PHYTOCHEMICAL SCREENING AND EVALUATION OF BIOMEDICAL PROPERTIES OF THE LEAF EXTRACT OF SACRED FIG *FICUS RELIGIOSA*

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Abstract

Ficus religiosa, the sacred fig tree, is widely recognized for its diverse ethnomedical and ecological importance. This study aimed to investigate the phytochemical constituents and biomedical properties of F. religiosa leaf extracts. Phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, proteins, tannins, and terpenoids in the leaf extracts. The leaf biomass and biochar were prepared and evaluated for their antibacterial, antioxidant, antidiabetic, anti-inflammatory, and wound healing properties. The biochar exhibited higher levels of flavonoids, phenolics, and vitamin A compared to the biomass. Both the biomass and biochar demonstrated significant antibacterial activity against Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa, with the biochar showing better efficacy. The antioxidant activity, assessed by DPPH free radical scavenging assay, increased with increasing concentrations of the samples and was comparable to the standard ascorbic acid. The biochar exhibited significant antidiabetic activity, as evidenced by the inhibition of α-glucosidase enzyme, comparable to the standard drug acarbose. The anti-inflammatory effect of both the biomass and biochar, evaluated by protein denaturation assay, was highly comparable to the standard drug diclofenac. The L6 cell line viability assay confirmed the non-cytotoxic nature and wound healing properties of the biomass and biochar. These findings highlight the potential of F. religiosa leaf extracts as a source of bioactive compounds with promising biomedical applications. Further in vivo studies are warranted to validate these properties and explore their therapeutic potential.

Keywords: *Ficus religiosa*, Antioxidant, Antidiabetic, Anti-Inflammatory, Wound healing.

Introduction

Plants and trees are essential resources for environmental and medicinal uses. They offer a wide range of bioactive substances that are utilized in both conventional and alternative medicine, and they also assist the environment by providing habitat, air purification, and carbon

sequestration. Medicinal plants have been essential to both conventional and contemporary treatment approaches. *Ficus religiosa* L., sometimes known as the sacred fig or peepal tree, is notable among them due to its many ecological and ethnomedical uses. Originally from the Indian subcontinent, *F religiosa* is now being studied scientifically for its potential as a medicine and for its ability to clean up the environment. It is also considered a sacred plant in Buddhism and Hinduism.

Under the phylums of Dicotyledone and Spermatophyte of the Plantae kingdom, which includes trees, vines, shrubs, epiphytes, and hemiphytes, the Moraceae family of the Urticales order includes the Ficus genera. *With a variety of traditional uses, Ficus religiosa is one of the most often planted Ficus species in the tropics. It is referred to locally as the Peepal tree (synonym: Pimpala) and is a member of the Urostigma subgenus.* It is a large tree and epiphytic when young, containing petioles of 5 to 10 cm in length, aspen-like lamina, sessile, and paired hypanthodia, with no male flowers and pedicellate or sessile [1]. The details of the plant are described below.

Domain: Eukaryota
Kingdom: Plantae
Subkingdom: Viridaeplantae
Phylum: Tracheophyta
Order: Urticales
Family: Moraceae
Genus: Ficus
Species: *Ficus religiosa*

Liver disorders are treated with a variety of medicinal herbs [2]. In clinical settings, phytocompounds found in medicinal plants are regarded as an alternative medicine since they have few adverse effects and great therapeutic potential [3,4]. According to this viewpoint, *F. religiosa* is an essential component of Ayurvedic and Unani medicines that are used to treat a variety of illnesses, including liver disorders [5,6].

Recent studies investigate the bioactive chemicals and therapeutic qualities of medicinal plant extracts, examining their action mechanisms and toxicological effects [7]. *Ficus carica*, another species of the Ficus genus, is particularly significant for its widespread use in food, industry, and medicine. Significant suppression of cancer cell lines, including Hep2 and HepG2, has been observed in some of these investigations [8], suggesting that fig leaves may play a role in the prevention or treatment of cancer. Furthermore, fig extracts have been investigated for their antioxidant and diabetes-management potential [9, 10]. It has also been discovered that all Ficus species contain these medicinally beneficial compounds. Therefore, this study advances our understanding of the phytochemical components of *Ficus religiosa* leaves as well as their biological advantages, such as their anti-inflammatory, antidiabetic, and antioxidant qualities as well as their capacity to promote wound healing.

Materials and Methods

Preparation of *Ficus religiosa* biochar

Ficus religiosa leaves were obtained from a tree located at DKM College for Women, Vellore. Double-distilled water was used to properly clean the *Ficus religiosa* leaves in order to get rid of dust and other surface impurities. Before being ground to the required size and stored, the leaves were first chopped into small bits and dried for 24 hours at 110 °C in a hot air oven to eliminate any remaining moisture.

Preparation of Ficus religiosa leaves biochar

The milled and powdered Ficus religiosa leaves were carbonized for three hours at 450 °C (heating rate = 15 °C/min) in a muffle furnace to produce the biochar. When the temperature reached room temperature, the carbon was taken out. NaOH was then used as an activating agent to chemically activate the carbon powder at a 1:3 impregnation ratio [11]. Deionized water was added to the active samples to fully clean them, and after 24 hours at room temperature, they were continuously swirled to enhance mixing. For 12 hours, the granular carbon powder from *Ficus religiosa* was dried at 120 °C in a hot air oven.

Phytochemical Screening

Detection of alkaloids

One milliliter of aqueous extract was stirred and placed in 1% aqueous hydrochloric acid on a steam bath. Then, 1 mL of the filtrate was treated with Dragedorff's reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids [12].

Detection of carbohydrates

A few drops of Benedict's reagent (an alkaline solution containing cupric citrate complex) were added to an aqueous extract, which was then heated in a water bath. The production of a reddish-brown precipitate indicated the presence of carbohydrates [13].

Detection of saponins

In a water bath, roughly 2 g of the powdered material was cooked in 20 ml of distilled water before being filtered. For a stable, long-lasting foam, 10 milliliters of the filtrate were combined with 5 milliliters of distilled water and shaken briskly. After adding three drops of olive oil to the mixture and giving it a good shake, the creation of an emulsion was monitored [14].

Detection of phenols

Three to four drops of ferric chloride solution were added to the extracts. The presence of phenols is indicated by the formation of a bluish black color [13].

Detection of flavonoids

In each instance, 10 milliliters of ethyl acetate were used to heat a part of the powdered plant sample for three minutes over a steam bath. After filtering the mixture, 1 milliliter of diluted ammonia solution was mixed with 4 milliliters of the filtrate. When diluted acid is added, the yellow coloration that was seen indicates the presence of flavonoids and indicates a positive test for them [14].

Detection of proteins

The ninhydrin reagent (2,2-dihydroxyindene-1,3-dione) was added to the extract and brought to a boil for a short while. The presence of an amino acid is indicated by the production of blue color.

Detection of tannins

In a test tube, roughly 0.5 g of the extract was heated in 10 ml of water before being filtered. After adding a few drops of 0.1% ferric chloride, the coloration was checked for brownish green or blue-black [15].

Detection of terpenoids

After combining 5 mL of each extract with 2 mL of chloroform, 3 mL of sulfuric acid concentrate was carefully added to create a layer. Terpenoids are indicated by a reddish-brown color at the contact [16].

Antibacterial Activity

The disc diffusion method and Muller-Hinton agar (MHA) medium were used to assess the *Ficus religiosa* leaves' biomass and biochar's antibacterial activity against the five bacterial strains. To investigate the antibacterial activity, the following bacteria were chosen: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa.*

A sterile spreader was used to evenly disperse the germs in Petri plates after they had been inoculated in Muller-Hinton Agar (MHA) medium to assess the biomass and biochar's antibacterial activity. After the MHA medium set, a small amount of the *Ficus religiosa* leaves' biomass and biochar were put on several agar plates for the disc diffusion procedure. The plates were incubated on various racks at 37°C for 24 hours before the organisms proliferated. The

plates were left to stand at room temperature for an hour in order to allow the compounds to spread. The antibacterial activity was assessed by measuring the zone of inhibition diameter that formed around the discs with regard to the test microorganisms using a ruler.

Antioxidant activity by the DPPH free radical scavenging assay

Ayyanar et al.'s method [17] was used to evaluate the biomass and biochar of Ficus religiosa leaves' capacity to scavenge 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radicals. To different quantities of biomass and biochar (56.25, 122.5, 225, 450, and 900 µg/mL), 100 µL of DPPH solution (0.1 mM) in methanol was applied. After being dark-incubated at room temperature for 30 minutes, the absorbance was measured at 517 nm. The common antioxidant medication utilized to express the DPPH radical scavenging activity was ascorbic acid. The scavenging effect was calculated using the following formula.

% Inhibition= [(Abs of Control - Abs of Sample)/Abs of Control] x 100

Antidiabetic activity by α-amylase and α-glucosidase enzyme inhibition assays

Using Gangapriya et al.'s [18] method, the biomass of Ficus religiosa leaves and the α-amylase enzyme inhibitory activity of biochar were evaluated. The combination was preincubated for 10 minutes at 25ºC after 100 µL of the sample at different concentrations (56.25, 122.5, 225, 450, and 900 µg/mL) was mixed with a 1% starch solution that contained 20 mM phosphate buffer saline (pH 6.9) and 6 mM sodium chloride. After adding 100 µL of pancreatic α-amylase enzyme (0.5 mg/mL), the mixture was incubated under the same conditions. 200 µL of dinitrosalicylic acid was added, and the hydrolytic reaction was halted by incubation at 100◦C for five minutes. After the mixture was further diluted with deionized water and the samples were allowed to come to room temperature, the absorbance at 540 nm was measured using acarbose as a positive control. The inhibition of the α-amylase enzyme was calculated as follows:

% Inhibition= [(Abs of Control - Abs of Sample)/Abs of Control] x 100

Anti-inflammatory activity by protein denaturation assay

The experiment was carried out in accordance with Sam Arul Raj et al. [19] and evaluated the anti-inflammatory qualities of the biomass and biochar of Ficus religiosa leaves using the protein denaturation assay. 0.45 mL of bovine serum was mixed with 0.5 mL of test samples in varying amounts. The reaction was incubated for 25 minutes at 40°C. After incubation, 2.5 mL of Na_3PO_4 buffer saline (pH 6.3) was added to the mixture. The absorbance of the turbidity was then measured at 660 nm. The results were contrasted with those of diclofenac, the common medication. The following formula was used to determine the *Ficus religiosa* leaves' biomass and biochar's inhibition percentage:

% inhibition = [(Control Abs–Sample Abs)/Control Abs] × 100

All the experiments on biological activities were conducted in triplicate and were expressed as the Mean ± Standard deviation.

Results and Discussion

The current study aimed to carry out the phytochemical screening and to check in vitro antibacterial, antidiabetic, anti-inflammatory, and wound healing capacity of *Ficus religiosa* leaves' biomass and biochar.

Table 1. Phytochemical screening of crude extracts of *Ficus religiosa.*

Phytochemicals	**Aqueous extract of *Ficus religiosa***
Carbohydrates	-
Alkaloids	++
Saponins	+
Polyphenols	+++
Flavonoids	+++
Protein	-
Tannins	++
Terpenoids	++

Where + shows low and ++ high presence, and – shows absence of phytochemical activities.

According to **Table 1,** very useful phytochemicals such as phytochemicals and flavonoids are found in abundance, while alkaloids, terpenoids, and tannins are also found in a reasonable quantity in the leaves of *Ficus religiosa*. Numerous active ingredients in Ficus religiosa, such as flavonoids, phenolic compounds, alkaloids, glycosides, tannins, and terpenoids, have been discovered through in-depth phytochemical studies conducted over the last fifteen years. High concentrations of phenolics and flavonoids were cited by Mishra et al. [20] as the reason for the antioxidant potential of methanolic extracts of *F. religiosa* leaves. According to a thorough ethnopharmacological profile put out by Sharma and Choudhary [21], many plant sections have a variety of secondary metabolites with a range of biological functions. Significant amounts of bioactive chemicals were discovered in ethanolic leaf extracts by Kumar et al. [22], who also highlighted the durability and effectiveness of these preparations in comparison to aqueous ones. More recently, Kaur et al. [23] identified substances such gallic acid, quercetin, and rutin in the leaf extracts using high-performance liquid chromatography (HPLC).

Table 2: Biochemical Factors in Ficus religiosa biomass and Biochar

Biochemical Factors	***Ficus religiosa* biomass**	***Ficus religiosa* biochar**
Total Flavonoids (mg QE/g)	50.33±2.04	61.8±1.01
Total Phenolics (mg GAE/g)	51.24±1.04	59.6±1.11
Vitamin A (AAE/g extract)	109.41±1.06	116.7±2.02

Table 2 represents the quantity of total flavonoids to the extent of which is 50.33±2.04 and 61.8±1.01mg QE/g, while the total phenolics are 51.24±1.04 mg GAE/g and 59.6±1.11 mg GAE/g, and vitamin A to the extent of 109.41±1.06 and AAE/g in *Ficus religiosa* leaves' biomass and biochar, respectively. The results confirm that the biochar possesses higher levels of flavonoids, phenolics, and Vitamin A.

Antibacterial Activity

Microbes are microorganisms, including bacteria, viruses, fungi, and others, that may cause infectious and deadly diseases if acquired into any biological system. An antimicrobial agent refers to natural or synthetic components that can kill or inhibit the growth of microorganisms. The increased multidrug-resistant organisms have increased the search for novel antimicrobial agents from natural sources, plants [24].

Table 3: Antibacterial Activity

Organism	***Ficus religiosa* biomass**	***Ficus religiosa* biochar**	**Control (Ciprofloxacin)**
E.coli	21	24	25
Klebsiella pneumonia	16	19	22
Staphylococcus aureus	20	22	23
Bacillus cereus	22	23	26
Pseudomonas aeruginosa	21	22	24

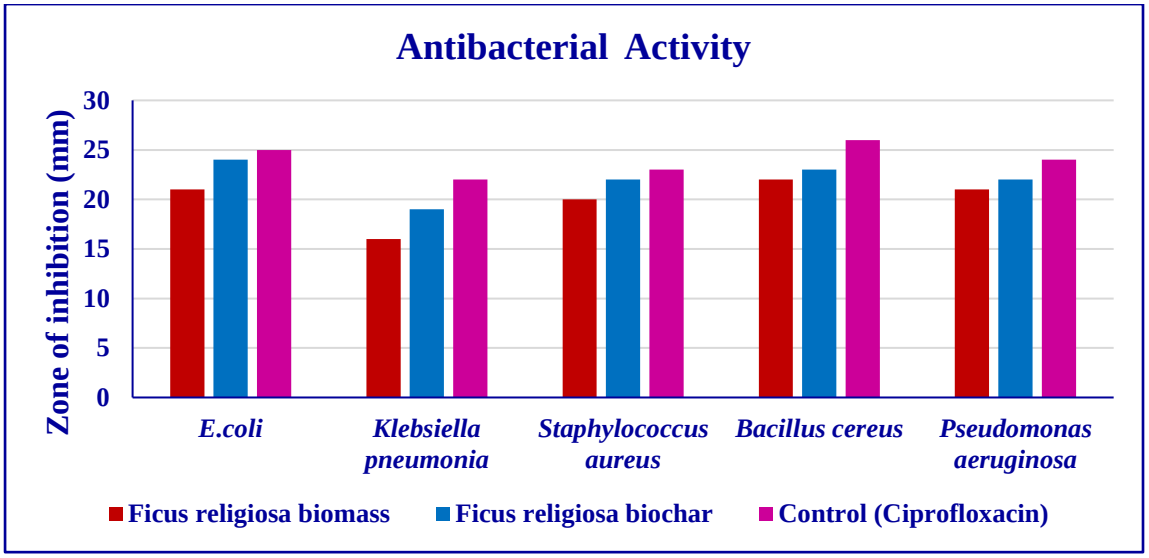

Figure 1: Antibacterial activity of *Ficus religiosa* biomass and biochar

Table 3 and Figure 1 represent the antibacterial activity of the prepared biomass and biochar of *Ficus religiosa* leaves. The results confirm that both the biomass and biochar possess good antibacterial activity against the selected bacterial strains, such as *E. coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa* on comparing with the standard drug ciprofloxacin. Among the two, the *Ficus religiosa* leaves' biochar possesses a better antibacterial activity than its biomass. Several studies have demonstrated the antibacterial properties of *Ficus religiosa* leaf extracts against a wide range of pathogens. Sharma et al. [25] reported that methanolic and ethanolic extracts exhibited inhibitory effects against *E. coli*, *S. aureus*, and *P. aeruginosa*. The mechanism was linked to cell membrane disruption and inhibition of bacterial enzymes. In a comparative study, Murugesu et al. [26] found that leaves possessed higher antimicrobial potential than bark and roots. Using minimum inhibitory concentration (MIC) techniques, they established that leaf extract could act as a promising agent against drug-resistant bacteria.

Antioxidant Activity

Plants are typically rich in diverse antioxidant compounds, which contribute to most of their biological effects in the human system. Antioxidants can inhibit oxidation via termination of radical reaction by donating at least one hydrogen atom to the free radical or by preventing the initiation of radical chain reaction via a substitutive reaction. Therefore, cells must preserve the levels of antioxidants through dietary and de novo synthesis [27,28].

Table 4: Antioxidant Activity (% Inhibition of DPPH)

Concentration (µg/mL)	% Inhibitory Activity		
	***Ficus religiosa* biomass**	***Ficus religiosa* biochar**	**Ascorbic Acid**
56.25	34.3	39.4	45.6
122.5	42.4	52.6	54.8
225	51.7	63.5	65.2
450	61.2	77.5	81.8
900	70.5	90.8	96.5

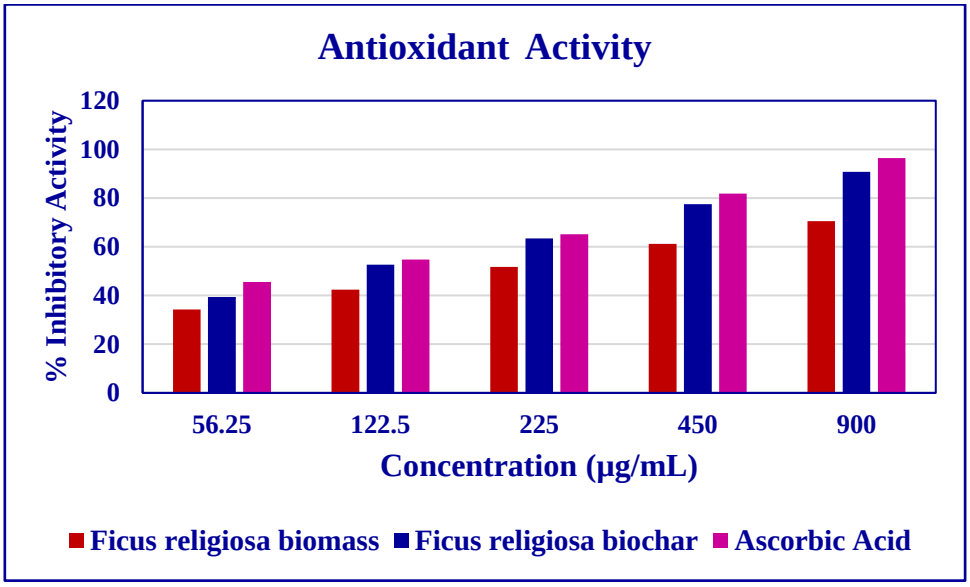

Figure 2: Antioxidant Activity of *Ficus religiosa* biomass and biochar

Table 4 and Figure 2 represent the percentage antioxidant activity of the *Ficus religiosa* leaf biomass and biochar. The results show that both the biomass and biochar have significant antioxidant activity, which increases with the increase in the concentration of the samples. The results are comparable to the standard ascorbic acid.

The ability of *F. religiosa* phytochemicals to scavenge free radicals has also been extensively researched. Using DPPH and FRAP tests, Mishra et al. [29] demonstrated a strong antioxidant capacity. Using ABTS assays, Kaur et al. [30] further confirmed these findings and connected the high flavonoid and phenolic content to antioxidant activity. Since dried leaf powder preserves these chemicals after harvest, its application has grown in popularity. Both fresh and oven-dried leaves retained antioxidant activity, albeit to differing degrees, according to Tan et al. [31]. The combined methanolic extracts of *F. religiosa* and *F. benghalensis* leaves were employed in a study. They demonstrated remarkably good DPPH scavenging action, with an inhibition percentage that was marginally greater than the standard (ascorbic acid) utilized. The fruit of the sacred fig, *F. religiosa*, contains flavonoids and phenolic compounds (1032 mg GAE/g extract and 63.31 mg QE/g extract, respectively), which are known to contribute to its strong antioxidant potential [32].

Antidiabetic Activity

Diabetes is managed by a variety of biologically influenced pathways that impact the disease's etiology. One way to lower the postprandial glucose level is to block the enzymes that hydrolyze glucose [33, 34]. This study used α-glucosidase enzyme inhibition experiments to investigate the antidiabetic benefits of both biochar and biomass from *Ficus religiosa.*

Table 5: Antidiabetic activity (% Inhibition of α-glucosidase enzyme)

Concentration (µg/mL)	% Inhibitory Activity		
	***Ficus religiosa* biomass**	***Ficus religiosa* biochar**	**Acarbose**
56.25	37.9	41.4	43.2
122.5	44.8	54.2	57.8
225	58.2	62.9	64.1
450	74.8	77.5	79.4
900	82.4	87.9	98.4

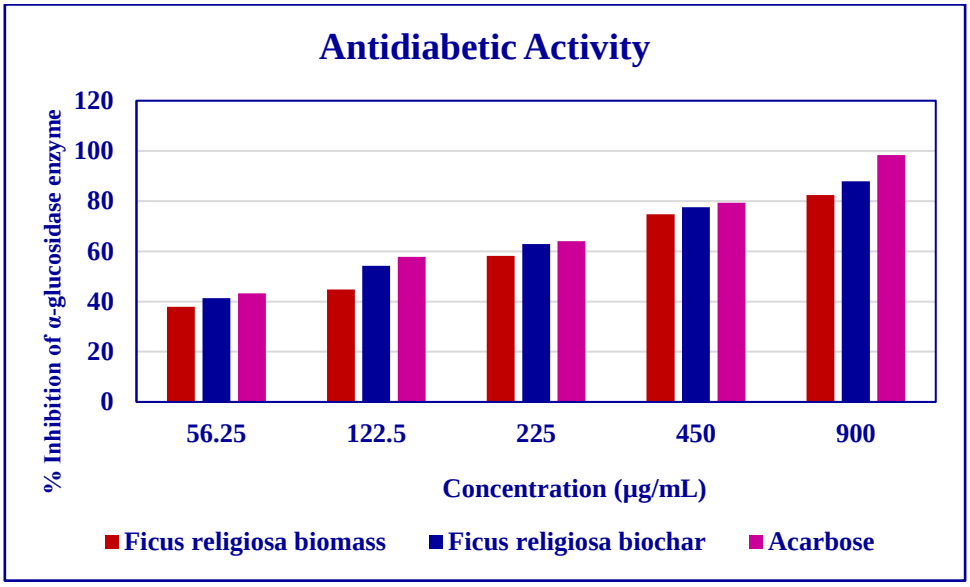

Figure 3: Antidiabetic Activity of *Ficus religiosa* biomass and biochar

The antidiabetic Activity of *Ficus religiosa* biomass and biochar was investigated as a % Inhibition of α-glucosidase enzyme, with acarbose as a standard, and presented in **Table 5 and Figure 3.** The results confirm the significant antidiabetic activity of the biochar when compared to the biomass. The results are comparable to those of the standard acarbose.

In diabetic animals, *Ficus religiosa* has consistently demonstrated hypoglycemic effects. Methanolic leaf extracts significantly decreased fasting blood glucose in rats with alloxan-induced diabetes, according to Garodia et al. [35]. Enhanced insulin secretion, decreased absorption of glucose, and inhibition of α-amylase and α-glucosidase are some of the mechanisms. A more recent study by Rajan et al. [36] examined ethanol and aqueous extracts and found that the ethanol extracts had a greater inhibitory effect on the enzymes

involved in glucose metabolism. This implies that selecting the right solvent is essential to optimizing therapeutic efficacy.

Anti-Inflammatory Activity

The healing process is triggered by inflammation, which is the body's defense mechanism against pathogens, damaged or injured cells, radiation, or poisonous substances [37]. Through a variety of pathways, inflammation is frequently linked to serious illnesses like diabetes, cancer, heart disease, etc. Numerous phytoconstituents may disrupt the processes and fight a number of inflammatory conditions [38].

Table 6: Anti-Inflammatory Activity

Concentration (µg/mL)	% Inhibitory Activity		
	***Ficus religiosa* biomass**	***Ficus religiosa* biochar**	**Diclofenac sodium**
56.25	38.6	41.7	45.2
122.5	48.3	50.6	51.4
225	58.9	61.8	64.8
450	69.7	75.9	78.6
900	89.2	92.6	98.2

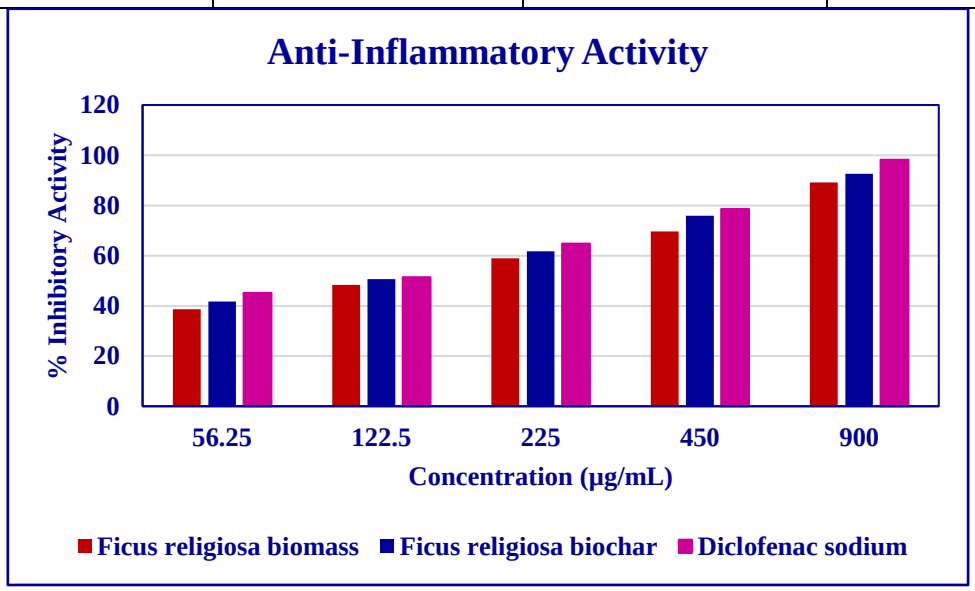

Figure 4: Anti- Inflammatory Activity of *Ficus religiosa* biomass and biochar

Anti-inflammatory studies have consistently shown promising outcomes. **Table 6 and Figure 4** represent the anti-inflammatory effect of *Ficus religiosa* biomass and biochar, with the standard as diclofenac drug. The results confirm the highly comparable anti-inflammatory effect of both the biomass and biochar of *F. religiosa* leaves, which increases with the increase in the sample quantity from 56.25µg/mL to 900µg/mL.

In rats, Singh et al. [39] showed that carrageenan inhibited paw edema. Significantly reduced levels of anti-inflammatory markers including COX-2, TNF-α, and IL-6 were seen. Gupta and Verma [40] demonstrated the ability of some phytochemicals in *F. religiosa* to bind with inflammatory mediators through the use of molecular docking. Additionally, in vitro models evaluating protein denaturation and HRBC membrane stability corroborated these computational results. Damage to the skin and other soft tissues, as well as the healing process of wounds, are among the mechanisms that entail inflammation. Injury triggers an inflammatory reaction that causes the cells beneath the dermis layer to produce more collagen. This is followed by the regeneration of epithelial tissue as a result of healing [41].

Wound Healing Property

Though less extensively studied, wound healing properties have emerged as a key area of research in the past five years. L6, the human keratinocyte cell line, is a biomarker to test the toxicity and the wound-healing properties of any material.

MTT Assay of *Ficus religiosa* biomass

24 Hrs **48Hrs** **72Hrs**

MTT Assay of *Ficus religiosa* biochar

24 Hrs **48Hrs** **72Hrs**

Figure 5: L6 Cell line viability (MTT Assay) of *Ficus religiosa* biomass and Biochar

Table 7: Cell Viability L6 Cell line

Incubation Time (Hrs)	% Cell Viability	
	***Ficus religiosa* - biomass**	***Ficus religiosa* - biochar**
24	69	81
48	88	95
72	97	100

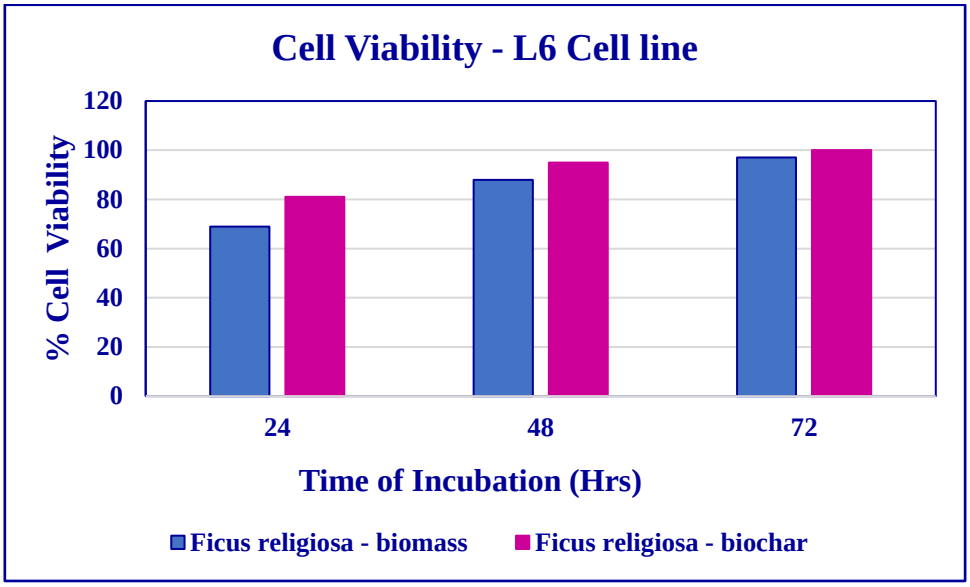

Figure 6: MTT Assay of L6 Cell line with *Ficus religiosa* leaf Biomass and Biochar

Figure 5 shows the L6 cell line morphology during MTT assay 24 hours, 48 Hrs, and 72 Hrs. The percentage viability of the L6 cell line in the *Ficus religiosa* leaf Biomass and Biochar is represented in **Table 7 and Figure 6.** The results show the non-cytotoxic nature of both the biomass and biochar for the growth of the L6 cell line. Hence, the materials are supposed to support wound healing properties.

Using rat excision and incision wound models, Nair et al. [42] found that topical treatment of Ficus religiosa leaf extract gel significantly improved wound contraction and epithelialization. Antioxidants and anti-inflammatory substances are thought to hasten the healing process. Fibroblast proliferation and collagen synthesis were studied by Saha et al. [43], which provided evidence in favor of *Ficus religiosa*'s function in fostering tissue regeneration. Cell culture, real-time polymerase chain reaction (PCR), astringent activity, and the wound healing assay were used to evaluate the wound healing qualities of aqueous extracts made from the bark, leaves, and aerial roots of *F. religiosa*. Using the bark and aerial root extracts, the metalloproteinase-1 (MMP) matrix was found to be downregulated in the PCR study. It was discovered that the bark and leaf extracts improved the area where wounds healed [44].

Conclusion

The current study aimed at investigating the qualitative phytochemical analysis of the leaves of *Ficus religiosa* and preparing the dried biomass and NaOH-activated biochar of the same. The biomass and the biochar were investigated for their antibacterial, antioxidant, antidiabetic, and anti-inflammatory properties. The studies confirmed the significant antibacterial, antioxidant, antidiabetic, and anti-inflammatory capacities of the biochar than the biomass. L6 cell line was used to test the *Ficus religiosa* biomass and biochar's cytotoxicity and the wound healing efficacy. The biochar showed better performance than the biomass in wound healing activity. Future research is needed for in vivo studies of the studied properties.

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