



Extraction, Phytochemical and Analytical Study of *Saccharum benghalense*

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ABSTRACT

Saccharum benghalense, a traditionally known medicinal shrub, lacks comprehensive scientific evaluation for its phytochemical profile and anti-inflammatory potential. This study aimed to extract, characterize, and evaluate the pharmacological activity of the methanolic extract of *Saccharum benghalense*. The whole shrub was collected from the UP-West region and authenticated (Ref. No. RU/PS/PI/2-25-31). Methanolic extraction was carried out using a Soxhlet apparatus, yielding 47.20% of dry extract. Preliminary phytochemical screening was conducted using standard methods. Bioactive compounds were isolated by Thin Layer Chromatography (TLC) and Column Chromatography. Structural identification was performed using Fourier Transform Infrared (FTIR) Spectroscopy, Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS). The anti-inflammatory activity was assessed *in vitro* using the inhibition of protein denaturation (egg albumin method) and cyclooxygenase (COX) enzyme inhibition. Phytochemical analysis confirmed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, steroids, terpenoids, and carbohydrates. The extract showed significant inhibition of protein denaturation. In COX assays, the extract exhibited 45% and 95% inhibition of COX-1 activity at concentrations of 50 µg/ml and 100 µg/ml, respectively, indicating selective COX-1 inhibition with limited COX-2 suppression. Methanolic extract of *Saccharum benghalense* demonstrated potent anti-inflammatory activity, particularly through selective inhibition of COX-1. This supports its potential for development into oral or topical anti-inflammatory formulations. Further preclinical and clinical investigations are recommended to assess safety, efficacy, and underlying molecular mechanisms.

KEYWORDS: *Saccharum benghalense*, Soxhlet extraction, TLC, FTIR, COX inhibition, anti-inflammatory activity.

1. INTRODUCTION

According to one definition, a sickness occurs when the body of a live animal or plant, or one of its sections, is not in its normal state and interferes with or alters the execution of essential duties [1]. Inflammation is a defense mechanism used by the body. The immune system uses it to recognise and get rid of harmful and unfamiliar stimuli and initiate the healing process. Inflammation can either be acute or persistent [2]. Nonsteroidal anti-inflammatory medicines (NSAIDs) are analgesic, antipyretic, and anti-inflammatory pharmaceuticals that have FDA approval. Because of these side effects, NSAIDs are used to treat a variety of conditions, including migraines, gout, chills, arthritic illnesses, muscular pain, and dysmenorrhea. In some cases of acute trauma, they are also utilised as opioid-sparing medications [3]. The suppression of the cyclooxygenase enzyme is the main way that NSAIDs work. Arachidonic acid is converted by cyclooxygenase (COX) into prostacyclins, prostaglandins, and thromboxanes. Lack of these eicosanoids is thought to be the cause of NSAIDs' therapeutic effects. Prostaglandins promote vasodilation, increase the temperature in the hypothalamus, and play a key role in anti-nociception, while thromboxanes are involved in platelet adhesion. COX-1 and COX-2 are the two cyclooxygenase isoenzymes [4].

Profile of a plant *Saccharum benghalense* is a synonym for *Tripidium bengalense*, commonly referred to as munj grass and found along riverbanks and in dry regions. The tall grass's panicles are smooth and greenish brown in hue. The grass is overgrown and reaches a height of seven feet. The leaf sheaths are villous and straight, with a pale straw color. The highest sheath may occasionally reach past the base of the panicle [5].

Taxonomy of plant

Kingdom	Plantae
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	Saccharum
Species	<i>Benghalense</i>

Saccharum benghalense exhibited a high level of presence of several moieties during the initial screening of phytoconstituents. There were significant amounts of alkaloids, terpenoids, flavonoids, phenols, coumarins, and betacyanins. In contrast, substantial amounts of cardiac glycosides, tannins, and steroids were found. It was discovered that there were no glycosides, saponins, or anthocyanins [6].

2. RESULTS AND DISCUSSION

Based on its practical yield, the *Saccharum benghalense* methanolic extract showed a percentage yield of 47.20%. During the initial screening of phytoconstituents, *Saccharum benghalense* showed a high degree of presence of several moieties. Alkaloids, terpenoids, flavonoids, phenols, coumarins, and betacyanins were present in considerable concentrations. On the other hand, significant levels of tannins, steroids, and cardiac glycosides were detected. There were no saponins, glycosides, or anthocyanins. Here are the phytoconstituents that *Saccharum benghalense* displayed:

Table 1: Phytochemicals in *Saccharum benghalense* extract

S.No.	Phytoconstituents	Methanolic extract of <i>Saccharum benghalense</i>
1	Alkaloids	++
2	Glycosides	-
3	Cardiac glycosides	++
4	Tannins	+
5	Saponins	-
6	Terpenoids	++
7.	Steroids	+
8.	Flavonoids	++
9.	Phenols	+
10.	Coumarins	+
11.	Anthocyanin	-
12.	Betacyanin	++

Absent (-), Present (+), Abundance (++)

Isolation of bioactive compounds

- TLC analysis of *Saccharum benghalense* extract

In the methanol: ethyl acetate (7:1.5) *Saccharum benghalense* extract, the R_f value was found to be 0.63. The 70:30 ratio of methanol to ethyl acetate Methanol: ethyl acetate (30:70) and the R_f value of 0.72 were displayed by the *Saccharum benghalense* extract. The R_f value of the extract from *Saccharum benghalense* was 0.84.

Table 2: TLC analysis of *Saccharum benghalense* extract

Herbal extract	Solvent system	R_f Value
<i>Saccharum benghalense</i> extract	Methanol + ethyl acetate (7:1.5)	0.63
	Methanol + ethyl acetate (70:30)	0.72
	Methanol + ethyl acetate (30:70)	0.84

- Column chromatography

Table 3: Column chromatography of *Saccharum benghalense* extract

Solvent Polarity	Solvent mixture/ Ratio (ml)	Fraction Color
0.50	n-hexane + methanol (90:10)	White
0.60	n-hexane + methanol (80:20)	Colorless
0.70	n-hexane + methanol (70:30)	White
0.78	n-hexane + methanol (60:40)	Colorless
0.86	n-hexane + methanol (50:50)	Colorless

Structural Characterization

- FTIR Spectroscopy of *Saccharum benghalense*

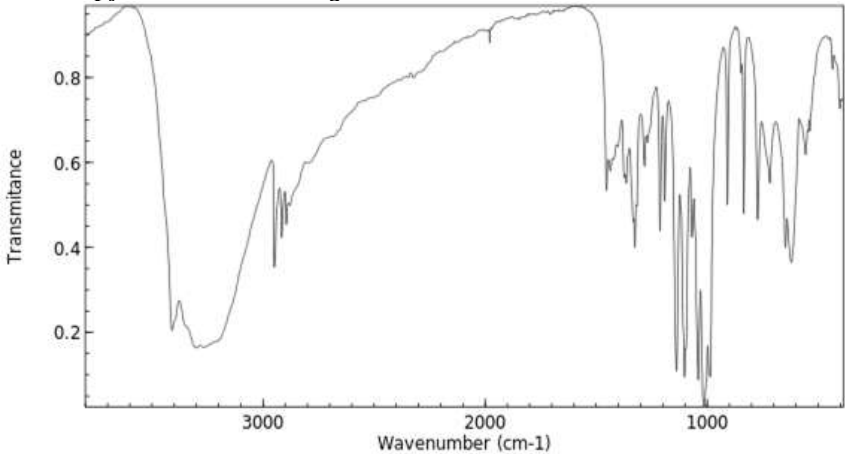


Figure 1: FTIR Spectrum of methanolic extract of *Saccharum benghalense*

FTIR study of *Saccharum benghalense*'s methanolic extract showed the presence of several functional groups that are suggestive of phytochemical components. Around 3400–3420 cm^{-1} , a large absorption band occurred, which is shown of O–H str. Vibrations. Presence of O–H group commonly highlights alcohols and phenolic compounds. Alkanes' C–H stretching vibrations are responsible for peaks in the 2920–2940 cm^{-1} range, which show the existence of saturated hydrocarbons. The existence of carbonyl-containing substances, such as carboxylic acids, esters, or ketones, is confirmed by the peak that appears at 1710–1740 cm^{-1} and in accord to the C=O str. vibration. The presence of flavonoids or tannins is indicated by a strong peak at 1600–1620 cm^{-1} , which is caused by C=C stretching in aromatic rings. N–O stretching is attributed to absorption bands at 1510–1540 cm^{-1} , which could be connected to nitro groups or compounds that resemble alkaloids. While the band at 1030–1070 cm^{-1} shows C–O–C str. vibrations typical of glycosides or polysaccharides, graph near 1240–1270 cm^{-1} in accord to C–O str. of ethers or phenolic compounds. Furthermore, bands that show up at 600–700 cm^{-1} might be the result of aromatic structures' C–H out-of-plane bending vibrations. By verifying the presence of multiple bioactive functional groups in the extract, including phenols, flavonoids, tannins, alkaloids, and glycosides, these results support the plant's potential medicinal properties.

NMR Spectroscopy of *Saccharum benghalense*

Multiple proton signals were detected in the ^1H NMR spectra of *Saccharum benghalense*'s methanolic extract, suggesting the existence of a complex mixture of phytoconstituents. Broad signals that show up in the δ 0.8–1.5 ppm range are ascribed to the aliphatic alkyl chains and fatty acids. Methylene groups next to carbonyls or unsaturated systems are shown as peaks in the δ 2.0–2.5 ppm range, which may indicate the existence of keto or esters. Protons on carbons bonded to oxygen are characterised by signals in the δ 3.2–4.5 ppm area; these are commonly found in sugars, glycosides, or alcohols. Multiple signals corresponding to aromatic and olefinic protons were observed in the aromatic area (δ 6.0–8.0 ppm), suggesting the presence of tannins, flavonoids, or other phenolic compounds. A broad singlet around δ 9.0–12.0 ppm indicates phenolic –OH or carboxylic acid protons.

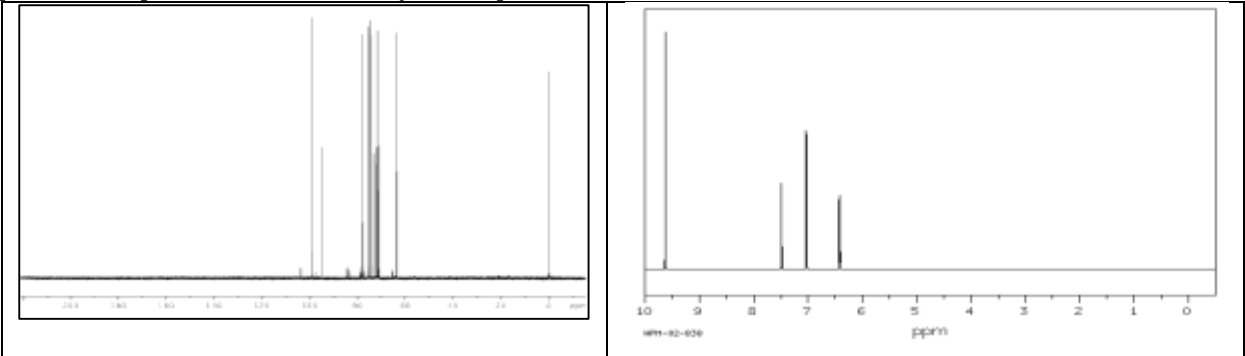


Figure 2: ¹HNMR & ¹³CNMR Spectrum of extract of *Saccharum benghalense*

Signals in the δ 15–40 ppm region in the ¹³C NMR spectrum indicate the existence of aliphatic carbons. Glycosides and sugars include oxygenated carbons, which are identified by peaks between δ 60 and 80 ppm. Aromatic and olefinic carbons are represented by signals at δ 100–130 ppm, while carbonyl carbons (esters, carboxylic acids, or flavonoid C=O groups) are identified by signals around δ 160–180 ppm.

The existence of numerous phytoconstituents, including as sugars, flavonoids, phenolic acids, tannins, and glycosides, which may support the plant's pharmacological potential, is confirmed by these spectrum features.

• **Mass spectroscopy of *Saccharum benghalense***

Mass spectra for different active constituents are as follows:

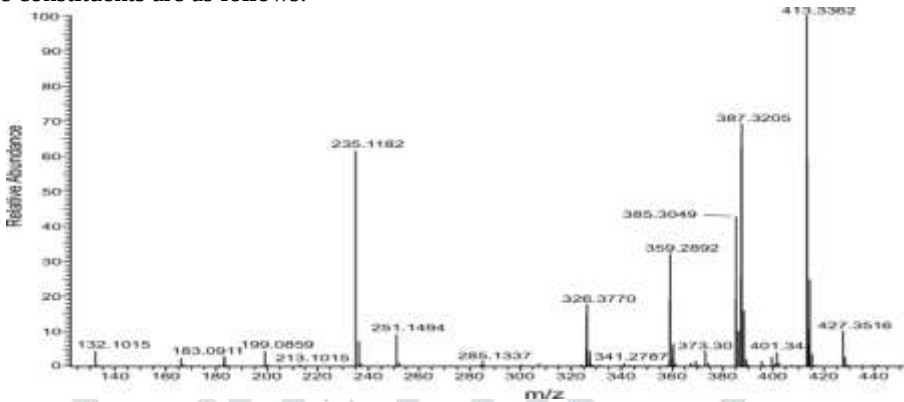


Figure 3:. Mass Spectrum of *Saccharum benghalense*

Saccharum benghalense's methanolic extract showed a complicated fragmentation pattern in the mass spectrometric analysis, suggesting the presence of several bioactive phytochemicals. Across a broad m/z range, prominent peaks were seen, indicating the constituents' molecular diversity. Simple phenolic compounds or tiny flavonoids are suggested by a significant peak in the m/z 150–180 area, whereas flavonoid glycosides or tannins may be represented by peaks at m/z 270–310. The presence of bigger glycosidic chemicals, triterpenoids, or saponins is indicated by signals in the m/z 350–500 range. The most prevalent ionised component in the extract is indicated by the existence of a base peak (highest intensity) at a specific m/z value.

Pharmacological evaluation

• Using egg albumin to inhibit (%) protein denaturation: The percentage of protein denaturation inhibition for *Saccharum benghalense* methanolic extract at different concentrations—12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml, respectively was assessed using egg albumin. At 200 µg/ml, 400 µg/ml, and 800 µg/ml, the methanolic extract of *Saccharum benghalense* demonstrated suppression of protein denaturation of 79.3±0.2%, 84.6±0.5%, and 93.7±0.1%, respectively, using egg albumin.

Table 4: Inhibition (%) of protein denaturation with *Saccharum benghalense* methanolic extract and egg albumin

Treatment	Using egg albumin to inhibit (%) protein denaturation [Conc. (µg/ml)]						
	12.5	25	50	100	200	400	800
Methanolic extract of <i>Saccharum benghalense</i>	41.9±0.7	58.2±0.3	67.9±0.1	71.6±0.8	79.3±0.2	84.6±0.5	93.7±0.1

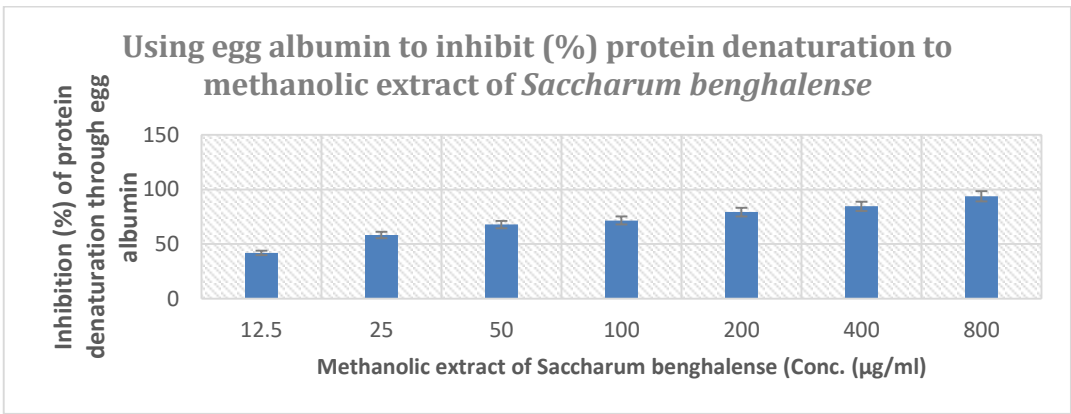


Figure 5: Graphical data of Using egg albumin to inhibit (%) protein denaturation of *Saccharum benghalense* extract

• **COX Activity Test**

COX-1 and COX-2 were identified by the COX activity test. 100% inhibition was seen with 1µM Diclofenac. The extract from *Saccharum benghalense* showed 45% and 95% inhibition of COX-1 at concentrations of 50µg/ml and 100µg/ml, respectively. *Saccharum benghalense* extract, on the other hand, showed 6% and 22% suppression of COX-2 at concentrations of 50µg/ml and 100µg/ml, respectively. *Saccharum benghalense* is therefore less effective in inhibiting the COX-2 enzyme and more selectively inhibits the COX-1 enzyme.

.Table 5: COX activity test of *Saccharum benghalense* extract

Enzyme	Source	% inhibition of diclofenac (1µM)	% Inhibition of <i>Saccharum benghalense</i> extract	
			50µg/ml	100µg/ml
COX-1	Ovine platelets	100.0	45	95
COX-2	Human recombinant	100.0	6	22

The ESR count in arthritic rats was dramatically lowered by *Calliobotrys* extract and all of its constituent parts in comparison to aspirin B. This suggests the significance of this plant in the management of arthritic conditions. The autoantibody RF, which targets the Fc region of IgG, is a significant serologic marker. Over time, it helps rheumatoid arthritis advance by forming immune complexes. Using *calliobotry* to treat RA. These haematological findings suggest that B. might be the contender. A debilitating consequence of rheumatoid arthritis, rheumatoid cachexia is characterised by muscle atrophy or weight loss [7].

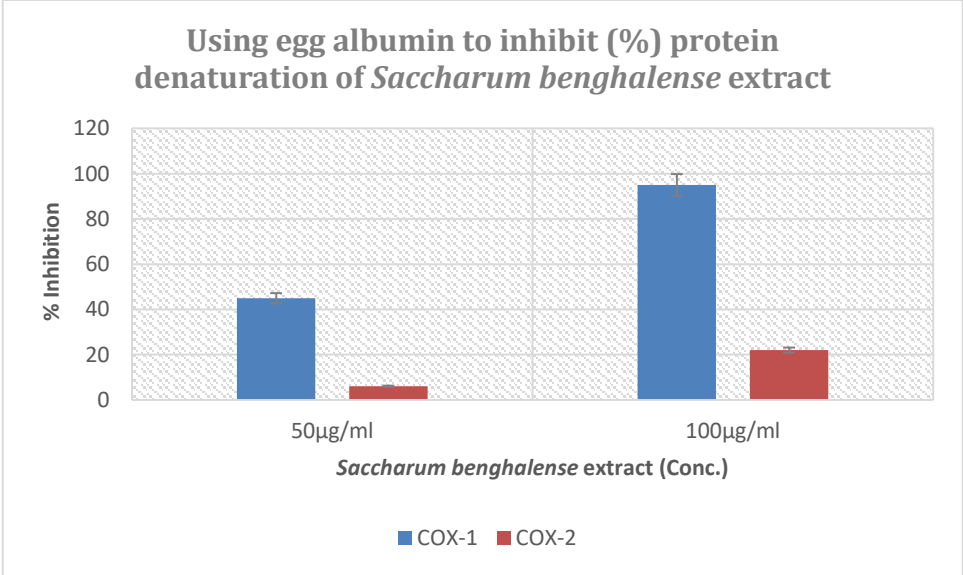


Figure 6: Graphical data of COX activity test of *Saccharum benghalense* extract

The effects of *B. calliobotrys* that prevent muscle atrophy can be inferred from the outcomes of the in vitro antiarthritic membrane stabilisation technique. Similarly, cytokine-mediated hypermetabolism is believed to be the aetiology of rheumatoid cachexia. Furthermore, previous studies have shown that anti-inflammatory medications can restore the decrease in 14C-glucose and 14C-leucine absorption in rats' stomachs caused by inflammation. The current study's findings demonstrate that the B aqueous fraction, n-butanol fraction, and methanolic extract. For rats with arthritis, the preventative impact of *calliobotrys* on body weight is especially advantageous. Additionally, the effectiveness of the B treatment was demonstrated by histology slides of the ankle joints. Between the drug-receiving groups and the negative control, there was no indication of ankle joint deterioration. Thus, the antiarthritic action of B has been confirmed by histological examinations. *Calliobotrys* reduces the inflammatory response, presumably as a result of inhibiting the cyclooxygenase enzyme and pro-inflammatory cytokines. Many plants in the genera *Berberis* and *Coptis* contain the pharmacologically powerful isoquinoline alkaloid berberine. Berberine has been demonstrated to have immunosuppressive and anti-inflammatory effects in a range of autoimmune illnesses by blocking Th17 and dendritic cell responses. Additionally, by preventing the most common causes associated with arthritis, berberine helps to reduce joint inflammation and severe pain [8]. The several active ingredients, including furfural, D-glucose, and D-galactose, from the methanolic extract of *Saccharum benghalense* were identified and characterised using FTIR, NMR, and mass spectroscopy.

Additionally, it was shown that at a dosage of 800 µg/ml, the percentage inhibition of protein denaturation using egg albumin was 93.7±0.1%. At 50µg/ml and 100µg/ml, respectively, the *Saccharum benghalense* extract inhibited COX-1 45% and 95%. It can be concluded that *Saccharum benghalense* inhibits the COX-1 enzyme selectively, while inhibiting the COX-2 enzyme less effectively.

3. MATERIALS AND METHODS

Collection, Identification, and Extraction of Plant Extract

A qualified botanist verified the authenticity of the entire *Saccharum benghalense* bush, which was gathered from the western part of Uttar Pradesh (UP-West), India. After giving the plant material a good cleaning to get rid of any dust, it was allowed to dry at room temperature in the shade. After drying, the material was first ground into a coarse powder and then ground even finer. Methanol was used to do a Soxhlet extraction on this powdered plant material. A rotary evaporator was used to concentrate the resultant extract, and conventional methods were followed to determine the percentage yield of the methanolic extract [9].



Figure 7: Soxhlet Extraction Process for *Saccharum benghalense*

Preliminary Phytochemical Screening

Phytochemical constituents were identified using standard procedures [10,11].

- **Alkaloids:** Mayer's Test: Alkaloids were present when a yellow precipitate formed following treatment with Mayer's reagent. Wagner's Test: Alkaloids are confirmed by a reddish-brown precipitate following a reaction with Wagner's reagent. Hager's Test: Alkaloids were confirmed by yellow precipitate after treatment with Hager's reagent.
- **Glycosides:** Fehling's Test: The presence of glycosides was indicated by the formation of a brick-red precipitate following heating with Fehling's solution.
- **Saponins:** Foam Test: Persistent froth after vigorous shaking suggested the presence of saponins.
- **Tannins:** Ferric Chloride Test: Tannins were suggested by a blue-black or green-black coloring. Lead Acetate Test: Tannins are confirmed by the formation of a white precipitate.
- **Flavonoids:** NaOH Test: Development of yellow-orange color upon treatment with NaOH and HCl confirmed flavonoids. H_2SO_4 Test: Orange coloration after treatment with concentrated sulfuric acid suggested flavonoids.
- **Terpenoids:** Grey coloration upon reaction with chloroform and concentrated H_2SO_4 indicated terpenoids.
- **Steroids:** Red coloration in the chloroform layer after addition of H_2SO_4 suggested steroids.
- **Carbohydrates:** Molisch's Test: Carbohydrates are confirmed by a violet ring at the interface with the addition of α -naphthol and H_2SO_4 . Fehling's Test: Reducing sugars were verified by the formation of a brick-red precipitate.

Isolation of Bioactive Compounds

Thin Layer Chromatography (TLC): Fine capillaries were used to apply a methanolic plant extract on TLC plates that had already been coated. The components of the mobile phase were acetone, ethyl acetate, and chloroform (7:1.5:1.5). Following development, plates were heated to 115°C for five minutes, sprayed with anisaldehyde-sulfuric acid reagent, and then dried and visualised. Spot counts, colours, and R_f values were recorded.

Column Chromatography: A slurry of silica gel (60–120 mesh) in n-hexane was put into the column. After being adsorbed onto silica, 10g of methanolic extract was put into the column. Gradient mixes of n-hexane and ethyl acetate (100:0 → 50:50) were used for elution. TLC was used to track the eluates using n-hexane:ethyl acetate (70:30).

Structural Characterization of Isolated Compounds

- **Infrared Spectroscopy:** To identify the functional groups of the isolated compounds, IR spectra were captured. Unique identification was obtained by the fingerprint area (1300–650 cm^{-1}) [12].
- **Spectroscopy using nuclear magnetic resonance (NMR)** To clarify the chemical structure, 1H -NMR spectra were captured using a Bruker Ultraspec 500MHz/AMX400MHz spectrometer with $CDCl_3$ and $DMSO-d_6$ as solvents [13].
- **Spectrometry by mass (MS)** A mass spectrometer was used to measure the ionised fragments' mass-to-charge ratio (m/z). To ascertain molecular weight and structural details, fragmentation patterns and molecular ion peaks were employed [14].

Pharmacological Evaluation

Inhibition of Protein Denaturation (Egg Albumin Method):

The inhibition of protein denaturation method was used to evaluate anti-inflammatory efficacy. 0.2 ml egg albumin, 2.8 ml phosphate-buffered saline (pH 6.4), and 2 ml of methanolic extract or diclofenac sodium at doses ranging from 12.5 to 800 $\mu g/ml$ were all present in the reaction mixture (5 ml). An same amount of distilled water was used to create a control. After 15 minutes of incubation at $37 \pm 2^\circ C$,

the samples were heated for five minutes at 70°C. At 660 nm, absorbance was measured. The following formula was used to get the percentage inhibition:

$$\% \text{ Inhibition} = [(Abs \text{ control} - Abs \text{ sample}) / Abs \text{ control}] \times 100 \text{ [15,16].}$$

Cyclooxygenase (COX) Inhibitory Assay:

A commercial COX inhibitor assay kit (Cayman Chemical, Kit No. 560131) was used to measure the inhibitory activity of COX-1 and COX-2. 100% DMSO (10 mg/ml) was used to create the methanolic extract, which was then evaluated at 30 and 100 µg/ml. As the standard, diclofenac sodium was used. After 15 minutes of enzyme preincubation at 37°C, 10 µl (10 mM) of arachidonic acid was added to each reaction mixture. After two minutes, 50 µl of 1N HCl and stannous chloride were used to terminate the process. Enzyme immunoassay (EIA) spectrophotometry was used to quantify prostaglandin F2α [17].

4. CONCLUSION

The current investigation demonstrates that *Saccharum benghalense* methanolic extract has the potential to be an effective anti-inflammatory agent. With few known adverse effects, the extract provides a reasonably priced and maybe safer substitute for traditional synthetic anti-inflammatory medications due to its plant origin. Comprehensive preclinical and clinical research are necessary to assess the effectiveness, pharmacokinetics, and safety profile of the preliminary findings. Furthermore, it is essential to clarify the cellular and molecular processes that underlie its anti-inflammatory properties. Following validation, the extract could be developed into appropriate topical or oral dose forms for use in the treatment of long-term inflammatory diseases such rheumatoid arthritis.

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Ethics and Consent: Nil

Conflicts of interest: None

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