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Original Article

Phytochemical Profiling of Unripe Fruits of *Ficus carica* and *Phyllanthus emblica* Grown in West Bengal and Evaluation of their *in vitro* Antidiabetic and Anti-Inflammatory Activity

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ABSTRACT

Background: Unripe fruits are promising sources of nutrition in the daily diet, which have gained attention for their rich phytochemical profile and associated health benefits. **Objectives:** The present study aimed to profile the phytochemical constituents of unripe fruits of *Ficus carica* and *Phyllanthus emblica* grown in West Bengal and to evaluate their *in vitro* antidiabetic and anti-inflammatory potential. **Materials and Methods:** Quantitative estimation of Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and pigment contents (anthocyanins, chlorophylls, and carotenoids) was performed using spectrophotometric methods. Antidiabetic activity was assessed by the α-amylase inhibition assay, while anti-inflammatory potential was evaluated using the egg albumin denaturation method at concentrations of 0.01, 0.05, and 0.1 mg/mL, with standard drugs for comparison. **Results:** Both fruits showed the presence of key phytoconstituents such as flavonoids, phenolics, tannins, carbohydrates, and saponins. *F. carica* exhibited higher flavonoid content (0.5296±0.001 mg QE/g) and higher tannin content (0.9982±0.0001 mg TAE/g), while *P. emblica* showed higher phenolic content (0.445±0.0004 mg GAE/g). Pigment analysis revealed appreciable levels of anthocyanins, chlorophylls, and carotenoids. Both extracts showed concentration-dependent α-amylase inhibitory activity, with IC_{50} values of 39.32 µg/mL for *F. carica* and 36.93 µg/mL for *P. emblica.* Additionally, significant inhibition of protein denaturation was seen in the anti-inflammatory assay, with IC_{50} values of 35.26 µg/mL for *F. carica* and 41.33 µg/mL for *P. emblica.* **Conclusion:** The findings confirm that unripe fruits of *F. carica* and *P. emblica* are rich in bioactive phytochemicals and exhibit notable antidiabetic and anti-inflammatory activities, supporting their potential use as natural therapeutic agents and functional dietary components.

Keywords: Antidiabetic Activity, Anti-inflammatory Activity, *Ficus carica*, *Phyllanthus emblica*, Phytochemical Profiling, Unripe Fruits.

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INTRODUCTION

Diabetes mellitus and inflammatory disorders represent two of the most widespread chronic health conditions worldwide and pose a significant burden on healthcare systems due to their long-term complications and management costs. These disorders are closely interconnected, sharing common pathological pathways such as oxidative stress, chronic low-grade inflammation, insulin resistance, and metabolic imbalance. Although several synthetic antidiabetic and anti-inflammatory drugs are currently available and effective in managing these conditions, their prolonged use is often associated with adverse effects, reduced patient compliance, and high economic costs. This has intensified global interest in the identification of safer, cost-effective, and plant-based therapeutic alternatives (Corathers *et al.*, 2013).

Medicinal plants have long been recognized as valuable reservoirs of bioactive phytochemicals, including flavonoids, phenolic compounds, tannins, glycosides, and alkaloids, which exhibit diverse pharmacological properties. These secondary metabolites play a crucial role in modulating oxidative stress, regulating glucose metabolism, and suppressing inflammatory mediators. Notably, unripe fruits are reported to contain higher concentrations of these phytoconstituents compared to their ripe counterparts, owing to active metabolic processes during early stages of fruit development. This enhanced phytochemical richness makes unripe fruits particularly attractive candidates for pharmacological and nutraceutical research (Tran *et al.*, 2020).

Ficus carica (fig) and *Phyllanthus emblica* (Indian gooseberry) are well-established medicinal plants extensively used in

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traditional systems of medicine for the management of metabolic and inflammatory disorders. The fruits of *F. carica* are rich in phenolics and flavonoids that influence carbohydrate-digesting enzymes, improve glycemic control, and attenuate inflammatory responses. Similarly, *P. emblica* is renowned for its high content of hydrolysable tannins, flavonoids, and vitamin C, which collectively contribute to its potent antioxidant, antidiabetic, and anti-inflammatory activities (Chakraborty *et al*., 2026; Sharma, 2022; Huang *et al*., 2023).

The phytochemical composition and biological activity of medicinal plants are influenced by geographical and climatic conditions. West Bengal offers a diverse environment that may affect the phytochemical profile of these fruits; however, limited studies have focused on unripe fruits grown in this region (Acharya, 2016).

Therefore, the present study aims to perform comprehensive phytochemical profiling of unripe fruits of *Ficus carica* and *Phyllanthus emblica* collected from West Bengal and to evaluate their antidiabetic and anti-inflammatory potential using relevant *in vitro* assays.

MATERIALS AND METHODS

Chemicals required

All chemicals used were of analytical grade and were procured from authorized suppliers. The chemicals were procured from Emplura and Emparta (Chennai). The standard chemicals were procured from Sigma-Aldrich, including quercetin, gallic acid, tannic acid, aspirin.

Instruments used

The instruments used included a UV-Visible Spectrophotometer (JASCO Corporation, V-630, Tokyo, Japan); Digital Weighing Balance (Wensar Weighing Scales Ltd., PGB-200, Maharashtra, India); pH meter (SYSTRONICS, 335, Ahmedabad, India); Incubator (Remi Elektrotechnik Ltd., Mumbai, India) and Hot Water Bath (SSFW, LWB-12H/D, Kolkata, India).

Fruit sample collection

About 25 fresh unripe fruits of each Fig (*Ficus carica*) and Amla (*Phyllanthus emblica*) procured from local market of Kanchrapara, West Bengal, India in September 2025. The unripe fruits washed properly, kept in shade dry and powdered.

Fruit extract preparation

About 2 g of the powdered sample of each unripe fruit was taken and macerated in 15 mL of ethanol for 7 days at a controlled temperature of 10±1°C with occasional shaking. The macerated product was filtered and dried to a residue at room temperature and stored.

Preliminary phytochemical testing

The presence or absence of phytoconstituents was determined using different testing procedures. Alkaloid was estimated by Dragendroff's test, Wagner's test, Carbohydrates by Iodine test, Molisch test, Fehling's test. The lead acetate test was performed for the identification of Tannin and Phenolics. Frothing's test was used for the identification of Saponin. Glycoside, Steroids test was also performed. Identification of proteins was done by performing Ninhydrin test (Kancherla *et al*., 2019).

Total Flavonoid Content determination

A calibration curve was prepared at 20, 40, 60, 80, and 100 mg/mL using Quercetin as reference standard, and the absorbance was recorded at 510 nm. The TFC of the plant extract was subsequently estimated at concentration of 0.1 mg/mL.

Total Phenolic content determination

The Total Phenolic Content (TPC) was determined using an established method (Martins *et al*., 2021). A calibration curve was constructed using gallic acid at concentrations of 20, 40, 60, and 100 mg/mL by reacting with 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 7% sodium carbonate (Na_2CO_3), followed by measurement of absorbance at 760 nm. The TPC of the plant extract was similarly evaluated at concentrations of 0.1 mg/mL.

Total Tannin content determination

The Total Tannin Content (TTC) was determined using an established procedure (Ononamadu *et al*., 2019). Tannic acid was employed as the reference standard to construct a calibration curve at concentrations of 20, 40, 60, 80, and 100 mg/mL, and the absorbance was recorded at 725 nm. The TTC of the plant extract was subsequently estimated at concentrations of 0.01, 0.05, and 0.1 mg/mL.

Total pigment content

Chlorophyll *a*, chlorophyll *b*, total chlorophyll content, and total carotenoid content were determined using 80% acetone as the solvent system. Anthocyanin content was estimated using a methanol-HCl-water mixture (90:1:9, v/v/v) according to the reported method and expressed as µg/g Fresh Weight (FW) (Chazaux *et al*., 2022). All measurements were performed in triplicate.

(i)Anthocyanin Content

$$(0.0821\,A_{534})\text{-}(0.00687\,A_{643})\text{-}(0.002426\,A_{661})\times 5$$

(ii)Chlorophyll a Content

$$(12.25\,A_{663})\text{-}(2.79\,A_{646.6})$$

(iii)Chlorophyll b Content

$$(21.3\,A_{646.6})\text{-}(5.1\,A_{663.6})$$

(iv)Total Chlorophyll Content

$$(7.15\,A_{663.6})+(18.71\,A_{646.6})$$

(v)Total Carotenoid Content

$$\frac{(1000 \times A_{470}) - (1.82 \times \text{Chlorophyll a}) - (85.02 \times \text{Chlorophyll b})}{198}$$

Anti diabetic potential

The α-amylase inhibitory activity was evaluated using an established method (Wickramaratne., 2016). The sample solution was prepared at a concentration of 0.1 mg/mL and further diluted to obtain 0.05 and 0.01 mg/mL. Reaction mixtures containing different concentrations of the plant extract (test), acarbose (positive control), or ethanol (control) were incubated with α-amylase solution (0.5 mg/mL), 1% starch solution, and 0.2 M phosphate buffer (pH 6.9) for 5 min. The reaction was terminated by the addition of 1% 3,5-dinitrosalicylic acid, and the absorbance was measured at 540 nm. The antidiabetic percentage was calculated by

(vi) % Antidiabetic Potential

$$\frac{A_c - A_t}{A_c} \times 100$$

Where Ac=Absorbance of control solution and At= Absorbance of test Solution.

Anti-inflammatory potential

The anti-inflammatory activity was assessed using an egg albumin denaturation assay following an established method. The reaction mixture consisted of 0.2 mL egg albumin, 2.8 mL phosphate buffer (pH 6.4), and 0.2 mL of the sample at concentrations of 0.1, 0.05, and 0.01 mg/mL (aspirin as the positive control, plant extract as the test sample, and appropriate control). The mixtures were incubated at 27°C for 10 min, followed by heating in a water bath at 70°C for 10 min. The absorbance was measured at 660 nm (Mustafa, 2023). Anti-inflammatory potential was estimated by using the formula:

(vii) % Anti-inflammatory Activity

$$\left(\frac{A_T}{A_C} - 1\right) \times 100$$

Where, $A_{C=}$Absorbance of control solution, $A_{T=}$Absorbance of test solution.

Statistical Analysis

All experiments were performed in triplicate. Data are presented as Mean±Standard Deviation (SD). Linear regression analysis was used to calculate IC_{50} values and calibration curves.

Ethical Statement

As this study involved the use of commercially available plant materials and did not involve human or animal subjects, ethical approval was not required.

RESULTS

Preliminary Phytochemical test

The phytochemical tests were conducted to determine the presence or absence of different classes of phytoconstituents. The results are shown in Table 1.

Phytochemical quantification

Standard curve of quercetin, gallic acid and Tannic acid was prepared at different concentration. Calibration curves of standard quercetin, gallic acid and tannic acid and demonstrated high regression correlation(R^2) of 0.9044, 0.9395,0.9987 with linear equations:

$$y=0.0003x + 0.1957,\ y=0.0061x - 0.0214 \text{ and } y=0.0014x + 0.023$$

Respectively, as presented in Figures 1-3.

The estimated value of TFC, TPC and TTC content of the unripe fruits is represented in Table 2.

Pigment content estimation

Pigment content estimation identified that *F. carica* has higher amount of Chlorophyll b, Total Chlorophyll content and Total carotenoid content, whereas *P. emblica* is rich in Chlorophyll a and anthocyanin.

Quantification of different pigment contents like anthocyanin, Chlorophyll, and Carotenoid is represented in Table 3.

Anti diabetic activity

The antidiabetic activity of both fruits was calculated and compared against the standard compound (Acarbose). The antidiabetic potential of the fruits is represented in Table 4.

Anti-inflammatory activity

The egg albumin denaturation assay was performed to evaluate the ability of the fruit extract to inhibit protein denaturation, a key mechanism involved in inflammation. The anti-inflammatory activity of the plant samples was assessed at three different concentrations (0.1, 0.05, and 0.01 mg/mL) and compared with aspirin as the positive control. The results revealed a significant difference in anti-inflammatory activity among the tested concentrations and between the fruit extracts and aspirin. The anti-inflammatory potential of the fruits is represented in Table 4.

DISCUSSION

The present study provides a comprehensive evaluation of the phytochemical composition and *in vitro* pharmacological activities of unripe fruits of *Ficus carica* and *Phyllanthus emblica* cultivated in West Bengal. The findings demonstrate that unripe fruits are rich sources of bioactive secondary metabolites and exhibit significant antioxidant, antidiabetic, and anti-inflammatory potential, supporting their traditional use and emerging role as functional foods.

Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, carbohydrates, glycosides, and saponins in both fruits, although variations in individual phytoconstituents were observed. These differences may be attributed to species-specific metabolic pathways and environmental factors such as soil composition and climatic conditions prevalent in West Bengal. The absence of proteins and alkaloids in both fruits suggests that the observed biological activities are primarily mediated by polyphenolic compounds rather than nitrogenous constituents.

Table 1: Estimation of Phytoconstituents in Unripe Fruits.

Sl. No.	Identification	Test Name	*F. carica*	*P. emblica*
1.	Alkaloid	Dragendroff's Test	-	-
		Wagner's Test	+	+
2.	Carbohydrate	Iodine Test	+	+
		Molisch Test	+	+
		Fehling's Test	+	+
3.	Protein	Ninhydrin Test	-	-
4.	Terpenes Test		+	-
5.	Tannin and Phenolics	Lead Acetate Test	+	-
6.	Glycoside Test		-	+
7.	Test for Tannin		+	+
8.	Steroids Test		-	+
9.	Saponin	Frothing's Test	+	+

'+' indicates presence of phytoconstituent; '-'indicates absence of phytoconstituent.

Table 2: Phytochemical Quantification of Unripe Fruits.

Sl. No.	Fruit Name	TFC (mg QE/g)	TPC (mg GAE/g)	TTC (mg TAE/g)
1.	*F. carica*	0.5296±0.001	0.100±0.0140	0.9982±0.0001
2.	*P. emblica*	0.2608±0.3388	0.445±0.0004	0.991±0.0048

Values are expressed Mean±Standard deviation.

Table 3: Quantification of Pigments of Unripe fruits.

Sl. No.	Fruit Name	Chlorophyll a content (µg/g)	Chlorophyll b content (µg/g)	Total Chlorophyll content (µg/g)	Total anthocyanin content (µg/g)	Total carotenoid content (µg/g)
1.	*F. carica*	2.2707±0.3336	4.2013±0.2568	3.3165±2.1963	0.1093±0.0043	1.5451±0.0458
2.	*P. emblica*	0.3316±0.1196	1.8559±1.3502	3.0482±0.0274	0.2199±0.0002	1.4191±0.0107

Values are expressed Mean±Standard deviation.

Table 4: Antidiabetic and Anti-inflammatory activity of Unripe fruits.

Activity	Fruit Name	0.01 mg/mL	0.05 mg/mL	0.1 mg/mL	IC_{50} (µg/mL)
Antidiabetic	F. carica	35.489±0.0564	32.8934±0.0978	49.5922±0.0523	39.3249
	P. emblica	13.6116±1.1999	27.0348±0.0499	70.1486±0.5721	36.9317
Anti-inflammatory	F. carica	23.71±0.3019	20.2166±0.7746	61.79±0.1908	35.2588
	P. emblica	32.02±0.1452	15.5766±0.0611	76.39±0.7313	41.3288

Values are expressed Mean±Standard deviation.

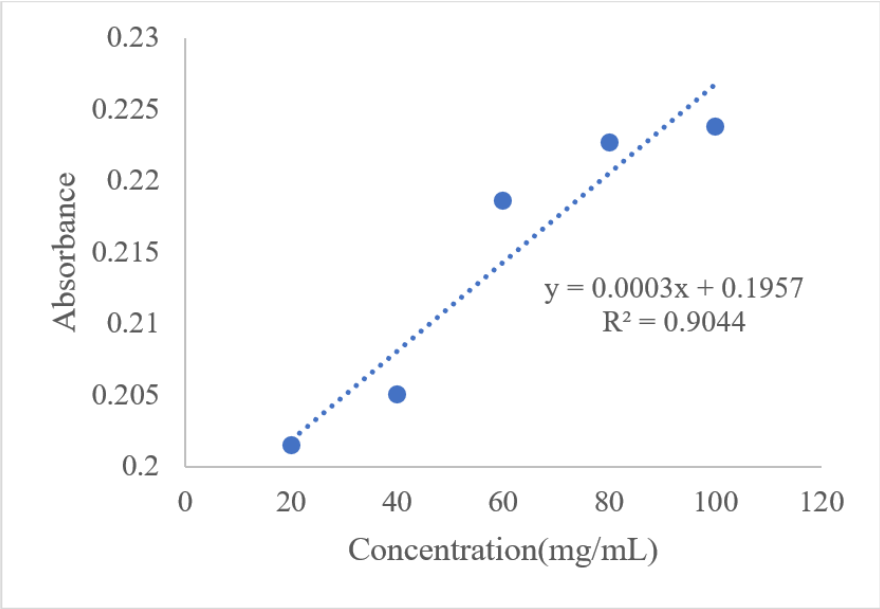

Figure 1: Calibration Curve of standard Quercetin.

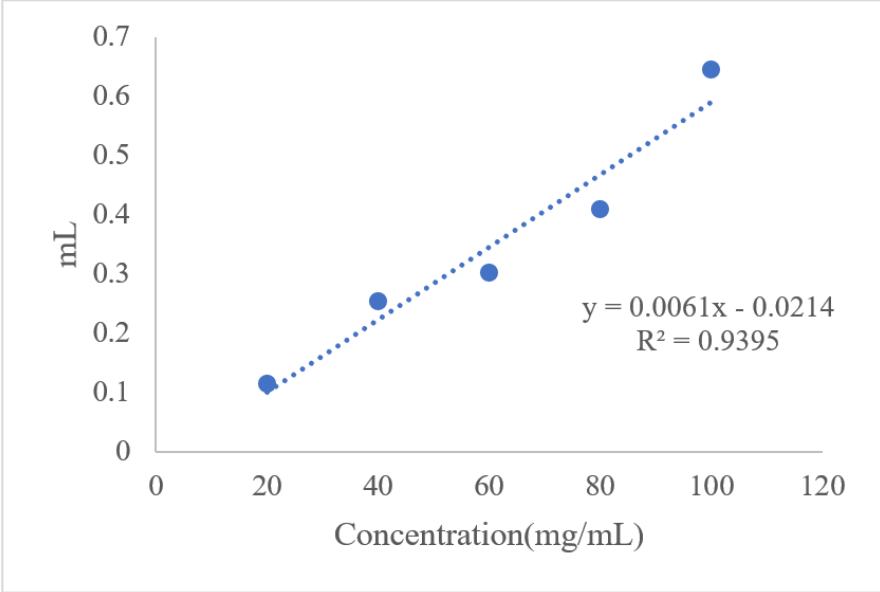

Figure 2: Calibration Curve of standard Gallic acid.

Quantitative analysis revealed that *F. carica* possessed a higher total flavonoid content and higher tannin content, whereas *P. emblica* exhibited significantly greater total phenolic content. Phenolics and flavonoids are well-known for their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals and reducing oxidative stress. The appreciable pigment content, including anthocyanins, chlorophylls, and carotenoids, further contributes to the antioxidant potential of the extracts and may synergistically enhance their biological efficacy.

The α-amylase inhibitory assay demonstrated concentration-dependent antidiabetic activity for both fruits, indicating their ability to delay carbohydrate digestion and reduce postprandial glucose levels. Such enzyme inhibition is a validated therapeutic approach in the management of type 2 diabetes. The observed activity may be attributed to the interaction of phenolics and flavonoids with the enzyme's active site, leading to reduced starch hydrolysis.

Anti-inflammatory activity assessed through the egg albumin denaturation assay showed significant inhibition of protein denaturation, a key mechanism involved in inflammation. Both fruit extracts exhibited notable activity, with *F. carica* showing comparatively lower IC_{50} values than *P. emblica*. The anti-inflammatory effects can be linked to the stabilization of proteins and suppression of inflammatory mediators by polyphenolic compounds.

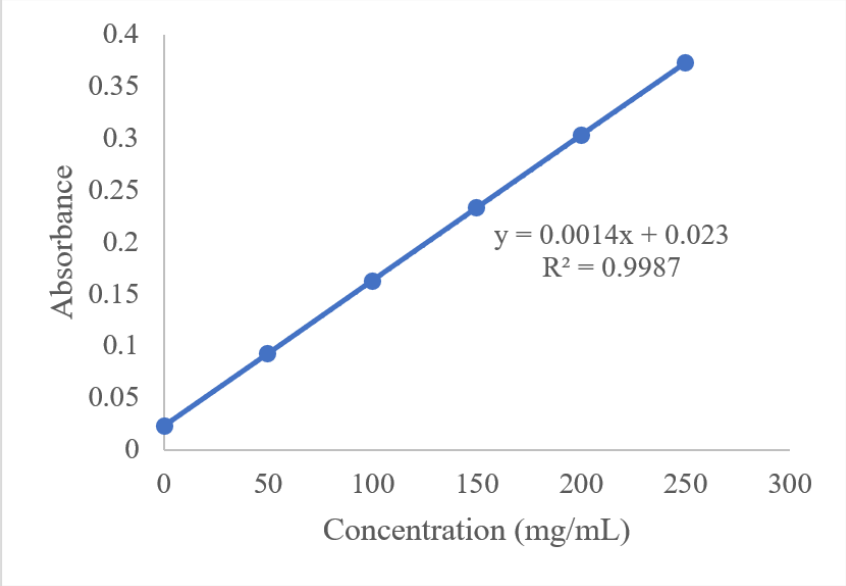

Figure 3: Calibration Curve of standard Tannic acid.

Overall, the results suggest that unripe fruits of *F. carica* and *P. emblica* possess multifunctional bioactivities mediated by their rich phytochemical profile. These findings highlight their potential application as natural therapeutic agents for managing oxidative stress-associated metabolic and inflammatory disorders. However, further *in vivo* studies, bioavailability assessments, and standardization protocols are necessary to validate their clinical relevance and support their development into nutraceutical or pharmaceutical products.

CONCLUSION

The diverse phytochemical composition of unripe fruits, including phenolics, flavonoids, anthocyanins, carotenoids, and chlorophyll, highlights their potential to modulate glucose metabolism, and inflammatory pathways *in vitro*. The findings provide strong evidence supporting the inclusion of unripe fruits in the daily diet to promote overall human health and well-being. This study pioneers the quantitative assessment of phytoconstituents and explores the therapeutic potential of selected unripe fruits cultivated in the soil of West Bengal, India. *F. carica* and *P. emblica* are potent antidiabetic and anti-inflammatory agents respectively. Further research can be extended up to examine the *in vivo* pharmacological activities shown by these unripe fruits and conduct bioavailability studies, and assessment of challenges in standardization and commercialization.

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ABBREVIATIONS

fw: Fresh Weight; **GAE:** Gallic Acid Equivalent; **HCl:** Hydrochloric Acid; **IC_{50}:** Half-maximal Inhibitory Concentration; **QE:** Quercetin Equivalent; **SD:** Standard Deviation; **TAE:** Tannic Acid Equivalent; **TFC:** Total Flavonoid Content; **TPC:** Total Phenolic Content; **TTC:** Total Tannin Content; **UV:** Ultraviolet; **nm:** Nanometer; **mg:** Miligram; **mL:** Milimeter; **µg:** Microgram; v/v/v: Volume per volume per volume; **M:** Molar; **Na_2CO_3:** Sodium carbonate; **Ac:** Absorbance of control solution; **At:** Absorbance of test solution.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Swarnabha Mukherjee (Research concept and design, Critical revision of the article), Neha Mondal (Collection and/or assembly of data), Shreya Mondal (Data analysis and interpretation), Gargi Banerjee (Data analysis and interpretation, Writing the article), Tushar Adhikari (Research concept and design, Critical revision of the article, Final approval of the article).

SUMMARY

- Unripe fruits of *Ficus carica* and *Phyllanthus emblica* from West Bengal were studied for their phytochemical composition and *in vitro* bioactivities.
- Both fruits had large amounts of flavonoids, phenolics, tannins, carbohydrates, and saponins.
- *F. carica* had a higher total flavonoid and tannin content. In contrast, *P. emblica* showed a higher total phenolic content.

- Significant amounts of anthocyanins, chlorophylls, and carotenoids were found in both unripe fruits.
- Ethanolic extracts showed that their ability to inhibit α-amylase depends on their concentration. This suggests they may have potential as antidiabetic agents.
- There was a considerable reduction in protein denaturation, which confirms strong anti-inflammatory activity.
- The findings support the possible use of unripe fruits as useful dietary components and natural treatment options.
- Further *in vivo* studies and bioavailability assessments are required to validate clinical applicability.

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