

Original Research

# Anti-Diabetic, Anti-Cholinesterase, and Anti-Inflammatory Potential of Plant Derived Extracts and Column Semi-Purified Fractions of *Ficus benghalensis*

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## Abstract

**Background:** The present study aimed to investigate the *in-vitro* anti-diabetic, anti-cholinesterase, and anti-inflammatory potential of extracts from different parts of *Ficus benghalensis*, including leaves, stem, and roots, as well as isolated column fractions (F-B-1 C, F-B-2 C, F-B-3 C, and F-B-4 C). **Methods:** The extracts and subsequent fractions were evaluated for their inhibitory activity against key enzymes involved in diabetes [ $\alpha$ -glucosidase and  $\alpha$ -amylase], neurodegenerative diseases [acetylcholinesterase and butyrylcholinesterase], and inflammation (cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX)). **Results:** The results showed that *F. benghalensis* leaf extract exhibited the highest  $\alpha$ -glucosidase inhibitory activity (73.84%) and  $\alpha$ -amylase inhibitory activity (76.29%) at 1000  $\mu$ g/mL. The stem extract (65.50%) and F-B-2 C fraction (69.67%) also demonstrated significant  $\alpha$ -glucosidase inhibitory activity. In terms of anti-cholinesterase activity, the extracts of roots, leaves, and stem showed promising inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), with half maximal inhibitory concentration (IC<sub>50</sub>) values ranging from 50.50 to 474.83  $\mu$ g/mL. The derived fractions (F-B-1 C, F-B-2 C, F-B-3 C, and F-B-4 C) also exhibited notable inhibition of AChE and BChE, with IC<sub>50</sub> values from 91.85 to 337.94  $\mu$ g/mL. Moreover, the F-B-3 C fraction demonstrated the highest COX-2 inhibitory potential (85.72%), followed by F-B-1 C (83.13%), the stem extract (80.85%), and the leaves extract (79.00%). The F-B-1 C fraction showed the highest 5-LOX inhibitory activity (87.63%), while the root extract exhibited the lowest inhibition (73.39%). **Conclusions:** The results demonstrated promising bioactivity, suggesting the potential of *F. benghalensis* as a source of natural compounds with therapeutic applications. Further studies are required to identify and isolate the active components responsible for these effects and to evaluate their *in-vivo* efficacy and safety.

**Keywords:** *Ficus benghalensis*;  $\alpha$ -amylase;  $\alpha$ -glucosidase; AChE; BChE; COX-2; 5-LOX

## 1. Introduction

Nowadays, health professionals are seriously concerned regarding the escalating prevalence of diabetes mellitus in both developing and developed countries. World Health Organization (WHO) stats reveal that 30 million people were diagnosed with diabetes in the year 1985, which increased to 135 million by 1995. It has been further projected that nearly 300 million population will be suffering from diabetes by the year 2025 [1]. This rise in the prevalence of diabetes is causing substantial financial problems in healthcare settings of underdeveloped countries

[2,3]. There are two main categories of diabetes: type-I accounts for nearly 5 to 10% of cases, while type-II diabetes accounts for approximately 90% of cases [4]. Impaired secretion of insulin results in type-I diabetes, whereas insulin resistance causes type-II diabetes [5,6]. Hyperglycemia is a state associated with uncontrolled diabetes that results in elevation of blood sugar levels. Prolonged hyperglycemic conditions may result in significant deterioration of different body organs, specifically blood vessels and the central nervous system. Presently, diversified synthetic drugs have been developed for the management of diabetes,



such as meglitinides, biguanides, incretin mimetics, thiazolidinediones, sodium/glucose co-transporter 2 (SGLT2) inhibitors, and dipeptidyl peptidase-IV inhibitors [7]. Even though various developed anti-diabetic drugs have demonstrated significant results, they are associated with different side effects [8]. These adverse effects include visual complications, headache, diarrhea, impotency, and hypoglycemia [9]. Therefore, research and development of innovative anti-diabetic medications that have significant effects against diabetes along with the least adverse effects remains a vital opportunity for scientists [10,11]. For this purpose, investigations on alternative natural medications have attained significant importance owing to their safe nature. Various folk medications, such as administration of different herbal plants and their resultant extracts, have been efficient alternatives to synthetic drugs in managing diabetes. Herbal plants have been a potential source of novel drug development, as nearly 90% of pharmaceuticals are directly or indirectly derived from them [12,13].

Alzheimer's disease (AD) is characterized as a neurodegenerative ailment known for its deteriorating effect on the central nervous system [14]. AD is related to different factors such as neuroinflammation, oxidative stress, aggregation of amyloids, and cholinergic deficiency [15–18]. To date, the underlying origin and optimum treatment of AD is still unclear and unidentified, while managing AD might include targeting its associated conditions. Effective management of the progression of AD can be possible by significantly dealing with oxidative stress, cholinergic deficiency, aggregation of amyloids, and neuroinflammation [19]. Mostly, the treatments of AD focus on easing the symptoms associated with its progression rather than providing an ultimate cure. These therapies help in improving cognitive functionality, managing behavioral symptoms, and improving overall quality of life [19]. Even though these treatments are of significant benefits, continual research to hunt for a definitive cure is needed [20]. As a result, recent investigations focus on exploring improved and alternative therapies for effective treatment of neurodegenerative ailments. Acetylcholine-a, neurotransmitter that is consumed by cholinergic neurons via the central nervous system, plays a vital role in normal functionality [21]. However, acetylcholinesterase (AChE), an enzyme, is considered to be responsible for the reduction of acetylcholine (ACh), hence causing problems in neurotransmission. Recently, AChE inhibitors have been used as an effective alternative approach for the management of AD as they elevate the levels of acetylcholine in the brain [22]. Various investigations have shown the anti-cholinesterase potential of different plant extracts. In spite of recent advancements in the field of modern medication, plant constituents play a significant part in the health and well-being of the community. Pharmaceutical companies are increasingly interested in exploring higher plants as potential sources for discovering new lead compounds and developing effective drugs.

This renewed interest highlights the potential therapeutic value of plant-based medicines in addressing various health conditions [23].

Inflammation is a complex biological process that plays a significant role in the development and progression of various disorders, including arthritis and cardiovascular disease [24]. The current approach for symptomatic treatment of inflammation primarily relies on nonsteroidal anti-inflammatory drugs (NSAIDs), which exert their anti-inflammatory effects by inhibiting cyclooxygenase (COX). COX exists in two isoforms: cyclooxygenase-1 (COX-1), which is constitutively expressed in most cells under normal conditions, and cyclooxygenase-2 (COX-2), which is induced by pro-inflammatory agents like tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Lipopolysaccharide (LPS), and tumor-promoting factors [25]. As a consequence, novel dual COX-2/5-LOX inhibitors with anti-inflammatory properties have been identified through investigations involving both natural and synthetic sources. In an effort to contribute to advancements in this area, studies have been conducted to provide a comprehensive summary of the structural characteristics and pharmacological activities of heterocyclic scaffolds and natural products that have been investigated as dual COX-2/5-LOX inhibitors [26]. Another limitation of COX inhibition is that it can lead to an increased production of leukotrienes (LT) by 5-lipoxygenase (5-LOX). While 5-lipoxygenase (5-LOX) inhibitors have demonstrated a protective effect, their usage is hindered by several limitations. These limitations include poor bioavailability, lack of inhibitor specificity, potential hepatic and renal abnormalities, myelosuppression, gastrointestinal disturbances, and an inability to provide protection in chronic inflammation models [27]. The complex and potentially adverse side effects associated with COX and 5-LOX inhibitors have indeed restricted their long-term use as treatments for inflammatory disorders [28]. The limitations and risks associated with these inhibitors highlight the need for alternative therapeutic approaches that can effectively manage inflammation with a more favorable side effect profile.

*Ficus benghalensis*, a member of the Moraceae family, is widely recognized as the Banyan tree in English. It is native to a vast region of Asia. This plant is grown in several botanical gardens throughout different tropical areas across the world [29]. Studies have been performed to assess the phytochemistry and health-promoting benefits of *F. benghalensis*. Nevertheless, very few investigations have been conducted on leaves of *F. benghalensis*, which is of great importance owing to its medicinal applications [30]. *F. benghalensis* was reported to own different health-promoting benefits such as antibacterial [31], anti-diabetic, anticancer, and anti-inflammatory [32]. Moreover, it has demonstrated beneficial effects against skin ailments, gastric ulcers, and other gastrointestinal problems [33]. *F. benghalensis* methanolic extract was found to be effective against inhibiting acetylcholinesterase activity [21]. The

composition of the leaves of *F. benghalensis* reveals the presence of crude protein (9.63%), Crude fiber (2.53%), phosphorous (0.4%), and calcium oxalate (2.53%). Phytochemical screening of leaves shows the presence of tannins, sterols, phenolic acids, saponins, and flavonoids. On the other hand, compounds like triterpenoids, volatile oils, and aromatic acids were not present in this plant [30]. In Ayurveda medications, this plant has been consumed for treating piles, dysentery, and diarrhea. Additionally, it is also employed as a hypoglycemic agent, indicating its potential in managing blood sugar levels [34]. Different extracts of *Ficus benghalensis* were assessed for their potential anti-allergic and anti-stress effects in asthma using milk-induced leucocytosis and milk-induced eosinophilia as indicators [35]. Literature reveals the potential of different *Ficus* species as anti-diabetic, antimicrobial, anticancer, and anti-inflammatory activities [36,37]. The current study is unique as we have compared the *in-vitro* anti-diabetic, anticholinesterase, and anti-inflammatory potential of extracts and sub-fractions of different parts of *F. benghalensis*. In light of this, current study was designed to evaluate the *in-vitro* anti-diabetic [ $\alpha$ -amylase and  $\beta$ -glucosidase inhibition], anti-inflammatory (5-LOX and COX-2 inhibition), and anti-cholinesterase (AChE and butyrylcholinesterase [BChE] inhibition) activity of different extracts and sub-fractions from stem, leaves, and roots of *F. benghalensis*.

## 2. Materials and Methods

### 2.1 Plants Collections

In June 2022, plant samples of *F. benghalensis* were gathered from Anbar Swabi. The collected specimen was taken to the Department of Botany, where it was examined and identified by Dr. Muhammad Ilyas, a member of the Botany Department at the University of Swabi. The voucher specimen number UOS-BOT/103 was then placed in the herbarium of the aforementioned department.

### 2.2 Extractions and Preparation of Subfractions

All information about software/equipment/drugs/reagents can be found in **Supplementary Material** and article. After collecting the plant material, consisting of stems, leaves, and roots, each weighing 2.2 kg, they were dried in the shade. Subsequently, the dried plant material was subjected to extraction using methanol. The obtained extracts were concentrated using a rotary evaporator (Rotavapor® R-300, BUCHI Corporation, New Castle, DE, USA), resulting in crude extracts of 18.00 g for the stems, 14.76 g for the leaves, and 16.1 g for the roots. Thin Layer Chromatography (TLC) profiling revealed that stem extract possessed the highest number of constituents, which were subjected to chromatographic analysis yielding different sub-fractions like F-B-1 C: CC chloroform: methanol (7:3); F-B-2 C: (chloroform: methanol (7.5:3.5); F-B-3 C: chloroform: methanol (6:4) and F-B-4 C: chloroform: methanol

(6.5:3.5). The extracts and sub-fractions obtained were carefully stored in a freezer to maintain their integrity and preserve their biological activity. Later, these stored samples were utilized for various biological studies and investigations.

### 2.3 In-Vitro Anti-Diabetic Activity

#### 2.3.1 $\alpha$ -amylase Inhibitory Activity Assay

An investigation was conducted to examine the impact of extracts and column semi-purified fractions of *F. benghalensis* on  $\alpha$ -amylase activity. This study employed an enzyme-starch system to assess the effects of extracts and column semi-purified fractions of *F. benghalensis* on  $\alpha$ -amylase activity [38]. Purposely, to prepare the experimental mixture, each extract, and column of semi-purified fractions of *F. benghalensis* powder (1%) was thoroughly stirred with 25 mL of a 4% potato starch solution in a beaker. Following that, 100 mg of  $\alpha$ -amylase was added to the starch solution, and the mixture was vigorously stirred. Subsequently, the prepared mixture was incubated at a temperature of 37 °C for a duration of 60 minutes. At the end of the incubation period, 0.1 M NaOH was introduced to halt the enzymatic activity. The mixture was subjected to centrifugation at 3000  $\times$ g for a duration of 15 minutes. Following centrifugation, the supernatant was collected, and the glucose content within it was measured.

#### 2.3.2 $\alpha$ -glucosidase Inhibitory Activity Assay

The  $\alpha$ -glucosidase inhibitory activity of extracts and column semi-purified fractions obtained from *F. benghalensis* was evaluated utilizing the methodology outlined by Pistia-Brueggeman and Hollingsworth in 2001 [39]. In order to assess the  $\alpha$ -glucosidase inhibitory activity of extracts and fractions derived from *F. benghalensis*, varying concentrations of the extracts/fractions (62.5–1000  $\mu$ g/mL) were combined with a solution containing 10  $\mu$ L of  $\alpha$ -glucosidase (1 U/mL) and 125  $\mu$ L of phosphate buffer (pH: 6.8; 0.1 M). The mixture was subjected to an incubation period of 20 minutes at a temperature of 37 °C. To initiate the reaction, a 20  $\mu$ L solution of 1 M pNPG (4-Nitrophenyl- $\beta$ -D-glucopyranoside) substrate was added, and the mixture was further incubated for 30 minutes. To halt the reaction, 50  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> (0.1 N) was introduced into the reaction mixture. A UV-Vis spectrophotometer was utilized to measure the optical density of both the sample and the control at 405 nm wavelength. The following formula was employed to calculate the inhibitory activity:

$$\text{Inhibitory Activity (\%)} : \left( \frac{OD(\text{control}) - OD(\text{sample})}{OD(\text{control})} \right) \times 100$$

$$\text{Inhibitory Activity (\%)} : \left( \frac{AbsC - AbsS}{AbsC} \right) \times 100$$

Here: OD (control) = absorbance of control, while OD (sample) = absorbance of extracts/fractions (AbsC: Absorption of control; AbsS: Absorption of sample).

## 2.4 In-Vitro Anti-Cholinesterase Activity

The anti-cholinesterase activity of both the extracts and column semi-purified fractions of *F. benghalensis* was assessed using the Ellman method [40]. In summary, the cholinesterase enzyme breaks down the acetyl thiocholine substrate, resulting in the formation of thiocholine. Thiocholine then reacts with Ellman's reagent (DTNB), generating 2-nitrobenzoic-5-mercaptothiocholine (thiocholine-thionitrobenzoate disulfide) and 5-thio-2-nitrobenzoic acid (thionitrobenzoate). These reaction products can be detected at a wavelength of 405 nm. For this assay, the reaction mixture had a total volume of 1 mL. Within a 1 mL cuvette, 50 µL of 3.5 mmol L<sup>-1</sup> acetyl thiocholine iodide (ATCI) in a buffer was combined with 920 µL of a 0.125 mmol L<sup>-1</sup> DTNB/buffer mixture. Subsequently, 20 µL of cholinesterase enzyme was added, and the absorbance was measured at 405 nm every 30 seconds for a total of 3 measurements. This process allowed the determination of the enzyme's overall activity prior to inhibition. Afterward, 10 µL of the sample [extract/fractions] was introduced into the mixture, and the absorbance was once again recorded at the same wavelength, with three measurements taken at 30-second intervals. Following the addition of the sample, the enzyme activity inhibition percentage was calculated. To evaluate the inhibition of BChE (Butyrylcholinesterase), a similar method as described earlier was employed with slight adjustments. In this case, 25 µL of 5 mM butyrylthiocholine iodide was utilized as the substrate, while the enzyme concentration was set at 0.05 U/mL of BChE. Galantamine served as the positive control for both enzymes. The experiments were conducted in triplicate to ensure the accuracy and reliability of the results. The IC<sub>50</sub> values, which represent the concentration at which there is 50% enzyme inhibition, were determined for the selected samples [extracts/fractions] using GraphPad Prism-7 software. Galantamine, a standard reference, was utilized for comparison and analysis.

## 2.5 In-Vitro Anti-Inflammatory Activity

### 2.5.1 COX-2 Inhibitory Assay

In this study, an *in-vitro* assay was performed to assess the anti-inflammatory effectiveness of extracts and column semi-purified fractions derived from *F. benghalensis*. In this study, the evaluation of COX-2 inhibitory potential was conducted using the methods outlined by Jan *et al.* [41] in 2020. Initially, 300 U/mL of COX-2 enzyme was prepared, from which 10 mL of COX-2 solution was ice-cooled for activation of enzymatic activity. To activate the enzymatic activity, the whole procedure was carried out for 5 to 10 minutes. Moreover, a co-factor solution (hematin: 1 mM, N,N,N,N-tetramethyl-p-phenylenediamine (TMPD):

0.24 mM, and glutathione: 0.9 mM) containing 50 mL that was prepared in Tris-HCl buffer (pH: 8, 0.1M) was mixed with this activated enzymatic solution. Afterwards the plant extracts and fractions were mixed with enzyme solution at varied concentrations (62.5–1000 µg/mL). Later, this mixture was incubated at 25 °C for 5 minutes. To initiate the reaction, 20 mL of a 30 mM arachidonic acid solution was introduced to the mixture. The resulting solution was then incubated for a duration of 4–5 minutes. In this assay, celecoxib was employed as a reference drug to enable comparison with the tested samples. Finally, absorbance was noticed at 540 nm wavelength using a UV-vis spectrophotometer.

$$\text{Inhibitory Activity(\%)} : \left( \frac{\text{AbsC} - \text{AbsS}}{\text{AbsC}} \right) \times 100$$

Here: AbsC = absorbance of control, while AbsS = absorbance of sample [extract/fractions].

### 2.5.2 5-LOX Inhibitory Assay

Additionally, this study included a 5-LOX (5-lipoxygenase) assay to assess the *in-vitro* anti-inflammatory activity of extracts and column semi-purified fractions obtained from *F. benghalensis* [42]. For the 5-LOX assay, different doses (ranging from 62.5 to 1000 µg/mL) of extracts and fractions derived from *F. benghalensis* were prepared. Subsequently, a solution of the 5-LOX enzyme was prepared with a concentration of 10,000 units per milliliter. To initiate the enzymatic reaction, an 80 mM linoleic acid (L1376-5G, Sigma-Aldrich, St. Louis, MO, USA) substrate was added to the solution. To create the desired reaction mixture, 250 µL of crude extracts were combined with a phosphate buffer (50 mM, pH 6.3). Next, an enzyme solution of 250 mL was introduced to the mixture, and the resulting mixture was incubated for a duration of 5 minutes. Following the incubation, the substrate solution was added to the enzyme mixture and thoroughly mixed together. The absorbance (Abs) of both the control and test samples was measured at a wavelength of 234 nm using a UV-Vis spectrophotometer. A graph was constructed to examine the correlation between different extract concentrations and the extent of enzyme inhibition. This analysis allowed for the calculation of the IC<sub>50</sub> values, which represent the concentration at which the enzyme inhibition reaches 50%. Finally, the percentage inhibition was determined using the following formula:

$$\text{Percent inhibition (\%)} : \left( \frac{\text{AbsC} - \text{AbsS}}{\text{AbsC}} \right) \times 100$$

Here: AbsC = absorbance of control, while AbsS = absorbance of sample (extract/fractions).

## 2.6 Statistical Analysis

The results in this study were expressed as mean  $\pm$  S.D. Level of significance ( $p < 0.05$ ) was assessed using two-way ANOVA. Tukey's HSD (honest significant difference) was performed to evaluate the mean pairwise comparison among groups.

## 3. Results

### 3.1 In-Vitro Anti-Diabetic Potential

**Supplementary Table 1** shows the results of the anti-diabetic activity of extracts from different parts and isolated column fractions of *F. benghalensis*. At 1000  $\mu\text{g/mL}$ , the highest  $\alpha$ -glucosidase inhibitory was demonstrated by *F. benghalensis* leaves extract (73.84%) followed by F-B-2 C (69.67%), *F. benghalensis* stem extract (65.50%), F-B-3 C (65.50%), F-B-1 C (61.22%), and *F. benghalensis* roots extract (55.44%). In the case of  $\alpha$ -amylase inhibitory activity, *F. benghalensis* leaves extract showed maximum (76.29%) potential, while *F. benghalensis* root extract had minimum inhibitory activity (57.33%). The standard (acarbose) used in this study showed a percent reduction of 81.85 and 83.53% in the activity of  $\alpha$ -glucosidase and  $\alpha$ -amylase, respectively.

### 3.2 In-Vitro Anti-Cholinesterase Potential

In this study, extracts of *F. benghalensis* roots, leaves, and stem, along with derived fractions (F-B-1 C, F-B-2 C, F-B-3 C, and F-B-4 C) were analyzed for their potential anti-cholinesterase activity. Glutamine was used in this study as a reference standard drug. Results revealed a significant reduction in activity of AChE and BChE in a concentration-dependent manner (**Supplementary Table 2**). It is evident from **Supplementary Table 2** that extracts of roots, leaves, and stems demonstrated potential inhibition of AChE and BChE with  $\text{IC}_{50}$  values ranging from 50.50 to 474.83  $\mu\text{g/mL}$ . In comparison, four different fractions (F-B-1 C, F-B-2 C, F-B-3 C and F-B-4 C) inhibited the activity of AChE and BChE with  $\text{IC}_{50}$  values in the range of 91.85 to 337.94  $\mu\text{g/mL}$ .

### 3.3 In-Vitro Anti-Inflammatory Potential

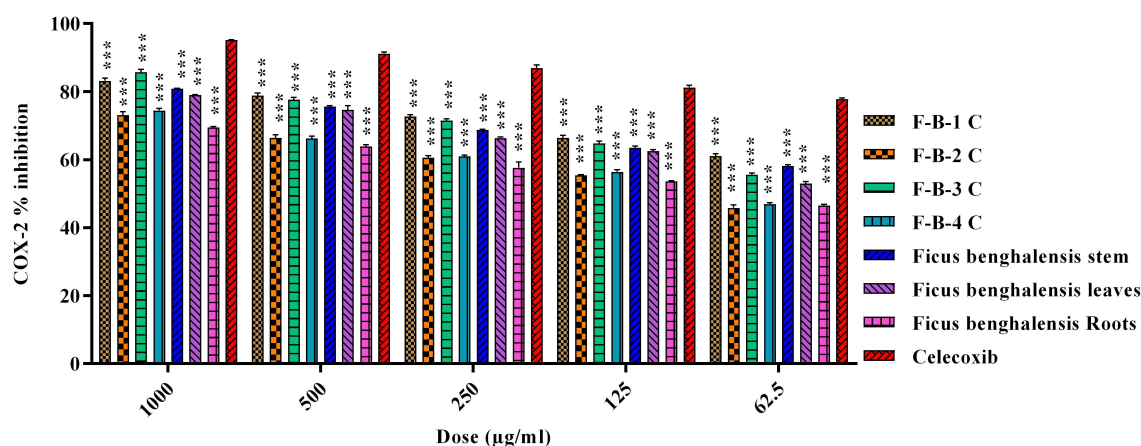
The results of the anti-inflammatory activity of extracts from different parts and isolated column fractions *F. benghalensis* against COX-2. At 1000  $\mu\text{g/mL}$ , the highest COX-2 inhibitory potential was demonstrated by F-B-3 C (85.72%), followed by F-B-1 C (83.13%), *F. benghalensis* stem extract (80.85%), *F. benghalensis* leaves extract [79.00%], F-B-2 C (73.08%), and *F. benghalensis* roots extract (69.47%). In the case of 5-LOX inhibitory activity, F-B-1 C showed maximum (87.63%) potential, while *F. benghalensis* root extract had minimum inhibitory activity (73.39%). The standard (Montelukast and Celecoxib) used in this study showed a percent reduction of 95.20 and 93.55% in the activity of COX-2 and 5-LOX, respectively

as shown in Figs. 1,2. The  $\text{IC}_{50}$  values of the crude extracts/fractions and isolated column fractions of *F. benghalensis* were shown in Figs. 3,4, respectively.

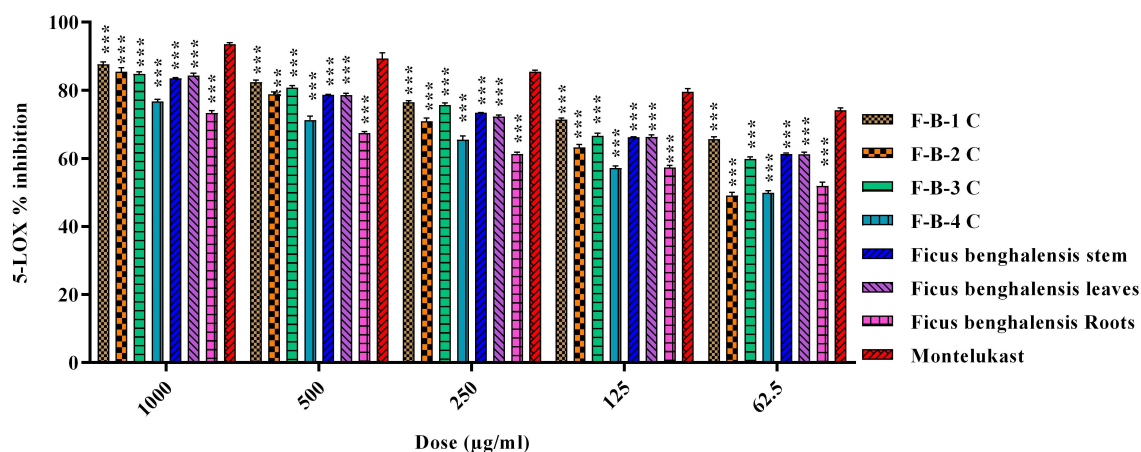
## 4. Discussion

The global population's reliance on plant-based remedies is on the rise, primarily attributed to their easy accessibility and affordability [43,44]. Consequently, researchers are persistently engaged in the pursuit of significant natural-based remedies that can be employed in the treatment, diagnosis, mitigation, or prevention of various disorders [45]. Validating the therapeutic potential of these readily accessible natural products holds the promise of offering more cost-effective treatment alternatives, particularly in light of the prevailing high inflation rates globally. It is worth noting that the pharmacodynamics of many drugs are associated with the inhibition of enzymes present in various biological compartments. Local physicians have recognized the therapeutic properties of the *Ficus* plant. The local physicians have long recognized the therapeutic properties of various parts of the *F. benghalensis* plant. The milky fluid obtained from the plant is known for its external application in alleviating pain, rheumatism, bruises, backaches, and swollen soles of the feet. In India, the roots of *F. benghalensis* are traditionally used to treat conditions such as dysentery, biliousness, gonorrhea, and liver swelling. Additionally, the aerial roots and tips of the plant are employed for their medicinal benefits in the treatment of dysentery and vomiting [46]. The infusion of small branches is consumed to alleviate hemoptysis, while the bark is believed to possess potent tonic properties and is used as a cure for diabetes [47]. Different components of *F. benghalensis* are employed in the treatment of diarrhea, leucorrhea, wound healing, and skin diseases [48]. According to folkloric practices, the aerial parts of *F. benghalensis* are employed to alleviate persistent vomiting and as an anti-asthmatic remedy [49]. Additionally, *F. benghalensis* leaves and stems have been documented for their therapeutic applications in various ailments [50]. However, there is a limited exploration of the phytochemical composition of this plant. Some compounds identified in its leaves include  $\beta$ -sitosterol, psoralen,  $\beta$ -amyrin, quercetin-3-galactoside, leucopelargonon, rutin, and leucodelphinidin derivatives [51].

The present study was performed to assess the anti-cholinesterase, anti-inflammatory, and anti-diabetic properties of extracts/fractions of *F. benghalensis*. *In-vitro* anti-diabetic assay revealed a significant [ $p < 0.05$ ] concentration-dependent reduction in the activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase due to the application of different extracts and fractions of *F. benghalensis*. In  $\alpha$ -glucosidase and  $\alpha$ -amylase assays, as compared to standard (Acarbose,  $\text{IC}_{50}$ : 26.58 and 21.30  $\mu\text{g mL}^{-1}$ ), the  $\text{IC}_{50}$  values of different extracts and fractions were in the range of 50.50–474.83 and 91.85–337.4  $\mu\text{g mL}^{-1}$ , respectively. The results of our study are in accordance with the findings



**Fig. 1.** Percent COX-2 inhibition activity of crude extracts/fractions and isolated column fractions of *Ficus benghalensis*. Data is represented as mean  $\pm$  Standard Error of the Mean (S.E.M);  $n = 3$ ; Two Way ANOVAs followed by the Bonferroni test were followed for test sample comparison to the standard drug. Values significantly different as compared to positive control; \*\*\* =  $p < 0.001$ . COX-2, cyclooxygenase-2.

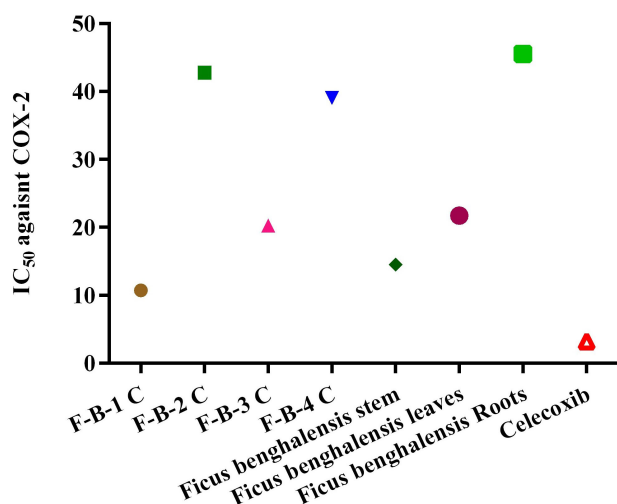


**Fig. 2.** Percent 5-LOX inhibition of crude extracts/fractions and isolated column fractions of *Ficus benghalensis*. Data is represented as mean  $\pm$  S.E.M;  $n = 3$ ; Two Way ANOVAs followed by the Bonferroni test were followed for test sample comparison to the standard drug. Values significantly different as compared to positive control; \*\*\* =  $p < 0.001$ . 5-LOX, 5-lipoxygenase.

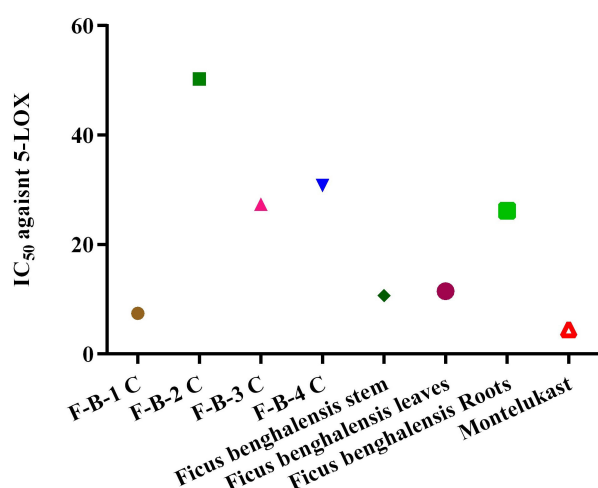
of Blicke in 2008 [51]. They reported that the bark of *F. benghalensis* significantly inhibited the activity of both  $\alpha$ -glucosidase and  $\alpha$ -amylase [52]. Glucosidases play a vital role in various biological processes, including the breakdown of dietary carbohydrates [53].  $\alpha$ -glucosidase is one of several glucosidases found on the brush-border surface membrane of intestinal cells, and it plays a crucial role in the digestion of carbohydrates [54]. Daniel *et al.* [55] in 1998 reported that the presence of phenolics and flavonoids in *F. benghalensis* bark contributes to its inhibitory effect on  $\alpha$ -glucosidase. These compounds possess potential antioxidant activity, and various investigations have shown that phenolic-enriched extracts of *F. benghalensis* demonstrate moderate free radical scavenging ability and strong inhibitory activity against  $\alpha$ -glucosidase. In a study con-

ducted by Madiwalar *et al.* [56], it was reported that 17 phytoconstituents derived from *F. benghalensis* demonstrated blood glucose-lowering effects. Notably, the highest drug-likeness score has been shown by 4-methoxybenzoic acid, while maximum ( $-8.02$  kcal/mol) binding affinity was demonstrated by lupeol acetate. Lupeol acetate formed nine pi-interactions with Ala451, Ile319, Phe323, Phe24, Ile200, Tyr324, and Ile28. Additionally, the extract displayed the most significant glucose uptake efficacy in yeast cells at a concentration of  $500 \mu\text{g/mL}$  [56]. Aqueous extract of *F. benghalensis* bark had inhibitory ( $\text{IC}_{50}$ :  $4.4 \mu\text{g/mL}$ ) effects of  $\alpha$ -amylase as experimented by Ponnusamy *et al.* [57].

In this study, the extracts and fractions of *F. benghalensis* were also examined for their potent anti-inflammatory properties. These two assays, namely COX-



**Fig. 3.** The IC<sub>50</sub> (half-maximal inhibitory concentration) values of various tested samples against COX-2. Data is represented as mean  $\pm$  S.E.M; n = 3; Values significantly different as compared to positive control.



**Fig. 4.** The IC<sub>50</sub> values of various tested samples against 5-LOX. Data is represented as mean  $\pm$  S.E.M; n = 3.

2 and 5-LOX inhibitory assays, were performed in this study. Results of both assays demonstrated a significant [ $p < 0.05$ ] reduction in the activity of COX-2 and 5-LOX activity. According to a study conducted by Kothapalli *et al.* [58], extracts of *F. benghalensis* leaves showed potential anti-inflammatory activity [58]. Moreover, an *in-vivo* study further investigated the anti-inflammatory potential of *F. benghalensis* bark extract in a rat model. They administered varied content of extract [50–200 mg/kg] of *F. benghalensis* bark in carrageenan-induced paw edema. Results showed that administration of this extract reduced the inflammation in a concentration-dependent manner. They were of the view that tannins and flavonoids present in the bark extract of *F. benghalensis* were responsible for this anti-

inflammatory potential. Oxidative stress induced by induction of carrageenan injection was also reduced on the application of experimented extracts [59]. Likewise, bark and leaves extract of *F. curtipipes*—another species of genus *Ficus*—have been reported to downregulate the activity of 5-LOX in a dose-dependent manner. The bark extract of *F. curtipipes* significantly inhibited 5-LOX activity with an IC<sub>50</sub> value of 10.75  $\mu$ g/mL. The inhibitory potential of bark was reported to be more owing to the presence of more phenolic compounds in stem (5374.1 mg/kg dry extract) as compared to leaves (611.5 mg/kg dry extract) [60]. In general, phenolic constituents are recognized as predominant inhibitors of 5-LOX activity [61]. The results of these studies are in accordance with the findings of the current study, which revealed the inhibitory potential of *F. benghalensis* extracts and fractions against the activity of 5-LOX and COX-2. Bengalensinone and benganoic acid have been isolated from roots of *F. benghalensis* and possessed inhibitory (164.5 and 154.5  $\mu$ M) action against acetylcholinesterase, respectively. Both these compounds inhibited the activity of butyrylcholinesterase with an IC<sub>50</sub> value of 224.9 and 120  $\mu$ M, respectively [62]. Moreover, Hassan *et al.* [21] have shown the anti-acetylcholinesterase (IC<sub>50</sub>: 194.6  $\mu$ g mL<sup>-1</sup>) activity of *F. benghalensis*.

Phytochemical profiling of *F. benghalensis* extracts have indicated the presence of diverse phytomolecules like steroids, tannins, flavonoids, alkaloids, anthraquinones, and glycosides [63]. Roots extract of *F. benghalensis* inhibited the activity of acetylcholinesterase in an *in-vitro* model. The ethyl acetate extract of roots showed inhibitory action with an IC<sub>50</sub> value of 67  $\mu$ g/mL as compared to the control (Donepezil), i.e., 33  $\mu$ g/mL. The results of this study were comparable to the outcomes reported by Ramasamy *et al.* [63]. Moreover, Hassan *et al.* [21] 2020 also assessed the *in-vitro* AChE inhibitory potential of extracts (methanolic), fractions (ethyl acetate), and isolated compounds of *F. benghalensis*.

## 5. Conclusions

The findings of this study revealed the *in-vitro* potential of *Ficus benghalensis* extracts and fractions in various pharmacological activities. The leaf extract exhibited the highest alpha-glucosidase and alpha-amylase inhibitory activities among all the tested samples, indicating its potential as an anti-diabetic agent. Furthermore, the roots, leaves, and stem extracts, as well as the derived fractions, exhibited significant anti-cholinesterase activity, highlighting their potential for the management of neurodegenerative diseases. Additionally, the F-B-3 C fraction showed the highest COX-2 inhibitory activity, suggesting its potential as an anti-inflammatory agent. Overall, this study provides valuable insights into the medicinal potential of *F. benghalensis* and supports its traditional use in the treatment of diabetes, neurodegenerative diseases, and inflammatory conditions. Further studies are warranted to identify

and characterize the active compounds responsible for these observed bioactivities and to evaluate their *in-vivo* efficacy and safety profiles.

## Availability of Data and Materials

The data generated in the present study are included in the figures and/or tables of this article.

## Author Contributions

AR was responsible for conceptualization, visualization, supervision, project administration and writing-original draft. Mibr and Mlri contributed by conducting formal analysis, interpreting data, investigation and writing-original draft. AAK, NA, TSA, TK and MUK contributed by writing-original draft, analyzing, data curation, reviewing and editing of manuscript. KB, MSJ, and RS made significant revisions, visualization, and editorial changes. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Plant samples of *F. benghalensis* were gathered from Anbar Swabi. The collected specimen was taken to the Department of Botany, where it was examined and identified by Dr. Muhammad Ilyas, a member of the Botany Department at the University of Swabi. The voucher specimen number UOS-BOT/103 was then placed in the herbarium of the aforementioned department.

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## Conflict of Interest

The authors declare no conflict of interest. Given his role as Guest Editor and Editorial Board Member of Frontiers in Bioscience-Landmark, Marcello Iriti had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Jen-Tsung Chen.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2905183>.

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