DOI: https://doi.org/10.36094/sc.v91.2025.Phytochemical_Composition_and_Bioactive_Potential.Sood.509

ARTICLE

Sci. and Cult. 91 (9–10) : 509-516 (2025)

PHYTOCHEMICAL COMPOSITION AND BIOACTIVE POTENTIAL OF *Zanthoxylum nitidum, Piper nigrum,* AND *Cinnamomum tamala*

KAUSHAL SOOD

This study provides a comprehensive evaluation of the phytochemical composition and bioactivities of ethanol extracts of Zanthoxylum nitidum, Piper nigrum, *and* Cinnamomum tamala, *focusing on their antioxidant, enzyme inhibitory, and anti-inflammatory properties. The total phenolic, flavonoid, and alkaloid contents of each extract were quantified, and multiple bioassays were employed to assess their therapeutic potential. Antioxidant activities were analyzed using three distinct assays: DPPH, ABTS, and total antioxidant capacity.* Piper nigrum *showed the strongest DPPH free radical scavenging activity (IC_{50}= 98.38 ± 1.97 µg/mL), while* Cinnamomum tamala *excelled in the ABTS radical scavenging assay (IC_{50}= 634.25 ± 12.68 µg/mL).* Zanthoxylum nitidum *exhibited the highest TAC, correlating with its phenolic content. The enzyme inhibitory assays revealed* C. tamala *as the most potent α-amylase inhibitor, while* P. nigrum *was the strongest α-glucosidase inhibitor, establishing their potential for glycemic control. Anti-inflammatory activity was also significant, with* P. nigrum *showing the highest inhibition of protein denaturation. Although strong trends were observed between bioactivities and phytochemical contents, many correlations were not statistically significant, indicating the need for larger sample sizes in future studies. These findings highlight the therapeutic potential of these plants, particularly for managing oxidative stress, diabetes, and inflammation. Further research may explore bioactive constituents and their mechanisms of action to deepen the understanding of the therapeutic properties of these plants.*

Introduction

The global prevalence of diabetes mellitus, particularly Type 2 diabetes mellitus (T2DM), continues to rise at an alarming rate. According to the World Health Organization (WHO), the number of people living with diabetes has nearly quadrupled since 1980, with over 422 million people currently affected worldwide. T2DM accounts for approximately 90% of all cases and is a major contributor to the global burden of disease, increasing the risk of serious complications such as heart disease, stroke, kidney failure, and lower limb amputations[1]. T2DM is characterized by chronic hyperglycemia, insulin resistance, and impaired insulin secretion, often leading to long-term complications such as neuropathy, nephropathy, and retinopathy [2]. This growing health crisis underscores the urgent need for effective treatments, particularly those that are safe, affordable, and accessible. In addition to pharmacological treatments, there is growing interest in natural plant-based remedies, driven by their relative safety and minimal side effects compared to synthetic drugs. Among the various medicinal plants explored for antidiabetic potential, *Piper nigrum*, *Zanthoxylum nitidum*, and *Cinnamomum tamala* have garnered attention for their multifaceted therapeutic properties. These plants, used extensively in traditional medicine systems across Asia, are known for their

Department of Zoology, Tinsukia College, Assam, India-786125

* e-mail: kaushalsoodresearch@gmail.com

bioactive compounds, including alkaloids, polyphenols, and flavonoids, which have demonstrated antioxidant, anti-inflammatory, and antidiabetic effects[3]. *Piper nigrum* (black pepper) contains piperine, an alkaloid with documented α-glucosidase inhibitory activity, making it a promising candidate for controlling postprandial blood glucose levels. Studies have shown that piperine can significantly reduce blood glucose levels, inhibit enzymes like α-amylase, and enhance insulin sensitivity, contributing to its potential as a natural antidiabetic agent[4]. Piperine has also been shown to possess anti-inflammatory and antioxidant properties, mitigating oxidative stress, which plays a central role in the pathogenesis of T2DM and its complications[5]. Similarly, *Cinnamomum tamala* (Indian bay leaf), rich in polyphenols, has exhibited significant antioxidant activity by scavenging free radicals and reducing oxidative stress. Polyphenolic compounds, such as cinnamaldehyde, are known to inhibit α-amylase and α-glucosidase, reducing postprandial hyperglycemia[6]. The essential oils of the plant have also been reported to possess potent anti-inflammatory effects, further highlighting its potential in managing inflammation-linked metabolic disorders[7]. Regular consumption of *C. tamala* extracts has been shown to improve lipid profiles, reduce fasting blood glucose, and mitigate oxidative damage, making it a valuable plant for T2DM management[8]. *Zanthoxylum nitidum* (Chinese prickly ash), traditionally used in Asian medicine for its analgesic and anti-inflammatory effects, contains bioactive compounds that have shown inhibitory activity against enzymes like α-amylase and α-glucosidase, which are crucial in carbohydrate metabolism[3,9]. The alkaloid nitidine chloride, isolated from *Z. nitidum*, has been reported to enhance glucose uptake in adipocytes, and its anti-inflammatory and antioxidant properties position it as a promising natural intervention in diabetes management[10].

The present study investigates the antidiabetic, antioxidant, and anti-inflammatory properties of these three plants *in vitro*, aiming to elucidate their potential as therapeutic agents for managing diabetes and its associated oxidative and inflammatory complications. By exploring the inhibitory effects of these plant extracts on key metabolic enzymes and assessing their antioxidant capacity, this study seeks to provide a scientific foundation for the traditional use of these plants in ethnomedicine. With the increasing interest in plant-based therapies as adjunct treatments for diabetes, this research could pave the way for developing novel, plant-derived therapeutic formulations with minimal side effects compared to conventional pharmacological agents.

Materials and Methods

Plant Material and Extract Preparation: The plant material was harvested from its natural habitat, thoroughly washed with distilled water, and air-dried. The dried leaves were then pulverized into a fine powder. This powder was subjected to extraction with absolute ethanol at a ratio of 1:4 (w/v) under constant shaking conditions (150 rpm) for 48 hours at ambient temperature. The resultant extracts were filtered, and the solvent was subsequently evaporated using a rotary evaporator (IKA RV 10, Germany). The dried extract was reconstituted in dimethyl sulfoxide (DMSO) for subsequent analyses.

Determination of Total Phenolic Content (TPC): The total phenolic content (TPC) of the extract was quantified using the method described by Singleton et al.[11]. A 0.5 mL aliquot of the sample was mixed with 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent. Following a 2-minute incubation at room temperature, 2 mL of 7.5% (w/v) sodium carbonate was added. After 30 minutes, absorbance was measured at 765 nm. The TPC was calculated from a regression equation obtained using gallic acid as a standard and expressed as mg gallic acid equivalents (GAE) per gram of extract.

Determination of Total Flavonoid Content (TFC): Total flavonoid content (TFC) was determined following the method described by Meda et al.[12] with modifications. A 5 mL aliquot of the extract was combined with 5 mL of 2% (v/v) aluminium trichloride in methanol. After a 10-minute incubation at room temperature, absorbance was recorded at 415 nm. The TFC was calculated using a regression equation derived from rutin as standard and expressed as mg rutin equivalents (RE) per gram of extract.

Quantification of Alkaloids: Alkaloid content was quantified using the method described by Harborne[13]. Each 50 g sample was mixed with 200 mL of 10% (v/v) acetic acid in ethanol and incubated for 4 hours at 28°C. The mixture was concentrated to one-fourth of its original volume, and alkaloids were precipitated by adding ammonium hydroxide drop-wise. The precipitate was washed with ammonium hydroxide, filtered, and weighed. Alkaloid content was expressed as mg/g of sample.

DPPH Free Radical Scavenging Activity: The DPPH free radical scavenging activity of the extracts was assessed using the method by Molyneux[14]. Extract concentrations were prepared and mixed with an equal volume of 0.1 mM DPPH solution in methanol. The mixtures were then incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm.

The free radical scavenging activity was calculated using the formula:

DPPH radical scavenging activity (%) = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$

Where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample or standard. The IC_{50} value, which corresponds to the concentration of the extract required to scavenge 50% of DPPH free radicals, was determined from the graph of percent inhibition versus sample concentration.

ABTS Free Radical Scavenging Activity: The ABTS radical scavenging activity of the extracts was assessed using the method described by Alam *et al.*[15]. Equal volumes of ABTS free radical solution and varying concentrations of the extracts were mixed and incubated for 10 minutes at room temperature. Absorbance was measured at 734 nm, and scavenging activity was calculated as a percentage:

ABTS radical scavenging activity (%) = $[(A_{control} - A_{sample})/(A_{control})] \times 100$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample/ standard.

The IC_{50} value was calculated as described in the preceding method.

Total Antioxidant Capacity: The total antioxidant capacity was assessed using the phosphomolybdenum assay described by Prieto et al.[15] with slight modifications. A 500 µg/mL sample (0.3 mL) was incubated with a reagent solution containing 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate at 95°C for 90 minutes. Absorbance was measured at 765 nm, and total antioxidant activity was expressed as ascorbic acid equivalent.

Enzyme Inhibition Assays: *In-vitro Amylase Inhibition Assay:* The amylase inhibitory activity was determined by the method of Carrasco *et al.*[16] based on the DNSA method first described by Miller[17]. The assay involved varying concentrations of the extracts (25-800 µg/mL) and 500 µL of 0.1 mg/mL α-amylase solution, incubated at 37°C for 10 minutes. Following this, 500 µL of 1% (v/v) substrate was added. After 5 minutes, 500 µL DNSA reagent was introduced, and the tubes were boiled for 5 minutes. Absorbance was measured at 540 nm. Acarbose was used as a positive control. Enzyme inhibition was calculated using the formula:

% Inhibition in α-amylase activity = [(A_control – A_test)/ A_control) × 100]

The IC_{50} value was calculated from regression plot of percent inhibition against extract concentration.

In-vitro α-Glucosidase Inhibition Assay: The α-glucosidase inhibitory activity was evaluated using the method by Brueggeman and Hollingsworth [18] with modifications. A 50 µL aliquot of 1 U/mL α-glucosidase was added to varying concentrations of the extracts (25-800 µg/mL) and incubated with 250 µL of 0.1 M phosphate buffer (pH 6.8) at 37°C for 30 minutes. Subsequently, 100 µL of 1 M pNPG substrate was added and incubated for 30 minutes at 37°C. Afterward, 100 µL of 0.1 N Na_2CO_3 was added, and absorbance was recorded at 405 nm. Acarbose served as a positive control. Enzyme inhibition was calculated using the formula:

% Inhibition in α-glucosidase activity = [(A_control – A_test)/ A_control) × 100]

The IC_{50} value was calculated from regression plot of percent inhibition versus against extract concentration.

In-vitro Anti-inflammatory Activity Assays: *Inhibition of Protein Denaturation:* Protein denaturation was assessed using the method described by Chaiya *et al.*[19] with slight modifications. A series of extract dilutions (2 mL, 12.5-800 µg/mL) were mixed with 0.2 mL of fresh egg albumin and 2.8 mL of phosphate-buffered saline (pH 6.4). The mixture was first incubated at room temperature for 15 minutes and then temperature was increased to 70°C for 5 minutes. After gradually cooling the tubes to room temperature, turbidity was measured at 660 nm. Distilled water served as the control. Diclofenac sodium was used as the standard. Inhibition was calculated as:

% inhibition of protein denaturation = $100 \times \{(A_{test}/ A_{control}) - 1\}$

The IC_{50} value, representing the concentration required for 50% inhibition, was calculated using regression plot analysis of % inhibition versus sample concentration.

Human Red Blood Cell (HRBC) Membrane Stabilization Assay: The HRBC membrane stabilization assay was performed according to the method described by Gandhidasan *et al.*[20]. Blood from a healthy volunteer was mixed with sterilized Alsever's solution and centrifuged at 3000 rpm for 5 minutes. Packed cells were washed with isosaline to prepare a 10% (v/v) RBC suspension. The assay mixture contained 1 mL phosphate buffer, 2 mL hyposaline, 0.5 mL of the sample, and 0.5 mL RBC suspension, and was incubated at 37°C for 30 minutes. After centrifugation, absorbance of the supernatant was measured at 560 nm. Diclofenac sodium was used as the

positive control. HRBC membrane stabilization was calculated using the formula:

% stabilization = $\{(A_{Control} - A_{Test})/ A_{Control}\}$ x 100

The IC_{50} was calculated from the percentage stabilization and was defined as the concentration required to inhibit HRBC lysis by 50%.

Statistical Analysis: Results were expressed as mean ± standard deviation (SD) of triplicates. Correlation analysis was performed with Pearson Product Moment Correlation. Significance was tested at $\alpha = 0.05$. IC_{50} values were calculated using SigmaPlot 10.0 (Systat Software, San Jose, CA).

Results

Total phenolic, flavonoid and alkaloid contents: The total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) of the plant extracts were measured and compared (Table 1). The TPC was determined using the regression equation $y = 0.0168x - 0.106$ ($R^2 = 0.99$) from the standard graph of gallic acid and the TFC was calculated using the regression equation $y = 0.0071x - 0.0139$ ($R^2 = 0.99$) based on the standard graph of rutin. Among the plant extracts, *Zanthoxylum nitidum* exhibited the highest TPC (38.51 ± 0.63 mg gallic acid equivalent/g extract), followed by *Cinnamomum tamala* (34.44 ± 0.84 mg GAE/g extract) and *Piper nigrum* (33.95 ± 1.2 mg GAE/g extract). These results indicate that *Z. nitidum* contains significantly higher phenolic content compared to the other samples. In terms of TFC, *P. nigrum* demonstrated the highest TFC (27.40 ± 0.13 mg rutin equivalent/g extract), while *Z. nitidum* and *C. tamala* exhibited much lower values, with 6.53 ± 1.51 mg RE/g extract and 5.90 ± 0.55 mg RE/g extract, respectively. These data suggest that *P. nigrum* is a richer source of flavonoids. Furthermore, *Z. nitidum* also exhibited the highest TAC (10.58 ± 0.21 mg/g extract), followed by *C. tamala* (6.80 ± 0.14 mg/g extract) and *P. nigrum* (3.47 ± 0.07 mg/g extract). Overall, the results indicate that the ethanol extract of *Z. nitidum* had the highest total phenolic and alkaloid contents, while that of *P. nigrum* demonstrated the highest flavonoid content, suggesting diverse phytochemical compositions across the plant extracts.

Table 1: Total Phenolic Content, Total Flavonoid Content and Total Alkaloid Content of plant extracts

Plant extract	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg RE/g extract)	Total Alkaloid Content (mg/g extract)
P. nigrum	33.95 ± 1.2	27.40 ± 0.13	3.47 ± 0.07
Z. nitidum	38.51 ± 0.63	6.53 ± 1.51	10.58 ± 0.21
C. tamala	34.44 ± 0.84	5. 90 ± 0.55	6.80 ± 0.14

Results reported as mean ± sd of triplicates

Table 2: Antioxidant Activity of Plant Extracts

Plant Extract	IC_{50} DPPH (in µg/mL)	IC_{50} ABTS (in µg/mL)	TAOC (in AAE)
P. nigrum	98.38 ± 1.97	5871.50 ± 117.43	1.73 ± 0.03
Z. nitidium	750.18 ± 15.00	1972.18 ± 39.44	2.68 ± 0.05
C. tamala	227.84 ± 4.56	634.25 ± 12.68	0.80 ± 0.02

Results reported as mean ± sd of triplicates

Antioxidant Activity Assays: The antioxidant activity of the plant extracts was evaluated using DPPH and ABTS free radical scavenging assays, and the total antioxidant capacity (TAOC) was measured by the phosphomolybdate assay. The IC_{50} values for DPPH and ABTS radical scavenging activities, along with TAOC, are summarized in Table 2. During the DPPH radical scavenging assay, *P. nigrum* exhibited the lowest IC_{50} value (98.38 ± 1.97 µg/mL), indicating the highest free radical scavenging antioxidant potential among the plant extracts tested. *C. tamala* followed with an IC_{50} value of 227.84 ± 4.56 µg/mL, while *Z. nitidum* showed the weakest DPPH free radical scavenging activity, with an IC_{50} value of 750.18 ± 15.00 µg/mL. The graph in Figure 1 illustrates the DPPH free radical scavenging activity of *P. nigrum*, *Z. nitidum*, and *C. tamala* as a function of extract concentration. *P. nigrum* demonstrated the highest free radical scavenging activity, achieving nearly 90% inhibition at concentrations above 300 µg/mL.

C. tamala followed, reaching around 80% inhibition at 500 µg/mL. In contrast, *Z. nitidum* displayed significantly lower activity, with a maximum inhibition of less than 60% even at the highest concentration tested (1000 µg/mL). These results align with the calculated IC_{50} values, where *P. nigrum* had the lowest IC_{50} (98.38 µg/mL), indicating the highest DPPH scavenging ability, while *Z. nitidum* had the highest IC_{50} (750.18 µg/mL), confirming its lower DPPH free radical scavenging potential. The ABTS radical scavenging assay showed a different trend.

The ABTS scavenging activity of the ethanol extract of the three plants is shown as a function of varying concentrations (0–1000 µg/mL) in Figure 2. *C. tamala* exhibited the highest activity among the three plant extracts, showing a dose-dependent increase in percentage

Table 3: Inhibition of α- amylase by Plant Extracts and Acarbose

Plant Extract	% Inhibition of α-amylase						IC_{50} (in µg/mL)
	25	50	100	200	400	800	
P. nigrum	1.40 ± 0.03	5.20 ± 0.10	8.20 ± 0.16	10.90 ± 0.22	14.60 ± 0.29	22.60 ± 0.45	1885.76 ± 37.72
Z. nitidum	4.80 ± 0.10	5.20 ± 0.10	7.50 ± 0.15	9.70 ± 0.19	11.20 ± 0.22	15.18 ± 0.30	3470.39 ± 69.41
C. tamala	9.40 ± 0.19	13.70 ± 0.27	13.70 ± 0.27	14.00 ± 0.28	21.30 ± 0.43	25.00 ± 0.50	823.04 ± 16.46
Acarbose	15.61 ± 0.31	22.60 ± 0.45	31.73 ± 0.63	35.10 ± 0.70	60.98 ± 1.22	79.29 ± 1.59	299.43 ± 5.99

Results reported as mean ± sd of triplicates

Table 4: Inhibition of α- glucosidase by Plant Extracts and Acarbose

Plant Extract	% Inhibition of α-glucosidase activity						IC_{50} (in µg/mL)
	25	50	100	200	400	800	
P. nigrum	56.43 ± 1.13	64.99 ± 1.30	69.05 ± 1.38	69.50 ± 1.39	69.95 ± 1.40	76.25 ± 1.53	10.18 ± 0.20
Z. nitidum	35.14 ± 0.70	36.94 ± 0.74	46.40 ± 0.93	54.05 ± 1.08	67.12 ± 1.34	79.73 ± 1.59	147.26 ± 2.95
C. tamala	24.54 ± 0.49	35.44 ± 0.71	45.89 ± 0.92	57.25 ± 1.14	67.24 ± 1.34	72.65 ± 1.45	136.55 ± 2.73
Acarbose	10.52 ± 0.21	15.49 ± 0.31	21.22 ± 0.42	32.12 ± 0.64	45.89 ± 0.92	55.26 ± 1.11	531.28 ± 10.63

Results reported as mean ± sd of triplicates

Table 5: Inhibition of HRBC lysis by Plant Extracts

Plant extract	% Inhibition of HRBC lysis					
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	IC_{50}(in µg/mL)
P. nigrum	2.10 ± 0.04	3.20 ± 0.06	6.97 ± 0.14	10.50 ± 0.21	15.20 ± 0.30	173.23 ± 3.46
Z. nitidum	12.14 ± 0.24	26.15 ± 0.52	38.26 ± 0.77	49.17 ± 0.98	51.32 ± 1.03	33.26 ± 0.67
C. tamala	4.77 ± 0.10	4.77 ± 0.10	5.32 ± 0.11	5.50 ± 0.11	6.97 ± 0.14	3077.05 ± 61.54

Results reported as mean ± sd of triplicates

Table 6: Inhibition of Protein Denaturation by Plant Extracts and Diclofenac sodium

Plant Extract/ Standard	% Inhibition of Protein Denaturation					
	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	IC_{50} (in µg/mL)
P. nigrum	25.14 ± 0.50	30.82 ± 0.62	38.91 ± 0.78	45.36 ± 0.91	59.21 ± 1.18	58.36 ± 1.17
Z. nitidum	11.11 ± 0.22	16.67 ± 0.33	27.78 ± 0.56	44.44 ± 0.89	55.56 ± 1.11	61.04 ± 1.22
C. tamala	11.10 ± 0.22	12.30 ± 0.25	13.20 ± 0.26	16.10 ± 0.32	20.00 ± 0.40	419.73 ± 8.39
Diclofenac Sodium	5.56 ± 0.11	22.22 ± 0.44	44.44 ± 0.89	66.67 ± 1.33	83.33 ± 1.67	30.12 ± 0.60

Results reported as mean ± sd of triplicates

inhibition. At a concentration of 1000 µg/mL, *C. tamala* reached over 75% inhibition, indicating a strong ability to neutralize ABTS radicals. In contrast, *P. nigrum* demonstrated much lower scavenging activity, with inhibition levels remaining relatively constant across the tested concentrations, peaking at approximately 25% inhibition at 1000 µg/mL. *Z. nitidum* displayed the lowest initial activity but showed a gradual increase in inhibition as the concentration increased, although it remained lower than *C. tamala* throughout the assay. *C. tamala* demonstrated the highest activity as evident from the lowest IC_{50} value (634.25 ± 12.68 µg/mL). *Z. nitidum* exhibited an IC_{50} value of 1972.18 ± 39.44 µg/mL, while *P. nigrum* had the highest IC_{50} value (5871.50 ± 117.43 µg/mL), indicating weaker ABTS scavenging potential. These results suggest that *C. tamala* has the most potent ABTS radical scavenging activity, as compared to the other two plant extracts. *Z. nitidum* exhibited the highest TAOC (2.68

± 0.05 AAE), followed by *P. nigrum* (1.73 ± 0.03 AAE), and *C. tamala* (0.80 ± 0.02 AAE). These results indicate that *Z. nitidum* has the greatest total antioxidant capacity among the tested extracts. In summary, *P. nigrum* showed the strongest DPPH radical scavenging activity, while *C. tamala* had the highest ABTS scavenging capacity. However, *Z. nitidum* exhibited the highest total antioxidant capacity, suggesting diverse antioxidant properties across the plant extracts.

Enzyme Inhibitory Assays: The α-amylase inhibitory activity of *P. nigrum*, *Z. nitidum*, *C. tamala*, and the standard inhibitor Acarbose was assessed at varying concentrations (25–800 µg/mL), and the IC_{50} values were calculated. The results, presented as mean ± SD of triplicates, indicate significant differences in the inhibitory potentials of the tested extracts (Table 3). At 800 µg/mL, *C. tamala* showed the highest inhibition of α-amylase (25.00 ± 0.50%), followed by *P. nigrum* (22.60 ± 0.45%) and *Z. nitidum* (15.18 ± 0.30%). Acarbose, the standard inhibitor, displayed superior inhibitory activity across all concentrations, achieving 79.29 ± 1.59% inhibition at 800 µg/mL, reflecting its strong potency compared to the plant extracts. The IC_{50} values, which represent the concentration required to inhibit 50% of α-amylase activity, further support this trend.

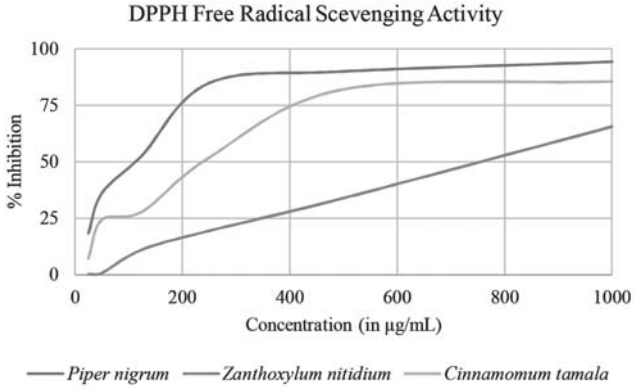

Fig. 1: DPPH Free Radical Scavenging Activity of plants.

C. tamala had the lowest IC_{50} (823.04 ± 16.46 µg/mL), indicating the highest α-amylase inhibitory capacity among the plant extracts. In contrast, *P. nigrum* and *Z. nitidum* demonstrated weaker inhibition with IC_{50} values of 1885.76 ± 37.72 µg/mL and 3470.39 ± 69.41 µg/mL, respectively. Acarbose showed the strongest inhibition, with an IC_{50} of 299.43 ± 5.99 µg/mL, confirming its greater effectiveness as a standard á-amylase inhibitor. These results suggest that *C. tamala* has the most potent α-amylase inhibition among the tested plant extracts, though Acarbose remains the most effective inhibitor overall. The inhibitory effects of *P. nigrum*, *Z. nitidum*, *C. tamala*, and Acarbose on α-glucosidase activity were evaluated at concentrations ranging from 25-800 µg/mL (Table 4). *P. nigrum* exhibited the highest α-glucosidase inhibition across all concentrations, achieving 76.25 ± 1.53% inhibition at 800 µg/mL. It also had the lowest IC_{50} of 10.18 ± 0.20 µg/mL, indicating strong inhibitory potential even at low concentrations. *Z. nitidum* followed, with a maximum inhibition of 79.73 ± 1.59% at 800 µg/mL and an IC_{50} of 147.26 ± 2.95 µg/mL. *C. tamala* displayed similar inhibition patterns, reaching 72.65 ± 1.45% inhibition at 800 µg/mL, with an IC_{50} of 136.55 ± 2.73 µg/mL, indicating moderate inhibitory activity. Interestingly, Acarbose, a standard α-glucosidase inhibitor, showed a much lower inhibition percentage compared to the plant extracts. It achieved only 55.26 ± 1.11% inhibition at the highest concentration tested (800 µg/mL) and had an IC_{50} value of 531.28 ± 10.63 µg/mL, significantly higher than all the plant extracts. This suggests that the plant extracts, particularly *P. nigrum*, are more potent α-glucosidase inhibitors than Acarbose. These findings highlight the potential of *P. nigrum* as a strong natural inhibitor of á-glucosidase, with *Z. nitidum* and *C. tamala* also exhibiting promising activity, albeit at higher concentrations than *P. nigrum.*

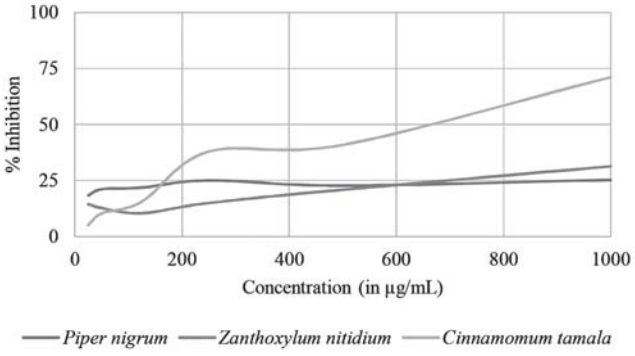

Fig. 2: ABTS Scavenging Activity of Plants.

Anti-inflammatory assays: The ability of *P. nigrum*, *Z. nitidum*, and *C. tamala* to inhibit hemolysis of human red blood cells (HRBC) was assessed at various concentrations (3.125–50 µg/mL), and the IC_{50} was calculated (Table 5). The results demonstrated significant differences in the anti-hemolytic activity of the plant extracts. *Z. nitidum* showed the highest HRBC lysis inhibition across all tested concentrations, with 51.32 ± 1.03% inhibition at 50 µg/mL and an IC_{50} value of 33.26 ± 0.67 µg/mL. This indicates a strong ability to protect HRBCs from lysis, suggesting potent membrane-stabilizing properties. *P. nigrum* exhibited lower inhibition levels, reaching only 15.20 ± 0.30% at 50 µg/mL, with an IC_{50} of 173.23 ± 3.46 µg/mL, reflecting moderate anti-hemolytic

activity. *C. tamala*, however, showed the weakest inhibition, with percentages remaining relatively low (6.97 ± 0.14% at 50 µg/mL) and an extremely high IC_{50} of 3077.05 ± 61.54 µg/mL, thus exerting a very weak protective effect on HRBC. The inhibition of protein denaturation by *P. nigrum*, *Z. nitidum*, *C. tamala*, and the standard anti-inflammatory drug Diclofenac Sodium was evaluated at various concentrations (6.25–100 µg/mL), and the IC_{50} were determined (Table 6). The results showed that *P. nigrum* exhibited the highest inhibition of protein denaturation among the plant extracts, achieving 59.21 ± 1.18% inhibition at 100 µg/mL with an IC_{50} of 58.36 ± 1.17 µg/mL, indicating strong anti-inflammatory potential. *Z. nitidum* showed moderate inhibition, reaching 55.56 ± 1.11% at the highest concentration, with an IC_{50} value of 61.04 ± 1.22 µg/mL. *C. tamala* demonstrated the weakest inhibition, with percentages remaining low (20.00 ± 0.40% at 100 µg/mL) and an IC_{50} value of 419.73 ± 8.39 µg/mL, suggesting minimal effectiveness in preventing protein denaturation. In comparison, Diclofenac Sodium showed the highest inhibition across all concentrations, reaching 83.33 ± 1.67% at 100 µg/mL. It had the lowest IC_{50} value of 30.12 ± 0.60 µg/mL, making it the most potent agent in this assay.

Discussion

The current study provides a comprehensive evaluation of the phytochemical composition and bioactivities of ethanol extract of *Zanthoxylum nitidum*, *Piper nigrum*, and *Cinnamomum tamala*, with a focus on their antioxidant, enzyme inhibitory, and anti-inflammatory properties. By quantifying their total phenolic, flavonoid, and alkaloid contents, and employing multiple bioassays, the study offers valuable insights into the therapeutic potential of these plants. Importantly, the use of three distinct antioxidant assays—DPPH, ABTS and TAOC provided a multifaceted assessment of antioxidant activities. Each assay targets different aspects of antioxidant mechanisms, with *P. nigrum* demonstrating the strongest DPPH radical scavenging activity, likely due to its high flavonoid content, while *C. tamala* excelled in the ABTS assay. Interestingly, *Z. nitidum* exhibited the highest total antioxidant capacity, which correlates with its high phenolic content. This diversity in assay outcomes highlights the necessity of using multiple methods to capture the full spectrum of antioxidant activity[21,22]. *C. tamala* exhibited the strongest α-amylase inhibition, while *P. nigrum* was the most potent α-glucosidase inhibitor. The findings suggest that flavonoids and phenolics in these plants may serve as natural alternatives to synthetic drugs for glycemic control, potentially offering fewer side effects [23,24]. *P. nigrum* exhibited the highest inhibition of protein denaturation, which aligns with its strong antioxidant capacity and suggests a role in preventing inflammation-related cellular damage [25]. The statistical analysis revealed that most correlations between bioactivities and phytochemical contents were not statistically significant, potentially due to the limited sample size. For instance, while a strong correlation was observed between TPC and DPPH scavenging activity and TFC and ABTS scavenging activity, the p-value indicated borderline significance rather than a definitive confirmation suggesting that larger datasets are needed to confirm these trends[11,26]. A strong correlation was observed between TAC and α-glucosidase inhibition indicating that higher antioxidant capacity may contribute to enzyme inhibition, aligning with prior research[2,27–29]. These observations underscore the importance of using larger datasets to confirm trends and explore the mechanistic relationships between phytochemicals and their bioactivities.

Conclusion

Overall, the findings demonstrate the diverse bioactive potential of these three plants, each excelling in different assays, suggesting their complementary roles in therapeutic applications. The strong antioxidant, enzyme inhibitory, and anti-inflammatory properties of *P. nigrum* and *Z. nitidum*, in particular, indicate their potential in developing natural therapies for managing oxidative stress, diabetes, and inflammation. Future research should focus on increasing the sample size and exploring additional bioactive compounds in these plants, which could strengthen the evidence for their use in medicinal applications. Furthermore, mechanistic studies are needed to elucidate the specific pathways through which these phytochemicals exert their bioactivities, providing deeper insights into their therapeutic potential. ❐

References

1. WHO, *World Heal. Organ. Website*, 1–16 (2023). at <https://iris.who.int/bitstream/handle/10665/374810/9789240081451-eng.pdf?sequence=1>
2. J. Blahova, M. Martiniakova, M. Babikova, V. Kovacova, V. Mondockova, and R. Omelka, *Pharmaceuticals* **14**, 1–32 (2021).
3. Y. Zheng, S. Liu, J. Xie, Y. Chen, R. Dong, X. Zhang, S. Liu, J. Xie, X. Hu, and Q.Yu, *LWT* **132** (2020).
4. H. Takooree, M.Z. Aumeeruddy, K.R.R. Rengasamy, K. N. Venugopala, R. Jeewon, G. Zengin, and M.F. Mahomoodally, *Crit. Rev. Food Sci. Nutr.* **59**, S210–S243 (2019).
5. Z.A. Damanhouri, *Med. Aromat. Plants* **03** (2014).
6. P.V. Rao and S.H. Gan, *Evidence-based Complement. Altern. Med.* **2014** (2014).
7. D. Gunawardena, N. Karunaweera, S. Lee, F. van Der Kooy,

D.G. Harman, R. Raju, L. Bennett, E. Gyengesi, N.J. Sucher, and G. Münch, *Food Funct.* **6**, 910–919 (2015).

8. R.K. Upadhyay, *Res. Rev. Pharm. Pharm. Sci.* **6**, 18–28 (2017).
9. J. Qu, S.S. Yu, D. Du, and Y.D. Wang, *RSC Adv.* **3**, 10078–10102 (2013).
10. F. Qin, C.Y. Wang, R. Hu, C.G. Wang, F. F. Wang, M.M. Zhou, D. Liang, H.B. Liao, S.K. Lee, and H.S. Wang, *Fitoterapia* **142**, 104486 (2020).
11. V.L. Singleton, R. Orthofer, and R.M. Lamuela-Raventós, *Methods Enzymol.* **299**, 152–178 (1999).
12. A. Meda, C.E. Lamien, M. Romito, J. Millogo, and O.G. Nacoulma, *Food Chem.* **91**, 571–577 (2005).
13. J. B. Harborne, *Phytochemical methods: a guide to modern techniques of plant analysis* (Chapman and Hall, 1998).
14. P. Molyneux, *Songklanakarin J. Sci. Technol.* **26**, 211–219 (2004).
15. P. Prieto, M. Pineda, and M. Aguilar, *Anal. Biochem.* **269**, 337–341 (1999).
16. M. Carrasco, J. Alcaíno, V. Cifuentes, and M. Baeza, *Microb. Cell Fact.* **16**, 1–10 (2017).
17. G.L. Miller, *Anal. Chem.* **31**, 426–428 (1959).
18. G.P. Brueggeman and R.I. Hollingsworth, **57**, 8773–8778 (2001).
19. P. Chaiya, S. Senarat, T. Phaechamud, and W. Narakornwit, *Mater. Today Proc.* **65**, 2290–2295 (2022).
20. R. Gandhidasan, A. Thamaraichelvan, and S. Baburaj, (1991).
21. F. Shahidi and P. Ambigaipalan, *J. Funct. Foods* **18**, 820–897 (2015).
22. H. Nechchadi, F.E. Kacimi, A. McDonald, S. Boulbaroud, H. Berrougui, and M. Ramchoun, *Plant Foods Hum. Nutr.* (2024).
23. E. Apostolidis, Y.I. Kwon, and K. Shetty, *Innov. Food Sci. Emerg. Technol.* **8**, 46–54 (2007).
24. R. Tundis, M.R. Loizzo, and F. Menichini, *Mini-Reviews Med. Chem.* **10**, 315–331 (2010).
25. E.O. Iwalewa, L.J. McGaw, V. Naidoo, and J.N. Eloff, *African J. Biotechnol.* **6**, 2868–2885 (2007).
26. F. Shahidi, *Antioxidants: Principles and applications Handb. Antioxidants Food Preserv.* (Elsevier Ltd, 2015).
27. U. Hossain, A.K. Das, S. Ghosh, and P.C. Sil, *Food Chem. Toxicol.* **145**, 111738 (2020).
28. F.S. Al Amri and M. A. Hossain, *Egypt. J. Basic Appl. Sci.* **5**, 245–251 (2018).
29. K. Bljajiæ, A. Brajkoviæ, A. Èaèiæ, L. Vujiæ, J. Jablan, I. Saraiva De Carvalho, and M. Zovko Konèiæ, *Horticulturae* **7**, 1–19 (2021).