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Preliminary Chemical Components and Bio-activities of *Ficus racemosa* Stem Bark Extracts

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Abstract

This study aimed to investigate the phytochemical composition and biological activities of Ficus racemosa bark. To achieve this, extracts were prepared using various solvents (acetone, hexane, methanol, ethanol, and aqueous mixtures) and subsequently evaluated for their antioxidant, enzyme inhibitory, and anti-inflammatory properties. The presence of bioactive compounds, including glycosides, steroids, tannins, polyphenols, and flavonoids was analyzed. Antioxidant potential was assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing power assays. In the DPPH assay, the IC_{50} values ranged from 5.55 to 9.89 mg L^{-1}, with the ethanol extract (S4) showing the lowest IC_{50} (5.55 mg L^{-1}), surpassing vitamin C (IC_{50} = 6.18 mg L^{-1}). The ferric reducing power assay indicated that the methanol extract (S2) exhibited the highest antioxidant capacity with an EC_{50} of 7.59 mg L^{-1}, 3.5 times lower than that of the butylated hydroxyanisole (BHA) standard (26.82 mg L^{-1}). In vitro α-glucosidase inhibition assays revealed that all the extracts effectively suppressed enzyme activity, with IC_{50} values from 0.049 mg L^{-1} (S4) to 0.66 mg L^{-1} (S1), significantly lower than acarbose (IC_{50} = 156.16 mg L^{-1}). Additionally, the extracts markedly reduced nitric oxide production in LPS-stimulated RAW 264.7 macrophages (Lipopolysaccharide-stimulated macrophage cell line 264.7), with IC_{50} values between 62.08 and 182.47 µg.mL^{-1}, compared to the positive control N^G-Monomethyl-L-arginine (L-NMMA) (IC_{50} = 11.91 µg.mL^{-1}). These findings highlight the potent antioxidant, anti-diabetic, and anti-inflammatory properties of *F. racemosa* bark extracts, supporting their potential as natural therapeutic agents.

Keywords

Phytochemical composition, antioxidant activity, α-glucosidase inhibition, nitric oxide inhibition, anti-inflammatory

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Introduction

The genus *Ficus* (family Moraceae) is comprised of approximately 850 species of trees, shrubs, vines, and woody plants widely distributed in tropical and subtropical regions (Veerapur *et al*., 2012; Donia *et al*., 2013; Singh *et al*., 2013). Many *Ficus* species have traditionally been used to treat skin diseases, ulcers, dysentery, asthma, and diabetes. In South Asia, particularly India and Sri Lanka, the bark, latex, and fruit juice of *Ficus racemosa* are valued for their antidiabetic and anti-inflammatory properties (Lansky *et al*., 2008; Thirumalai *et al*., 2012; Neamsuvan *et al*., 2015). Pharmacological studies have also demonstrated the genus's potential in addressing cancer and inflammation (Lansky *et al*., 2008).

Several studies have analyzed the chemical compositions of *F. racemosa* stem bark extracts. GC–MS analysis identified diverse bioactive compounds, including triterpenes, sterols, long-chain fatty acids, purine nucleosides, and alkenes (Bagga *et al*., 2016). Acetone and methanol extracts were shown to have antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis* (Faiyaz *et al*., 2010). An ethanol extract improved the biochemical parameters in diabetic rats induced by a high-fat diet and streptozotocin (Veerapur *et al*., 2012), enhancing glucose tolerance and HDL-cholesterol levels at 200 and 400 mg kg^{-1}. It also inhibited PTP-1B (IC_{50} = 12.1 µg mL^{-1}) and DPP-IV (42.5%) in bioassays.

Other investigations have identified antioxidant compounds from the root bark, including n-hexacosane, eight triterpenes (polypodatetraene, α-amyrin acetate, gluanol acetate, lupeol acetate, 24,25-dihydroparkeate, and lanost-20-en-3β-acetate), bergenin, and two phytosteroids (β-sitosterol and β-sitosterol-D-glucoside) (Jain *et al*., 2013). Acute toxicity studies confirmed that ethanol extracts of *F. racemosa* leaves are non-toxic at doses up to 2000 mg kg^{-1} (Patil *et al*., 2010; Zulfiker & Saha, 2011).

However, despite extensive studies from India and other regions, investigations on Vietnamese populations of this species remain limited, and environmental and geographical variations may influence its phytochemical profiles. Therefore, this study aimed to analyze the chemical compositions and evaluate the antioxidant, α-glucosidase inhibitory, and nitric oxide suppression activities of *F. racemosa* stem bark extracts obtained using solvents of varying polarities. The findings provide new insights into the bioactive potential of this species and support its future application as a natural source of therapeutic agents in Vietnam.

Materials and Methods

Materials

Reagents and chemicals

Folin-ciocalteu (FC) reagent and quercetin were purchased from Merck. Ascorbic acid, gallic acid, L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), potassium hexacyanoferrate ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), butylated hydroxyanisole (BHA), and ferric chloride ($FeCl_3$) were obtained from Macklin, China. All other reagents and solvents used in the study were of analytical grade.

Plant material

The stem bark of *F. racemosa* was collected in Hanoi, Vietnam. The plant material was identified and authenticated by Dr. Do Van Truong at the Vietnam National Museum of Nature. A voucher specimen (ĐVT-1041) was deposited in the museum's herbarium for reference. The collected stem bark was thoroughly washed with distilled water to remove any impurities, and then dried and ground into fine powder using an electric grinder for subsequent analyses.

Methods

The overall experimental workflow of this study, including the extraction and bioactivity assays, is summarized in **Figure 1**.

Preparation of extracts

Fifty gram samples of the dried *F. racemosa* stem bark powder were sequentially extracted with 750mL of different solvents: hexane, acetone, methanol, methanol:water (1:1), ethanol,

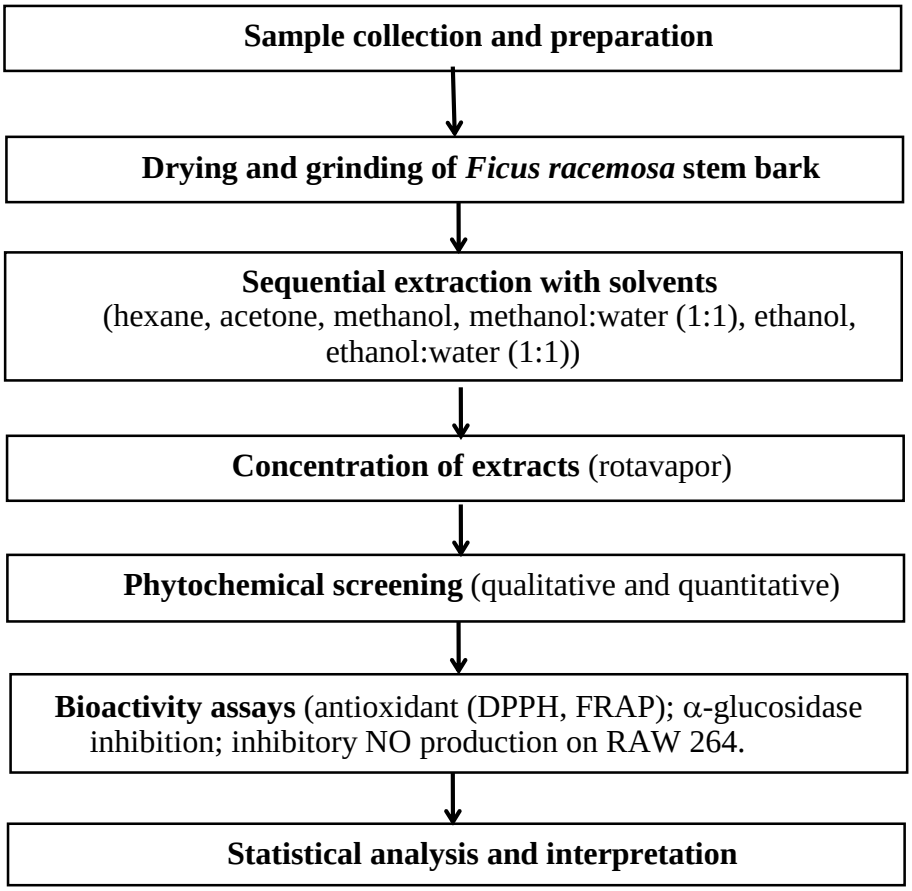

Figure 1. Schematic overview of the experimental workflow

and ethanol:water (1:1). The solvents were selected in increasing order of polarity to ensure the extraction of a broad range of compounds, from non-polar lipophilic to highly polar phenolic constituents. Extraction was performed using sonication at 30°C for 30 minutes, repeated three times. The solvents were then evaporated under reduced pressure using a Rotavapor (Buchi, Flawil, Switzerland). The resulting residues were then stored, and portions of these extracts were used for further experiments.

Phytochemical analysis

❖ *Qualitative analysis*

Preliminary phytochemical screenings were conducted to detect the presence of alkaloids, anthraquinones, flavonoids, phenolics, tannins, glycosides, and steroids using standard protocols (Harborne, 1984; Iyengar, 1995; Kokate *et al.*, 1998; Khandelwal, 2005).

(i) Alkaloids: Two grams of dried *F. racemosa* stem bark powder was shaken with 0.2M sulfuric acid for 15 minutes and filtered. The filtrate was washed with 0.2M sulfuric acid and then concentrated ammonia was added. The mixture was shaken with two portions of chloroform ($CHCl_3$) and treated with 1mL of Dragendorf reagent. A dark-red precipitate indicated the presence of alkaloids.

(ii) Anthraquinones: A mixture of 0.5g dried powder and 10mL of benzene was sonicated for 30 minutes, then filtered. The filtrate was shaken with a 10% ammonia solution. The appearance of a red, pink, or purple color in the ammonia phase confirmed the presence of anthraquinones.

(iii) Flavonoids: Three milliliters of the extract was mixed with 2mL of 10% sodium hydroxide solution. A yellow color, which disappeared upon adding two drops of 2M hydrochloric acid (HCl), indicated flavonoids.

(iv) Phenolics: One milliliter of the extract was mixed with 1mL of ferric chloride ($FeCl_3$) solution. A green color indicated the presence of phenolic compounds.

(v) Tannins: Two grams of extract were mixed with 10mL ethanol:water 1:1 and filtered. Half of the filtrate was treated with 2mL of $FeCl_3$ solution, where a dark blue color indicated tannins. The other half was treated with three drops of $Pb(CH_3COO)_2$, where a bright-yellow confirmed tannins.

(vi) Glycosides: Two milliliters each of acetic acid and chloroform were added to 10mL of stem bark extract. After cooling and the addition of a few drops of concentrated sulfuric acid (H_2SO_4), a green color confirmed glycosides. Alternatively, 2mL of concentrated H_2SO_4 was added to 10mL of the ethanol:water (1:1) extract where a dark-red color indicated glycosides (Iyengar, 1995).

(vii) Steroids: Five milliliters of extract was mixed with 2mL each of chloroform and concentrated H_2SO_4. A red color in the chloroform layer indicated steroids.

❖ *Quantitative analysis*

(i) Determination of total phenolic content

The total phenolics content of the *F. racemosa* stem bark extracts was determined using the Folin-Ciocalteu (FC) reagent method, with gallic acid as the standard (Singleton *et al*, 1999; Kaur & Kapoor, 2002). Briefly, 0.5mL of the extract (1 mg mL^{-1}) was mixed with 2.5mL of 1/10 diluted FC reagent. After mixing for five minutes, 2.0mL of 10% (w/v) sodium carbonate was introduced and the mixture was left for two hours in the dark. Absorbance was recorded at 765nm, and the total phenolic content was expressed as microgram of gallic acid equivalent (GAE) per gram of dry weight (mg GAE g^{-1}). A calibration curve was constructed using gallic acid standards at concentrations of 0, 5, 10, 20, 30, and 40 µg mL^{-1}.

(ii) Evaluation of the antioxidant activities of the *F. racemose* stem bark extracts

The antioxidant properties of the *F. racemosa* stem bark extracts were assessed using two assays: DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP).

DPPH free radical scavenging activity

The radical scavenging capacity of the *F. racemosa* stem bark extracts was determined using the DPPH method (Blois, 1958; Fu *et al.*, 2011). A 0.1mM solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH*) in methanol was prepared. One milliliter of this solution was combined with 3mL of methanol and the aqueous extracts at varying concentrations (1-250 µg mL^{-1}). Ascorbic acid served as a positive control. Following a 30 minute incubation in the dark, absorbance was recorded at 517nm. The inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_o - A_x}{A_o} * 100$$

Where, A_o is the absorbance of the blank (solvent), and A_x is the absorbance of the test sample. IC_{50} values (concentration of the sample required to neutralize 50% of the free radicals) were derived from the regression equation obtained for the methanol and aqueous extracts.

Ferric reducing power activity

The ferric reducing power was determined following the method outlined by Oyaizu (1986). The *F. racemosa* stem bark extracts at varying concentrations (10, 20, 40, 60, 80, and 100 mg L^{-1}) were combined with 2.5mL of 200mM phosphate buffer (pH 6.6) and 2.5mL of 1% $K_3Fe(CN)_6$. The mixtures were incubated at 50°C for 20 minutes, followed by the addition of 2.5mL of 10% TCA. The solution was centrifuged at 10,000rpm for 10 minutes. Five milliliters of the upper layer was combined with 5.0mL of distilled water and 1mL of 0.1% $FeCl_3$. Absorbance was measured at 700nm. BHA was used as a positive control and tested at the same concentrations.

(iii) Inhibitory activity enzyme α-glucosidase

The α-glucosidase inhibitory potential of the *F. racemosa* stem bark extracts was evaluated using a 96-well plate method based on the protocols described by Haimin *et al.* (2004), Kim *et al.* (2004), Li *et al.* (2005), and Hakamata *et al.* (2009). The extracts were diluted with DMSO and deionized water to obtain concentrations of 256, 64, 16, and 4 µg mL^{-1}. Acarbose was used as a reference inhibitor.

The reaction mixture included 100mM phosphate buffer (pH 6.8); 0.2 U mL^{-1} *α-*

glucosidase, 2.5mM *p*-nitrophenyl *α*-D-glucopyranoside; and the different extract concentrations. Reaction buffer replaced the extract in the control sample. The reaction was incubated at 37ºC for 30 minutes then halted with Na_2CO_3. Absorbance was recorded at 410nm using a BIOTEK microplate reader. The α-glucosidase inhibitory effect was calculated using the formula:

$$\text{Inhibition}\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} * 100 \quad (\%)$$

where A_o is the absorbance of the blank (solvent) and A_x is the absorbance of the test sample. IC_{50} (the concentration required to inhibit 50% of the α-glucosidase activity) was determined using Tablecurve software.

(iv) Inhibitory NO production on RAW 264.7 cells assay

The inhibitory effects of the extracts on nitric oxide (NO) production were assessed in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units mL^{-1} penicillin G sodium, 100 μg mL^{-1} streptomycin sulfate, 0.25 μg mL^{-1} amphotericin B, and 10% fetal bovine serum (FBS). Cells were seeded in 96-well plates at a density of 2×10^5 cells $well^{-1}$ and incubated for 24 hours at 37°C under 5% CO_2. After incubation, the cells were treated with the extracts dissolved in phenol red-free DMEM for 30 minutes, followed by 1 μg mL^{-1} LPS stimulation for 24 hours. The NO level in the cultured medium was quantified using Griess reagent. A standard curve was generated using sodium nitrite, and absorbance was recorded at 540nm.

Cytotoxicity was assessed using the MTT assay to confirm that the observed NO inhibition was not due to cell viability loss. For the MTT assay, cells were incubated with 10μL of MTT reagent (5 mg mL^{-1}) for four hours. The medium was discarded, and formazan crystals were solubilized in 100μL of dimethyl sulfoxide (DMSO). The optical density (OD) was recorded at 540nm using a spectrophotometer. Wells containing only culture medium were the negative control, while untreated wells (with 100% cell growth) served as the positive control (Cheenpracha *et al*., 2010; Marques *et al*., 2022*;* Nguyen *et al*., 2023).

The concentration of the sample that produced ≥80% cell survival was used to calculate the percentage of NO production inhibition.

$$\%\,\text{of surviving cells} = 100 - \frac{(OD_{control(+)} - OD_{sample})}{(OD_{control(+)} - OD_{control(-)})}.100 \quad (\%)$$

(v) Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Since the study involved comparisons among multiple extract samples, statistical differences were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine pairwise differences between groups. A significance level of $P < 0.05$ was applied.

For antioxidant assays and nitric oxide inhibition assays, IC_{50} and EC_{50} values were calculated using linear regression analysis in GraphPad Prism 8.0. Statistical groupings indicating significant differences among samples have been added to all relevant tables and figures.

Results and Discussion

Phytochemical components

The phytochemical analysis of the *F. racemosa* bark extracts identified various bioactive compounds in the extracts obtained using polar solvents such as acetone, methanol, ethanol, and the mixed solvents methanol:water (M:W 1:1) and ethanol:water (E:W 1:1). These included glycosides, steroids, tannins, polyphenols, and flavonoids, while the hexane extracts showed no such compounds (see **Table 1**).

Among these, polyphenols and flavonoids are of particular interest due to their well-known antioxidant, anti-inflammatory, and anticancer properties, which are essential for various therapeutic applications. The presence of these compounds in all five polar solvent extracts highlights the medicinal potential of *F. racemosa*

bark. This is consistent with previous studies emphasizing the bioactive properties of polyphenols and flavonoids (Singleton *et al*., 1999; Kaur & Kapoor, 2002).

Glycosides and steroids, found in some of the extracts, may also contribute to the anti-inflammatory and antidiabetic effects (Chhetri *et al*., 2005; Veerapur *et al*., 2012). The absence of anthraquinones and alkaloids suggests either their low concentration or complete absence in the bark, indicating that these compounds are not significant in *F. racemosa*.

The total polyphenol content of the extracts was determined using Folin-Ciocalteu reagent and expressed as GAE. The bark extract samples were diluted to 20 mL L^{-1}. The results are summarized in **Table 2**.

The masses of the extracts obtained using the E:W (1:1) and M:W (1:1) mixtures were more than twice that of the corresponding pure ethanol and methanol extracts. This suggests that a higher solvent polarity enhances the extraction of constituents from *Ficus racemosa* bark peel. Additionally, the presence of water molecules in hydrated form may facilitate interactions with polar compounds in the extract.

The results also demonstrated significant differences in the total polyphenol content across the five extracts, ranging from 466.67 mg GAE g^{-1} to 709.50 mg GAE g^{-1}. However, the extract yields varied considerably, with the lowest yield observed for acetone (4.94%) and the highest for the E:W (1:1) mixture (13.54%). Consequently, the total polyphenol content of *F. racemosa* bark varied markedly depending on the solvent used. Acetone exhibited the lowest polyphenol extraction efficiency (29.68 mg GAE g^{-1} DW), whereas both ethanol and methanol – particularly in aqueous mixtures – provided significantly higher efficiencies (63-65 mg GAE g^{-1} DW). This finding aligns with previous studies on polyphenol extraction efficiency from plant materials (Zhao *et al.*, 2008; Donia *et al*., 2013; Manohar *et al*., 2013).

The higher total phenolic content observed in the methanol and ethanol extracts can be attributed to the greater polarity of these solvents, which enhances the solubility of phenolic

Table 1. Phytochemical components of *F. racemosa*

	Components	Extracts					
		Acetone	Hexane	Methanol	M:W (1:1)	Ethanol	E:W (1:1)
1	Glycosides	+	-	+	+	+	+
2	Steroids	+	-	+	+	+	+
3	Anthraquinones	-	-	-	-	-	-
4	Alkaloids	-	-	-	-	-	-
5	Tannins	-	-	+	+	+	+
6	Polyphenols	+	-	+	+	+	+
7	Flavonoids	+	-	+	+	+	+

Table 2. Total polyphenol content of the extracts

Sample	Extract solvent	Extracts	Extract mass (g)	Extract content (%)	Total polyphenol content	
					(mg GAE g^{-1} Extract)	(mg GAE g^{-1} DW)
S1	Acetone	Brown powder	2.47	4.94	600.83^{b} ± 5.29	29.68^{c} ± 0.26
S2	Methanol	Brown crystals	3.05	6.10	675.90^{ab} ± 8.74	41.23^{b} ± 0.53
S3	M:W (1:1)	Brown crystals	6.35	12.70	512.91^{c} ± 14.35	65.14^{a} ± 1.82
S4	Ethanol	Brown crystals	3.00	6.00	709.5^{a} ± 32.28	42.57^{b} ± 1.94
S5	E:W (1:1)	Brown crystals	6.77	13.54	466.67^{d} ± 6.25	63.18^{a} ± 0.85

Note: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a–d) indicate statistically significant differences among samples according to one-way ANOVA followed by Tukey's post-hoc test (P <0.05).

hydroxyl groups and facilitates the extraction of polar antioxidant compounds. Similar patterns have been reported in other medicinal plants, where alcohol-based solvents, particularly methanol and ethanol, yielded extracts rich in phenolics and flavonoids (Jaiswal *et al*., 2014; Nguyen *et al*., 2020). Consequently, solvent polarity plays a critical role in the recovery efficiency of phenolic constituents from *F. racemosa* bark.

In contrast, the hexane extract contained negligible or undetectable levels of polyphenols, which is consistent with its non-polar nature that limits its ability to extract polar compounds. The aqueous mixtures (E:W 1:1 and M:W 1:1) exhibited higher total extraction yields due to their enhanced solvent penetration and interaction with plant matrices. However, the presence of water molecules in these mixtures may form hydrogen bonds or complexes with certain organic constituents, leading to a dilution effect and slightly lower polyphenol concentrations compared with the pure alcohol extracts.

Overall, these findings suggest that solvent polarity and the presence of water influence both the extraction efficiency and the composition of the phytochemicals obtained. The strong polyphenol recovery in the methanol and ethanol extracts highlights their suitability for subsequent bioactivity evaluations. Further comparative studies on solvent composition and extraction kinetics are warranted to optimize the recovery of bioactive compounds from *F. racemosa* bark.

Antioxidant activity

DPPH free radical scavenging activity

The DPPH assay showed a concentration-dependent increase in free radical inhibition across all the *F. racemosa* bark extracts. Higher concentrations led to stronger inhibition indicating significant antioxidant potential. All the extracts exhibited antioxidant activity comparable to vitamin C, as indicated by their IC_{50} values, which were lower than 10 mg L^{-1}. The IC_{50} values for the extracts were 8.08 mg L^{-1} (S1), 7.44 mg L^{-1} (S2), 7.21 mg L^{-1} (S3), 5.55 mg L^{-1} (S4), and 9.89 mg L^{-1} (S5), compared to 6.18 mg L^{-1} for vitamin C. suggesting strong antioxidant properties (**Figure 2**).

The ethanol extract (S4), with the highest polyphenol content, demonstrated the strongest antioxidant activity (IC_{50} = 5.55 mg L^{-1}, even lower than that of vitamin C, IC_{50} = 6.18 mg L^{-1}). This supports the role of polyphenols as key contributors to antioxidant capacity as reported by Jara *et al*. (2008).

Our results are consistent with earlier findings that extracts rich in polyphenols and flavonoids demonstrate strong DPPH scavenging activity (Kaur & Kapoor, 2002; Pérez-Jiménez *et al*., 2008). The comparable or superior antioxidant capacity of *F. racemosa* bark to vitamin C highlights its potential as a natural source of free radical scavengers.

The DPPH and ferric reducing power assays confirmed the potent antioxidant activity of the *F. racemosa* bark extracts. The methanol extract (S2) consistently exhibited the strongest antioxidant potential, surpassing both the ethanol extract (S4) and the BHA reference standard, indicating methanol's efficiency in extracting antioxidant compounds.

In contrast, *F. racemosa* leaf extracts had an IC_{50} of 150 mg L^{-1} (Aslam & Vijay, 2016), much higher than the bark extracts, reinforcing the superior antioxidant potential of the bark.

Ferric reducing power activity

The antioxidant efficiency of the *F. racemosa* bark extracts was evaluated based on the ferric reducing ability and expressed in μg mL^{-1} BHA equivalents. The BHA standard curve ($y = 0.0099x + 0.2345$, $R^2 = 99.87$), confirmed the reliability of the method.

Among all the extracts (**Table 3**), the methanol extract (S2) had the highest antioxidant potential (71.68 ± 1.29 μg mL^{-1} BHA at 20 mg L^{-1}), followed by the ethanol extract (S4), (63.75 ± 1.09 μg mL^{-1} BHA). These results confirm that methanol and ethanol are the most effective solvents for extracting antioxidant compounds from *F. racemosa* bark.

To further assess the antioxidant potential of the bark extracts, the EC_{50} values (concentration at $OD_{0.5}$) were determined, with

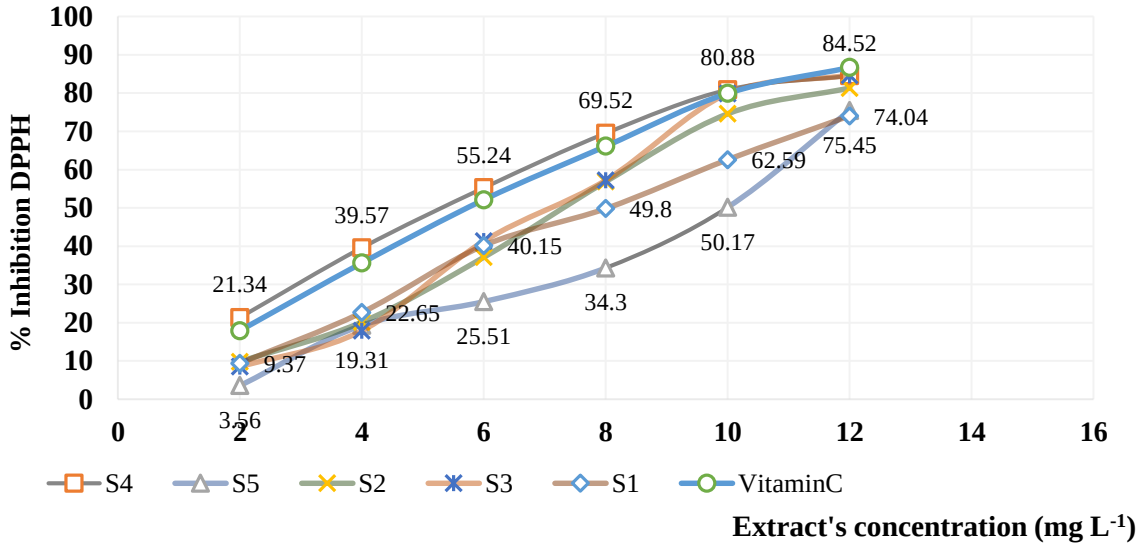

Figure 2. Antioxidant activity of the *F. racemosa* bark extracts

Table 3. Ferric reducing power capacity of the *F. racemosa* bark extracts

Extract's conc. (mg L^{-1})	Oxidant content equivalent to mg L^{-1} BHA				
	S1	S2	S3	S4	S5
2	$2.28^{c} \pm 0.03$	$2.67^{bc} \pm 0.17$	$3.16^{b} \pm 0.17$	$3.21^{b} \pm 0.06$	$3.58^{a} \pm 0.35$
4	$7.29^{c} \pm 0.06$	$15.30^{a} \pm 0.32$	$6.03^{c} \pm 0.40$	$8.12^{bc} \pm 0.28$	$6.19^{c} \pm 0.40$
8	$19.53^{c} \pm 0.23$	$29.36^{a} \pm 1.43$	$15.58^{c} \pm 0.67$	$24.61^{b} \pm 0.59$	$18.92^{c} \pm 0.44$
16	$39.71^{c} \pm 0.43$	$59.16^{a} \pm 1.01$	$37.41^{c} \pm 0.56$	$48.16^{b} \pm 1.34$	$43.67^{bc} \pm 0.5$
20	$56.88^{c} \pm 1.05$	$71.68^{a} \pm 1.29$	$43.57^{d} \pm 0.57$	$63.75^{b} \pm 1.09$	$56.51^{c} \pm 0.55$

Note: Values are expressed as mean ± standard deviation (n = 3). EC_{50} values and antioxidant efficiency. Different superscript letters within the same row indicate statistically significant differences among samples according to one-way ANOVA followed by Tukey's post-hoc test (P <0.05).

the results summarized in **Table 4**. The EC_{50} values ranged from 7.59 mg L^{-1} (S2) to 11.71 mg L^{-1} (S5). Comparatively, the EC_{50} value of BHA was 26.82 mg L^{-1}, indicating that the *F. racemosa* bark extracts exhibited superior antioxidant activity.

Among the tested extracts, the methanol extract (S2) demonstrated the highest antioxidant activity with the lowest EC_{50} value (7.59 mg L^{-1}), approximately 3.5 times more potent than BHA (EC_{50} of 26.82 mg L^{-1}). This finding highlights the strong antioxidant properties of *F. racemosa* bark, particularly in extracts obtained using polar solvents such as methanol.

In vitro α-glucosidase inhibiting activity

α-Glucosidase, an enzyme located in the small intestinal cell membranes, catalyzed the hydrolysis of oligosaccharides into glucose, facilitating absorption into the bloodstream. In individuals with impaired carbohydrate metabolism, such as diabetes, this process contributes to elevated blood glucose levels. Inhibiting *α-glucosidase* slows carbohydrate breakdown, helping regulate blood sugar levels, making it a potential therapeutic target (Nguyen *et al.*, 2020). Various natural compounds, including flavonoids, tannins, steroids, triterpenoids, curcuminoids, and phenolics, have demonstrated *α-glucosidase* inhibitor effects (Chhetri *et al.*, 2005; Veerapur *et al.*, 2012; Rahman, 2015).

The *α-glucosidase* inhibitory activity of the *F. racemosa* bark extracts was evaluated in comparison to acarbose, a commercially used drug for type 2 diabetes management. **Table 5** presents the inhibition assay results.

Table 4. EC_{50} (concentration at $OD_{0.5}$) of BHA and the *F. racemosa* bark extracts

No.	Extracts	Regression equation	$OD_{0.5}$ ($EC_{0.5}$) mg L^{-1}
1	S1	y = 0.0237x + 0.2752	9.49
2	S2	y = 0.0317x + 0.2593	7.59
3	S3	y = 0.0219x + 0.2466	11.57
4	S4	y = 0.0332x + 0.1443	10.71
5	S5	y = 0.0305x + 0.1427	11.71
6	BHA	y = 0.0099x + 0.2345	26.82

Table 5. *α-glucosidase* inhibition by acarbose and the *F. racemosa* bark extracts

Sample	Acarbose	S1	S2	S3	S4	S5
IC_{50} (mg L^{-1})	156.16 ± 5.43	0.66 ± 0.02	0.131 ± 0.006	0.55 ± 0.02	0.049 ± 0.002	0.61 ± 0.02

The results showed that all the *F. racemosa* bark extracts exhibited *α-glucosidase* inhibitory activity, with significantly lower IC_{50} values than acarbose, the positive control. The IC_{50} values ranged from 0.049 mg L^{-1} (S4) to 0.66 mg L^{-1} (S1), whereas acarbose had a much higher IC_{50} of 156.16 ± 5.43 mg L^{-1}, highlighting the extracts' potent inhibitory effects.

Notably, S4 and S2 exhibited the highest inhibitory effects, with IC_{50} values of 0.049 ± 0.002 mg L^{-1} and 0.131 ± 0.006 mg L^{-1}, respectively. These extracts also had the highest polyphenol contents among all the tested extracts, supporting the hypothesis that polyphenols play a crucial role in *α-glucosidase* inhibition. The S1 and S5 extracts also showed strong activity with IC_{50} values well below 1 mg L^{-1}, emphasizing the potential of *F. racemosa* bark as a natural source of *α-glucosidase* inhibitors.

These findings align with previous studies reporting the *α-glucosidase* inhibitory potential of plant-based compounds. Tadera *et al*. (2006) and Kumar *et al*. (2011) highlighted that flavonoids and polyphenols, commonly found in plant extracts, significantly inhibit *α-glucosidase* activity. Moreover, the efficacy of these extracts suggests that *F. racemosa* bark could serve as an alternative or complementary therapeutic agent for managing type 2 diabetes, particularly in functional food or nutraceutical applications.

The high *α-glucosidase* inhibitory activity observed in the *F. racemosa* bark extracts underscores the medicinal potential of this plant. Given the promising in vitro results, further in vitro evaluations are needed to assess their therapeutic potential and safety in diabetes management.

Nitric oxide (NO) production inhibitory activity

To assess the anti-inflammatory activity of the *F. racemosa* bark extracts, their ability to inhibit nitric oxide (NO) production was evaluated in LPS-stimulated RAW 264.7 macrophages. NO, a key mediator in inflammation, is commonly overproduced during inflammatory responses. Inhibition of NO production can be a critical approach to reducing inflammation (Cheenpracha *et al.*, 2010; Marques *et al.*, 2022; Nguyen *et al.*, 2023). The extracts were tested at concentrations of 256 µg mL^{-1}, 64 µg mL^{-1}, 16 µg mL^{-1}, and 4 µg mL^{-1} (**Table 6**). Cell viability remained above 82% at the highest concentration, indicating no significant cytotoxicity.

Four out of the five extracts from the *F. racemosa* bark exhibited NO production, though their activities were less potent than the positive control (NG-Methyl-L-arginine acetate, L-NMMA). The S2, S3, S4, and S5 extracts exhibited significant inhibition while the acetone extract (S1) showed no significant inhibition at concentrations below 256 µg mL^{-1}.

The inhibition of NO production by the *F. racemosa* bark extracts suggests notable anti-inflammatory activity. The ethanol (S4) extract demonstrated the most potent effect, with an IC_{50}

Table 6. NO Inhibitory activity of the *F. racemosa* bark extracts on LPS-stimulated RAW 264.7 cells

Extracts	Cell viability	IC_{50} (µg mL^{-1})
S1	> 88%	> 256
S2	> 82%	128.00 ± 5.12
S3	> 90%	118.00 ± 4.05
S4	> 90%	62.08 ± 3.5
S5	> 90 %	182.47 ± 6.0
L-NMMA		11.91 ± 0.7

of 62.08 ± 3.5 µg mL^{-1}. The acetone extract (S1) did not show significant NO inhibitory activity, suggesting that acetone may not efficiently extract bioactive compounds responsible for NO inhibition.For comparison, L-NMMA exhibited strong NO inhibitory activity with an IC_{50} of 11.91 ± 0.7 µg mL^{-1}, indicating that while the *F. racemosa* bark extracts have anti-inflammatory potential, they are less potent than the synthetic inhibitor.

These findings align with previous studies, reporting that flavonoids, tannins, and polyphenols in plant extracts inhibit NO production and reduce inflammation (Tadera *et al*., 2006; Altmann & Gertsch, 2007). The significant inhibition observed, particularly in the ethanol (S4) and methanol (S2) extracts, suggests that *F. racemosa* bark could serve as a natural anti-inflammatory agent.

In conclusion, the present study demonstrated that polar solvent extracts of *F. racemosa* stem bark, particularly methanol and ethanol, exhibit high levels of polyphenols and strong antioxidant, α-glucosidase inhibitory, and nitric oxide suppression activities. These results provide a scientific basis for the potential use of *F. racemosa* bark as a natural source of antidiabetic and anti-inflammatory agents. Further in vivo and chromatographic studies are recommended to isolate and identify the active compounds responsible for these effects.

Conclusions

This study highlights the bioactive potential of *Ficus racemosa* bark extracts in antioxidant, anti-diabetic, and anti-inflammatory applications. The results indicated that these extracts contain a diverse array of phytochemicals, including polyphenols, flavonoids, and glycosides, which contribute to their biological activities as demonstrated by their low IC_{50} values in both the DPPH radical scavenging and ferric reducing power assays. Additionally, the extracts showed remarkable α-*glucosidase* inhibition, with IC_{50} values significantly lower than acarbose, indicating potential for blood sugar management. Moreover, the extracts demonstrated significant NO production inhibition in LPS-stimulated macrophages, suggesting anti-inflammatory properties. These findings support the potential use of *F. racemosa* bark extracts as natural therapeutic agents for managing oxidative stress, inflammation, and diabetes. Future studies are needed to isolate and characterize the active compounds and evaluate their clinical safety and efficacy.

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