terminated with DNSA reagent. Absorbance was measured at 650 nm, with acarbose as the positive control.

## III. RESULTS AND DISCUSSION

### 3.1. Phytochemical contents of the *P. tectorius* fruits and leaves

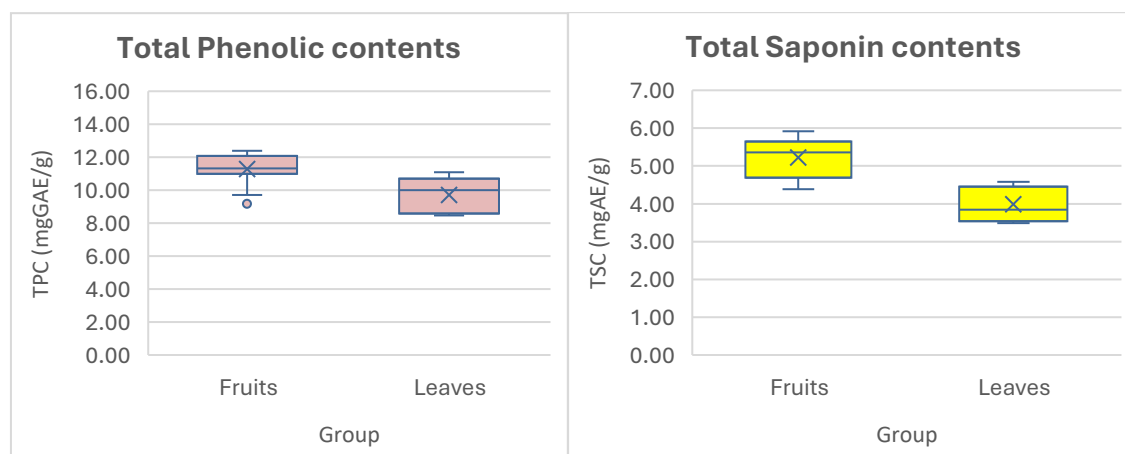
The extraction yields of *P. tectorius* ranged from 9.59% to 11.94% in fruits and from 11.51% to 14.51% in leaves, indicating that leaves generally provided higher extraction efficiency than fruits. In contrast, the distribution of secondary metabolites showed distinct patterns.

The total phenolic content (TPC) of fruits varied between 9.38 and 14.31 mg GAE/g sample, while the figures for the leaves ranged from 11.27 to 16.03 mg GAE/g sample. For total saponin content (TSC), fruits displayed higher values, ranging from 4.61 to 6.54 mg AE/g sample, compared to leaves, which contained 4.52–5.93 mg AE/g sample.

**Table 1: Extraction yields, total phenolic (TPC) and saponin (TSC) contents of the *P. tectorius* samples**

No.	Code	Part	Extraction yield	TPC (mg GAE/g)		TSC (mg AE/g)	
				Sample	Extract	Sample	Extract
1	F01	Fruits	9.78%	11.38 $\pm$ 1.34	116.32 $\pm$ 13.66	5.47 $\pm$ 0.57	55.96 $\pm$ 5.82
2	F02	Fruits	9.77%	11.17 $\pm$ 1.11	114.34 $\pm$ 11.38	5.63 $\pm$ 0.64	57.64 $\pm$ 6.51
3	F03	Fruits	11.51%	12.73 $\pm$ 1.56	110.62 $\pm$ 13.54	5.16 $\pm$ 0.59	44.82 $\pm$ 5.17
4	F04	Fruits	9.59%	12.15 $\pm$ 1.30	126.67 $\pm$ 13.51	4.61 $\pm$ 0.48	48.07 $\pm$ 5.02
5	F05	Fruits	10.15%	12.28 $\pm$ 1.46	120.97 $\pm$ 14.42	4.82 $\pm$ 0.52	47.45 $\pm$ 5.09
6	F06	Fruits	11.09%	11.02 $\pm$ 0.90	99.34 $\pm$ 8.08	6.41 $\pm$ 0.81	57.81 $\pm$ 7.27
7	F07	Fruits	11.00%	12.64 $\pm$ 1.38	114.92 $\pm$ 12.58	5.86 $\pm$ 0.74	53.27 $\pm$ 6.77
8	F08	Fruits	9.75%	11.26 $\pm$ 0.97	115.51 $\pm$ 9.90	4.94 $\pm$ 0.50	50.70 $\pm$ 5.14
9	F09	Fruits	10.00%	9.38 $\pm$ 1.13	93.75 $\pm$ 11.31	5.94 $\pm$ 0.58	59.43 $\pm$ 5.81
10	F10	Fruits	11.35%	14.31 $\pm$ 1.78	126.09 $\pm$ 15.69	6.38 $\pm$ 0.69	56.24 $\pm$ 6.05
11	F11	Fruits	11.94%	13.82 $\pm$ 1.44	115.74 $\pm$ 12.03	6.54 $\pm$ 0.55	54.78 $\pm$ 4.63
12	F12	Fruits	10.01%	12.66 $\pm$ 1.25	126.44 $\pm$ 12.54	6.06 $\pm$ 0.67	60.49 $\pm$ 6.65
13	F13	Fruits	11.17%	13.40 $\pm$ 1.45	119.93 $\pm$ 13.0	5.34 $\pm$ 0.64	47.85 $\pm$ 5.71
14	L01	Leaves	12.67%	11.27 $\pm$ 1.39	88.94 $\pm$ 10.99	4.52 $\pm$ 0.47	35.67 $\pm$ 3.73
15	L02	Leaves	14.51%	12.57 $\pm$ 1.46	86.63 $\pm$ 10.09	5.29 $\pm$ 0.52	36.45 $\pm$ 3.60
16	L03	Leaves	13.00%	12.38 $\pm$ 1.01	95.25 $\pm$ 7.78	5.53 $\pm$ 0.59	42.52 $\pm$ 4.51
17	L04	Leaves	14.15%	16.03 $\pm$ 1.60	113.31 $\pm$ 11.31	5.57 $\pm$ 0.55	39.34 $\pm$ 3.86
18	L05	Leaves	14.04%	12.15 $\pm$ 1.14	86.54 $\pm$ 8.11	5.43 $\pm$ 0.53	38.70 $\pm$ 3.76
19	L06	Leaves	12.18%	13.38 $\pm$ 1.72	109.84 $\pm$ 14.09	5.65 $\pm$ 0.66	46.41 $\pm$ 5.39
20	L07	Leaves	13.21%	14.39 $\pm$ 1.34	108.95 $\pm$ 10.17	4.75 $\pm$ 0.52	35.99 $\pm$ 3.96
21	L08	Leaves	11.51%	11.80 $\pm$ 1.46	102.55 $\pm$ 12.73	5.14 $\pm$ 0.44	44.66 $\pm$ 3.84
22	L09	Leaves	12.66%	12.96 $\pm$ 1.22	102.37 $\pm$ 9.60	5.93 $\pm$ 0.75	46.87 $\pm$ 5.96

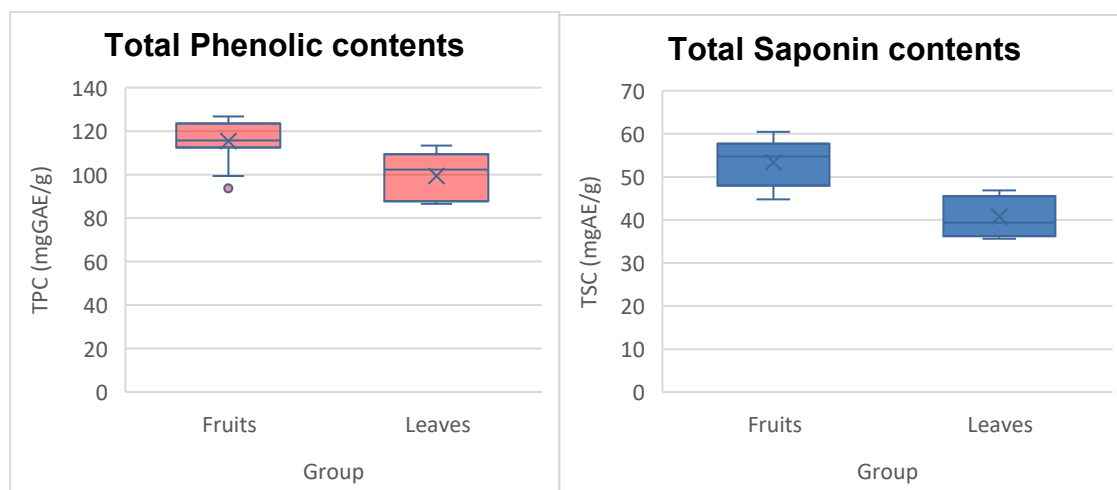
The corresponding boxplot (Figure 1) confirmed this trend, showing that fruits had higher median saponin levels and a more clustered distribution, suggesting greater stability in saponin accumulation relative to leaves. Boxplot analysis highlighted that fruits exhibited relatively consistent TPC values, whereas leaves showed broader variability, with some samples reaching comparatively higher phenolic concentrations.

**Figure 1: Boxplots of the TPC (left) and TSC (right) of the *P. tectorius* samples**

To statistically evaluate these observations, permutation tests were conducted on sample-based values. The mean TPC did not differ significantly between fruits (12.17 mg GAE/g) and leaves (12.99 mg GAE/g) (mean difference =  $-0.82$  mg GAE/g;  $p = 0.187$ ). Similarly, the mean TSC values of fruits (5.63 mg AE/g) and leaves (5.31 mg AE/g) were not significantly different (mean difference =  $+0.32$  mg AE/g;  $p = 0.214$ ). These findings are consistent with the overlapping distributions shown in the boxplots, indicating that although fruits tended to accumulate more saponins and leaves occasionally exhibited higher phenolic levels, such differences were not statistically robust.

The phenolic content of *Pandanus tectorius* extracts showed a clear difference between plant parts. Fruit extracts contained between 93.75 and 126.67 mg GAE/g extract, with a mean of 115.43 mg GAE/g extract, whereas leaf extracts ranged from 86.63 to 113.31 mg GAE/g extract, with a lower mean of 99.38 mg GAE/g extract. The boxplot (Figure 2) illustrates this separation, with fruits displaying higher median values and a narrower interquartile range, suggesting more consistent phenolic enrichment compared to leaves. A permutation test confirmed the statistical significance of this difference (mean difference =  $+16.06$  mg GAE/g extract;  $p = 0.002$ ), indicating that fruit extracts are consistently richer in phenolic compounds. A similar pattern

was observed for saponins. Fruit extracts displayed concentrations between 44.82 and 60.49 mg AE/g extract, with a mean of 53.42 mg AE/g extract, while leaf extracts contained 35.67–46.87 mg AE/g extract, averaging 40.73 mg AE/g extract.



**Figure 2: Boxplots of the TPC (left) and TSC (right) of the *P. tectorius* fruit and leaf extracts**

The boxplot also highlights this divergence, with fruit extracts showing higher medians and tighter distributions compared to the broader and lower range of leaves. The difference in saponin content between plant parts was even more pronounced than that observed for phenolics, with a mean difference of +12.69 mg AE/g extract, which was highly significant according to permutation testing ( $p < 0.0001$ ). Together, these results clearly demonstrate that while both fruits and leaves yield ethanol-soluble phenolics and saponins, fruit extracts consistently exhibit higher concentrations and more uniform distributions of these metabolites, as evidenced by both boxplot visualization and robust statistical testing.

### 3.2. $\alpha$ -amylase inhibitory effect of the extracts from the *P. tectorius* fruits and leaves

The  $\alpha$ -amylase inhibitory activity of *P. tectorius* extracts, expressed as  $IC_{50}$  values ( $\mu\text{g/mL}$ ), varied considerably among samples (Table 2). For fruit extracts,  $IC_{50}$  values ranged from  $130.48 \pm 10.51 \mu\text{g/mL}$  (F12) to  $200.45 \pm 26.01 \mu\text{g/mL}$  (F09), with an overall mean of approximately  $162 \mu\text{g/mL}$ . Leaf extracts displayed a similar variability, ranging from  $146.38 \pm 12.25 \mu\text{g/mL}$  (L06) to  $198.90 \pm 22.32 \mu\text{g/mL}$  (L02), with a mean of about  $171 \mu\text{g/mL}$ . These results indicate that both fruits and leaves possess moderate  $\alpha$ -amylase inhibitory potential, though the activity was not uniform across all samples. When compared to the positive control acarbose ( $IC_{50} = 88.96 \pm 9.69 \mu\text{g/mL}$ ), all extracts were less potent, requiring approximately 1.5–2 times higher concentrations to achieve 50% enzyme inhibition.

**Table 2:  $\alpha$ -amylase inhibitory effect of the *P. tectorius* extracts**

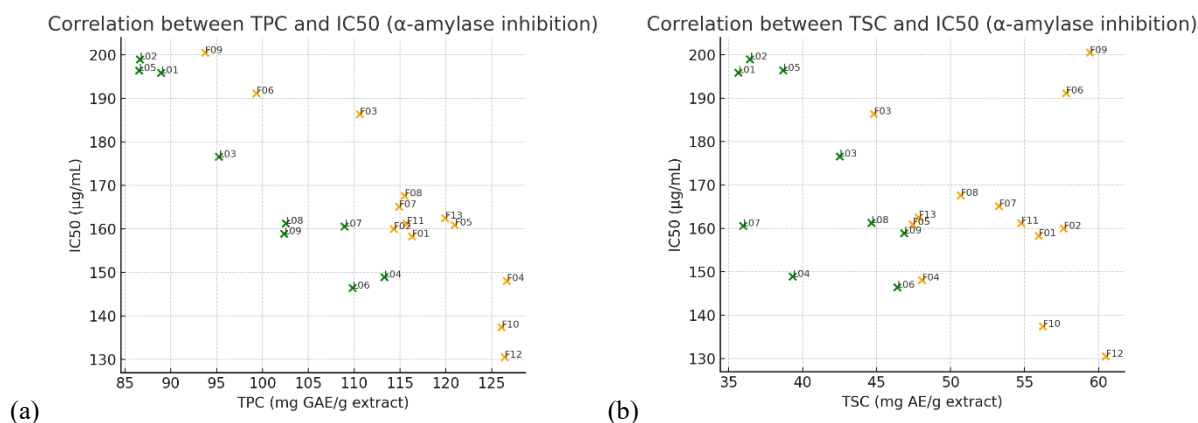
No.	Code	Part	$\alpha$ -amylase
1	F01	Fruits	$158.24 \pm 12.74$
2	F02	Fruits	$159.94 \pm 14.45$
3	F03	Fruits	$186.32 \pm 17.12$
4	F04	Fruits	$148.01 \pm 12.12$
5	F05	Fruits	$160.85 \pm 18.3$
6	F06	Fruits	$191.09 \pm 17.67$
7	F07	Fruits	$165.07 \pm 20.92$
8	F08	Fruits	$167.56 \pm 16.15$
9	F09	Fruits	$200.45 \pm 26.01$
10	F10	Fruits	$137.38 \pm 15.54$
11	F11	Fruits	$161.16 \pm 14.29$
12	F12	Fruits	$130.48 \pm 10.51$
13	F13	Fruits	$162.44 \pm 18.33$
14	L01	Leaves	$195.81 \pm 25.09$
15	L02	Leaves	$198.9 \pm 22.32$
16	L03	Leaves	$176.53 \pm 18.86$
17	L04	Leaves	$148.87 \pm 14.22$
18	L05	Leaves	$196.34 \pm 19.66$
19	L06	Leaves	$146.38 \pm 12.25$

20	L07	Leaves	160.52 $\pm$ 15.33
21	L08	Leaves	161.21 $\pm$ 15.96
22	L09	Leaves	158.85 $\pm$ 13.11
Acarbose			88.96 $\pm$ 9.69
Positive control			

Nevertheless, several fruit samples (notably F10 and F12) exhibited relatively strong inhibition ( $IC_{50}$  at 130–140  $\mu$ g/mL), approaching the activity of acarbose. The observed variability among extracts likely reflects differences in the abundance and composition of phenolic and saponin constituents, which are known contributors to  $\alpha$ -amylase inhibition. Overall, both fruit and leaf extracts of *P. tectorius* demonstrated promising  $\alpha$ -amylase inhibitory activity, with fruits generally showing slightly stronger effects than leaves, although the differences were not as pronounced as those observed in metabolite concentrations. These findings support the potential role of *P. tectorius* as a natural source of  $\alpha$ -amylase inhibitors, relevant for the management of postprandial hyperglycemia

### 3.3. Discussion

The correlation analysis revealed distinct relationships between metabolite levels and  $\alpha$ -amylase inhibitory activity. A strong negative correlation was observed between total phenolic content (TPC) and  $IC_{50}$  values (Pearson's  $r = -0.842$ ,  $R^2 = 0.709$ ), indicating that extracts richer in phenolics required markedly lower concentrations to achieve 50% enzyme inhibition. This strong inverse association, also illustrated in the scatter plot (Figure 3a), highlights the central role of phenolic compounds as key contributors to  $\alpha$ -amylase inhibition in *P. tectorius*. In particular, fruit samples such as F10 and F12, which exhibited the highest TPC levels, also showed the lowest  $IC_{50}$  values, approaching the potency of the positive control acarbose. In contrast, total saponin content (TSC) displayed only a weak negative correlation with  $IC_{50}$  ( $r = -0.319$ ,  $R^2 = 0.102$ ). Although higher TSC was generally associated with somewhat lower  $IC_{50}$  values, the relationship was inconsistent across samples, as reflected in the scattered distribution of data points in the TSC– $IC_{50}$  plot (Figure 3b). This suggests that while saponins may contribute to  $\alpha$ -amylase inhibition, their effect is minor compared to phenolic constituents.



**Figure 2: Correlation between  $\alpha$ -amylase inhibitory activity and the TPC (a) and TSC (b) of the *P. tectorius* fruit and leaf extracts**

Previous studies have demonstrated that phenolic compounds inhibit  $\alpha$ -amylase activity primarily through direct interactions with the enzyme's active site[24]. The hydroxyl groups of phenolics can form hydrogen bonds with catalytic residues, while their aromatic rings participate in hydrophobic and  $\pi$ – $\pi$  stacking interactions, thereby reducing substrate access and catalytic efficiency[25]. This mechanism is consistent with the strong negative correlation observed between TPC and  $IC_{50}$  in the present study, suggesting that phenolic enrichment in *P. tectorius* extracts directly enhances inhibitory potency. In contrast, the weaker relationship between TSC and  $IC_{50}$  implies that saponins may act through indirect mechanisms, such as modulating membrane permeability or protein conformations, but are less effective in directly blocking  $\alpha$ -amylase activity[26]. Collectively, these findings highlight the importance of phenolic constituents as the principal bioactive agents responsible for  $\alpha$ -amylase inhibition in *P. tectorius*. This provides a biochemical rationale for the potential application of fruit extracts, which are phenolic-rich, in the development of functional foods or phytopharmaceuticals aimed at managing postprandial hyperglycemia and related metabolic disorders.

#### IV. CONCLUSION

This study shows that fruits and leaves of *P. tectorius* provide similar levels of phenolics and saponins on a raw material basis, but fruit extracts are significantly richer in these metabolites when expressed per gram of ethanol extract. Phenolic content exhibited a strong negative correlation with  $\alpha$ -amylase IC<sub>50</sub> values, confirming its predominant role in the inhibitory effect, while saponins had a minor contribution. Although both plant parts have potential as herbal resources, fruit extracts appear to be superior candidates for the development of standardized phytopharmaceutical or nutraceutical products targeting postprandial hyperglycemia.

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