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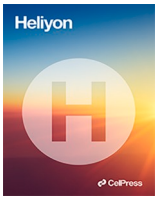

Research article

# Antibacterial, antidiabetic and antioxidant bioevaluation of *Calamus leptospadix* Griff. and isolation of a flavan type compound

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ABSTRACT

The plant based natural products have always been a rich source of bioactive molecules for drug discovery. The tender shoots of *Calamus leptospadix* Griff., an edible medicinal plant was extracted using methanol, water and ethanol as three different solvents to study the effect of the extracting solvents and temperature on their antioxidant, antidiabetic and antibacterial properties and total phenolic and flavonoid contents. The antioxidant properties were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) assay. The α-glucosidase inhibitory assay was carried out to determine the antidiabetic potential. The antibacterial properties of the extracts were determined against four strains of bacterial species viz. *Bacillus subtilis*, *Streptococcus pneumoniae*, *Escherichia coli* and *Citrobacter freundii* using Broth macro dilution method.

The methanolic extracts of the plant were found to possess the highest total phenolic and total flavonoid contents. In the antioxidant assays, the cold methanolic extract was found to exhibit the highest DDPH radical scavenging activity and ferric-reducing antioxidant power. In the antidiabetic assay, the extract exhibited better α-glucosidase inhibitory potential than that of the positive control acarbose. It was also found to be effective against both gram-positive and gram-negative strains in the antibacterial assay. A flavan-type compound 4-(5,7-dimethoxychroman-2-yl)phenol was isolated from the most bioactive cold methanolic extract of the plant and characterised from its XRD, $^{1}H$ and $^{13}C$ NMR, HRMS and IR data.

## 1. Introduction

All over the world, millions of individuals of all ages suffer from diabetes mellitus, a chronic metabolic disease. It is predicted that by 2030, 643 million people will be affected by this global burden of diabetes mellitus [1–3]. The current therapies available for the treatment of diabetes include the administration of insulin and oral agents like metformin, insulin secretagogues, glucosidase inhibitors, peroxisome proliferator-activated receptor gamma (PPARγ) activators, dipeptidyl peptidase-4 (DPP4) inhibitors, etc. However, most of these are expensive and associated with serious side effects and long-term complications such as hypoglycaemia, weight gain, gastrointestinal discomfort and diarrhoea [4–7]. Like diabetes mellitus, antibacterial resistance is also one of the greatest threats

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to humankind which defuncts the conventional antibiotics [8]. Failure or underperformance of conventional therapeutics compels the researchers to take clues from the recognised traditional medicinal systems like Ayurveda and traditional Chinese medicine and tend towards natural products for the discovery of new lead molecules [9–11].

Arunachal Pradesh-the largest northeastern state of India is located in the Eastern Himalayas-a biodiversity hotspot that is home to about 5800 plant species out of which roughly 2000 are endemic [12]. *Calamus leptospadix* Griff. a plant of the genus *Calamus*-the largest genus of rattan-comprising about 370 species belonging to the family of Arecaceae and sub-family Calamoideae is native to India (Assam and the Indian part of Eastern Himalaya), Bangladesh, Myanmar and Nepal [13]. *C. leptospadix* is known as *lejaibet* in the North East Indian state of Assam and as *jeying* in the East Siang district of Arunachal Pradesh. The tender shoots of *C. leptospadix* are used as vegetables in this region [14]. Many species of the *Calamus* are of economic importance as they find widespread application in the cottage industries in making furniture, fancy items and basketry [15]. The sheath-removed stem of these plants is known as cane which is the raw material for the cottage industries for its lightness, durability and flexibility [16]. The shoot extracts of *C. leptospadix* have been reported to possess antimicrobial [17], antioxidant [18], anthelmintic [19] and antidiabetic [20] properties. Food plants with medicinal properties are often advantageous for any kind of application in healthcare because of their lower or zero risk of toxicity. Emerging research indicates oxidative stress as a significant factor in the development of diseases like diabetes and cardiovascular ailments [21,22]. As such, dietary intake of antioxidant rich foods can be helpful in the management of the diseases like diabetes. The promising bioactivities and its applicability as a food make *C. leptospadix* a potential candidate for antidiabetic drug discovery studies.

The nature of compounds that get extracted from the plant biomass depends on the nature of the extraction solvent and the applied temperature and pressure. The use of solvents of different polarities and molecular sizes helps understand their effect on the phytochemical profile and bioactive properties. Small polar alcohols are advantageous because of their penetrating strength when it comes to the effectiveness of extracting plant secondary metabolites from the matrix. In general, water and alcoholic solvents such as methanol and ethanol are preferred for this purpose. Aqueous extraction in the case of food plants is typically preferred because the molecules within the cellular milieu will be in an aqueous medium. Non-polar compounds are not easily extracted by water at normal temperatures. However, at high temperatures, their dielectric constant falls and it may be more effective in extracting non-polar compounds [23]. Further, in the case of food plants that are used in cooked form, aqueous extraction under hot conditions enables the assessment of the effect of cooking on the bioactive potential. In continuation of our work on medicinally and economically weighty plants of North East India [23–27], we carried out the extraction of the edible tender shoots of *C. leptospadix* in three different solvents viz. water, ethanol and methanol under hot and cold conditions and determined their antimicrobial, antidiabetic and antioxidant properties. We further attempted the isolation and characterization of the active principles from the methanolic cold extract of the plant which was found to be the most active in the bioactivity studies. This resulted in the isolation of one flavan-type compound (Fig. 1), however the same was not found to possess any potential antidiabetic or antimicrobial properties.

## 2. Materials and methods

### 2.1. Chemicals, reagents and instruments

Sodium phosphate monobasic, sodium phosphate dibasic, sodium carbonate, ferric chloride, potassium persulphate, TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine), aluminium chloride, α-glucosidase, α-amylase, Folin-Ciocalteu reagent, DPPH, potassium iodide, rutin and *p*-nitrophenyl-α-D-glucopyranoside were bought from Sisco Research Laboratories Pvt. Ltd. located in Maharastra, India. Starch, ascorbic acid, glacial acetic acid, silica gel of different mesh sizes, sodium acetate, sodium bicarbonate and hydrochloric acid were procured from Avantor Performance Material India Pvt. Ltd., (Maharastra, India). Luria Bertani broth, Miller was purchased from HiMedia Leading Bio Sciences Company, Mumbai India. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, iodine and ABTS were purchased from TCI (Tokyo Chemical Industry Co. Ltd., Japan). Potassium phosphate dibasic, potassium phosphate monobasic and ferrous sulphate heptahydrate were purchased from Sigma-Aldrich (St. Louis, USA). Bacterial strains viz.,

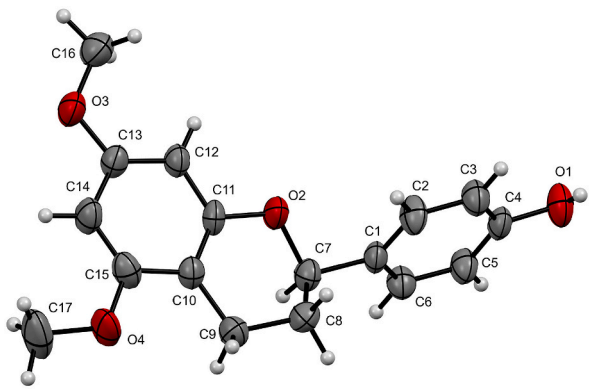


**Fig. 1.** ORTEP diagram of 4-(5,7-dimethoxychroman-2-yl)phenol with 50% probability ellipsoid.

*Bacillus subtilis* (MTCC 1134), *Streptococcus pneumoniae* (MTCC 1935), *Escherichia coli* (MTCC 40) and *Citrobacter freundii* (MTCC 3105) were purchased from microbial type culture collection and gene bank (MTCC), Chandigarh, India.

Aqueous extracts were lyophilized in a FreeZone 2.5 L, −84 °C benchtop freeze dryer (Labconco Corporation, USA). A multimode reader spectrophotometer (Multiskan Go) manufactured by ThermoFisher Scientific, USA was used to record the UV–vis spectra. $^{1}$H- and $^{13}$C- NMR spectra were recorded in a 600 MHz spectrophotometer (Bruker ASCEND 600) using tetramethylsilane (TMS) as the internal standard. The high-resolution mass spectrometry (HRMS) and the liquid chromatography-mass spectrometry (LCMS) data were recorded using a Thermo Scientific Orbitrap Exploris 120 spectrometer and an Agilent 6125 SQ LCMS system, respectively. SCXRD data were collected on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromator. FT-IR spectra were recorded using a Thermo Nicolet 380 spectrophotometer. The melting point was determined using BUCHI M − 560 apparatus.

### 2.2. Plant material

The tender shoots of the plant were collected from Sille village of East-Siang district (28°2′ 23.99″N and 95°20′59.99″E) of Arunachal Pradesh on 18$^{th}$ Feb 2020. The plant was identified by one of the authors (TP) of this manuscript and a voucher specimen was deposited at the herbarium of Rajiv Gandhi University with accession number HAU/AN-1943.

### 2.3. Extraction

The plant material was washed thoroughly with water, cut into small pieces and allowed to dry in shade. After grinding with a mechanical grinder to fine powder form, the dried plant materials were stored in air-tight containers until further use. Five distinct extracts were made using three solvents viz; methanol, 95% ethanol and water. Methanol and 95% ethanol were used in both hot and cold conditions and water in hot conditions. Each cold extract was prepared by macerating 20 g dried powder with 200 mL of the respective solvent in a 500 mL conical flask for 12 h. A sintered funnel was used to filter the contents, and the filtrate was subjected to centrifugation at 7000 rpm for 10 min. The solvent was evaporated over a rotary evaporator to obtain the extract. The procedure was repeated thrice and the residual water was removed from the combined extract using a lyophiliser.

For the preparation of ethanol and methanol hot extracts, 20 g dried powder was taken in a 500 mL round bottom flask and refluxed with 200 mL of the respective solvent for 12 h. This was followed by filtration, centrifugation and evaporation as described above.

To make the hot water extract, 20 g of the finely ground plant material and 300 mL of distilled water were boiled together for 2 h in a wide mouth flask of 1000 mL over a heating mantle. After cooling to room temperature the contents were filtered using a sintered funnel followed by centrifugation of the filtrate at 7000 rpm for 10 min. The supernatant liquid was then kept in a deep freezer at −20 °C overnight to solidify and lyophilized for 48 h to get the extract. A total of five extracts of the plant viz., methanol cold (CL-MC), methanol hot (CL-MH), ethanol cold (CL-EC), ethanol hot (CL-EH) and water (CL-W) were prepared.

### 2.4. Determination of total phenolic content

The Folin Ciocalteu (FC) method with certain modifications was used to determine the total phenolic content of the five different extracts [28–30]. Gallic acid was used as a standard for this assay. Extract solutions for the assay were prepared in methanol or water depending on their solubility with a concentration of 10 mg mL$^{-1}$ and gallic acid solutions were prepared in methanol with different concentrations (15.625, 31.25, 62.5, 125, 250, 500 and 1000 μg mL$^{-1}$). In a 96-well microplate, 20 μL of the extract/standard solution was mixed with 100 μL of FC reagent and incubated for 5 min. After the incubation period, to each well containing the mixture, 80 μL of 7.5% $NaHCO_3$ solution was added and incubated for an additional 30 min at room temperature. The absorbance was measured at wavelength 765 nm. A standard gallic acid calibration curve was used to calculate the total phenolic content of each extract. The results were shown in terms of mg gallic acid equivalent per gm of extract.

### 2.5. Determination of total flavonoid content

Aluminium chloride colorimetric assay was used to determine the total flavonoid content of the extracts following a reported procedure with rutin as the standard [31,32]. Rutin solutions with different concentrations (2000, 1500, 1000, 500 and 250 μg mL$^{-1}$) were prepared in methanol. All five extract solutions were prepared in water or methanol as per their solubility with a concentration of 10 mg mL$^{-1}$. In a 96-well plate, 25 μL of either the rutin solution or extract solution and 100 μL double distilled water were mixed to begin the assay. After 5 min, 15 μL of 10% $AlCl_3$ (w/v) solution was added to each well and incubated for 5 min and 50 μL NaOAc solution (1 M) was added to each well followed by 50 μL of double distilled water. After incubation for 40 min at room temperature, the absorbance of each reaction mixture was measured at 405 nm. Using a standard rutin calibration curve, the total amount of flavonoid present in each of the extracts was determined and expressed as milligram rutin equivalent per gm of extract.

### 2.6. Determination of antioxidant properties

#### 2.6.1. DPPH inhibitory assay

The DPPH free radical scavenging assay was carried out using Locatelli et al.'s methodology with some modifications [33]. The assay was carried out by taking 100 μL of the standard (ascorbic acid) or the extract with different concentrations (2000, 1500, 1000, 500, 250 and 125 μg mL$^{-1}$) in a 96-well plate. To every single well, 200 μL of a 0.1 mM DPPH methanolic solution was added. The

mixtures were gently shaken and incubated for 30 min at room temperature in the dark. This was followed by the recording of the absorbance at 517 nm. The percentage inhibition of the DPPH radical scavenging activity was calculated using equation (1). The $IC_{50}$ values were determined from the logarithmic plot of the extract's concentration vs the percentage inhibition at that concentration.

$$\%\ \text{Inhibition} = \left(\frac{OD_{control} - OD_{sample}}{OD_{control}}\right) \times 100 \quad (1)$$

Where, $OD_{control}$ = absorbance of control – absorbance of blank.

$OD_{sample}$ = absorbance of sample – absorbance of blank.

A mixture of methanol and DPPH solution was used as the control for ethanolic and methanolic extracts. The mixture of water and DPPH solution was used as the control for water extracts.

### 2.6.2. ABTS assay

The $ABTS^{\bullet+}$ radical scavenging assay was conducted using the protocol described by Ozgen et al. [34]. An ABTS stock solution of 7 mM was prepared for this by dissolving 3.8 mg ABTS in 1 mL of 10 mM sodium phosphate buffer of pH 7.4. Dilution of the ABTS solution was done using appropriate volumes of a potassium persulphate solution (2.5 mM) made in the same phosphate buffer to prepare the ABTS solutions of the required concentrations. After that, this mixture was left at room temperature for 16–18 h in the dark. By reducing the concentration of the $ABTS^{\bullet+}$ solution with phosphate buffer, the absorbance was calibrated to occur in the range of $0.70 \pm 0.02$ at wavelength 734 nm. Trolox was used as the standard antioxidant for the experiment at various concentrations (1000, 800, 600, 400, and 200 μM). The experiment was then carried out in a 96-well plate by combining 200 μL of $ABTS^{\bullet+}$ solution with 20 μL of different concentrations of Trolox or the extracts (1000, 500, 250, 125, 62.5, 31.2, and 15.625 μg $mL^{-1}$). After a gentle shake using a vortex, the mixture was further incubated for 5 min in the dark. 200 μL of $ABTS^{\bullet+}$ solution was combined with 20 μL of distilled water/methanol to be used as the control for the test. The absorbance values for all the samples were measured in triplicate. The inhibition percentage of the $ABTS^{\bullet+}$ radical by the extracts or the standard was estimated using equation (1). The $IC_{50}$ values were determined from the logarithmic plot of the extract's concentration vs the percentage of inhibition at that concentration.

### 2.6.3. FRAP assay

The Benzie and Strain method was used to conduct the ferricreducing antioxidant power (FRAP) assay [35]. For the assay, 300 mM sodium acetate buffer (pH = 3.6), 10 mM TPTZ solution prepared in 40 mM HCl, and 20 mM ferric chloride solutions were combined in a 10:1:1 ratio to create the standard FRAP solution. The pale yellow-orange FRAP solution was used within 2 h of preparation. Several concentrations of iron (II) sulphate solution (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8 and 3.9 μM) were made to obtain the standard calibration curve. The experiment was performed in a 96-well plate adding 200 μL of FRAP solution to 10 μL of different concentrations of freshly prepared iron (II) sulphate heptahydrate and the plant extracts or the ascorbic acid (standard for the experiment). The contents of the plate were thoroughly mixed before being incubated at 37 °C for 4 min. The absorbance was measured at wavelength 593 nm in the multimode reader prewarmed to 37 °C. Equation (2) was used to calculate the FRAP values of ascorbic acid and the extracts.

$$\text{FRAP} = \frac{\text{Absorbance of ascorbic acid or sample extract}}{\text{Absorbance of Iron(II) standard}} \times \text{Iron(II)standard concentration } (\mu M) \quad (2)$$

## 2.7. Antidiabetic properties

### 2.7.1. α-Glucosidase inhibitory activity

The procedure outlined by Goswami et al. was used to assess the extracts' ability to inhibit α-glucosidase [23]. Extract and standard (acarbose) solutions for this assay were prepared in distilled water and DMSO/water (2:8) depending on their solubility. A 0.1 M potassium phosphate buffer (pH = 6.8) was used to prepare the α-glucosidase (1 U $mL^{-1}$) solution. A 96-well plate containing 120 μL of the extract/standard at various concentrations was combined with 20 μL of α-glucosidase solution. The mixture was then incubated at 37 °C for 15 min and 20 μL of *p*-nitrophenyl-α-D-glucopyranoside (5 mM) solution was then added to start the reaction. The reaction mixtures were kept for an additional 15 min of incubation. To terminate the reaction 80 μL sodium carbonate (0.2 M) was added to each well and the absorbance was recorded at 405 nm. To determine the % inhibition of α-glucosidase, equation (3) was used.

$$\%\ \text{Inhibition of } \alpha - \text{glucosidase} = \left(\frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}}\right) \times 100 \quad (3)$$

## 2.8. Antibacterial study

The antibacterial properties of the *C. leptospadix* extracts were determined against two Gram-positive strains of bacterial species viz. *Bacillus subtilis* and *Streptococcus pneumoniae* and two Gram-negative strains of bacterial species viz. *Escherichia coli* and *Citrobacter freundii* using the Broth macro dilution method. The standard used in this test was Cefpodoxime Proxetil. The broth macro dilution method was performed for the investigation of the antibacterial properties by following the protocol reported by Wiegand et al. with slight variation [36]. A stock solution of 1 mg $mL^{-1}$ was prepared for each standard drug and test sample. Further, the stock solution was subsequently diluted to get a concentration of 100 μg $mL^{-1}$ to 20 μg $mL^{-1}$. The bacterial suspension was prepared in sterile

nutrient broth by transferring a few μL of 24-h-old bacterial stock solution and turbidity was adjusted to 0.5 McFarland standard ($1.5 \times 10^8$ CFU $mL^{-1}$). Further, 5 mL media containing 0.5 mL bacterial solution (0.5 McFarland standard), 300 μL of standard drug/test sample in sterile test tubes were incubated at 37 °C in a biochemical oxygen demand (BOD) incubator (% inhibition reading was taken at 12 h). Bacterial inoculated broth without test extract/standard drug served as positive control and a tube with only broth served as negative control. Bacterial growth turbidity was observed with the naked eye in bright light and absorbance was taken at 600 nm and the results were accordingly recorded. The $IC_{50}$ values were calculated using the linear regression plot of concentration versus percentage of inhibition of bacterial growth. The concentration at which the least turbidity of bacterial growth was observed was taken as the MIC of the tested sample.

#### 2.8.1. Preparation of media

5 g of Luria Broth was dissolved in 200 mL of distilled water. The mixture was heated in a heating mantle at 70 °C for 10 min and was autoclaved at 121 °C for 15 min.

#### 2.8.2. Preparation of bacterial cultures

Fresh cultures were prepared in broth and incubated at 37 °C for 24 h in a BOD incubator. The bacterial strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC, Chandigarh, India). The antibacterial potential of the extracts was investigated against each bacterial strain. For this purpose, distilled water was used for making solutions of the standard Cefpodoxime Proxetil and for extract CL-W, for other extracts, methanol was used as the solvent.

#### 2.8.3. Preparation of a working solution

The working solution was prepared by following the serial dilution method. 1 mL, 0.8 mL, 0.6 mL, 0.4 mL, and 0.2 mL were taken from the stock solution and the appropriate solvent was added to make the total volume of 10 mL to get solutions of concentration 100 μg $mL^{-1}$, 80 μg $mL^{-1}$, 60 μg $mL^{-1}$, 40 μg $mL^{-1}$ and 20 μg $mL^{-1}$.

#### 2.8.4. Preparation of reaction mixture

The reaction mixture was prepared by adding 2 mL of the media with 200 μL of working solution along with 10 μL of the specific strain of bacteria. The control was prepared by adding 2 mL media with 200 μL of distilled water along with 10 μL of each strain of the bacteria.

### 2.9. Isolation of compound

Since the methanolic extracts showed better efficacy in the antidiabetic, antioxidant and antibacterial assay, to isolate and characterise the active components, 900 g of dried plant material was macerated with cold MeOH (3.0 L, three times). The resulting extract (52 g) was fractionated using solvents ranging in polarity from low to high (hexane, 50% ethyl acetate in hexane, ethyl acetate, and 2% MeOH in ethyl acetate) using a short column with 100–200 mesh silica gel. According to the results of the TLC analysis of each fraction, ethyl acetate fraction and 50% ethyl acetate in hexane were combined. The combined fractions (4.5 g) were subjected to column chromatography over silica gel of 230–400 mesh size. Major fractions collected were further purified by preparative TLC. Some crystals started to appear at the bottom of the RB flask during the evaporation of one of the fractions. The fraction was kept undisturbed for 24 h, crystals were gathered, cleaned with hexane, and recrystallized from a 10% ethyl acetate-hexane solution to get needle-like shiny crystals (12 mg).

### 2.10. Statistical analysis

The bioactivity tests were carried out in triplicate ($n = 3$). Results are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed using the Originpro 2022b application. Levene's test and Bonferroni test were performed for homogeneity check and mean comparison respectively. Values of $p < 0.05$ were considered statistically significant. Using Pearson's method, a correlation analysis was carried out between the total phenolic, flavonoid content and antioxidant activities.

**Table 1**
TPC, TFC and antioxidant potential of the extracts.

| Standard/ Extract | TPC (mg GAE/g extract) | TFC (mg rutin equivalent/g extract) | DPPH assay ($IC_{50}$) (μg $mL^{-1}$) | ABTS assay ($IC_{50}$) (μg $mL^{-1}$) | FRAP assay (μM ascorbic acid/g extract) |
|---|---|---|---|---|---|
| Ascorbic acid | – | – | 6.54 ± 2.31 | – | 4839.17 ± 0.02 |
| Trolox | – | – | – | 17.93 ± 0.92 | – |
| CL-W | 38.19 ± 1.20 | 7.68 ± 0.48 | 297.00 ± 0.98 | 206.50 ± 0.58 | 334.63 ± 0.01 |
| CL-EC | 41.41 ± 1.78 | 9.37 ± 1.38 | 280.90 ± 0.61 | 125.62 ± 1.55 | 376.13 ± 0.03 |
| CL-EH | 50.01 ± 2.12 | 14.09 ± 1.17 | 216.10 ± 1.95 | 87.85 ± 0.85 | 351.49 ± 0.04 |
| CL-MC | 45.02 ± 2.39 | 11.94 ± 1.81 | 127.83 ± 1.74 | 145.57 ± 2.12 | 427.15 ± 0.02 |
| CL-MH | 53.55 ± 1.22 | 21.01 ± 2.86 | 172.75 ± 2.10 | 152.70 ± 1.26 | 452.66 ± 0.02 |

The values (mean ± SD) are the average of three independent experiments.

## 3. Results and discussions

### 3.1. Total phenolic content

Phenolics are one of the most prevalent secondary metabolites found in plants. Phenolics can scavenge free radicals by neutralising the radicals generated from lipids and halting the conversion of hydroperoxides into free radicals [37]. The regression equation of the calibration curve ($y = 0.003x + 0.0431$; $R^2 = 0.999$) was used to calculate the total phenolic content (TPC) values of the extracts. The absorbance of the gallic acid solution versus its concentrations (15.625–500 μg $mL^{-1}$) was plotted linearly to produce this equation. The TPC values of the extracts were calculated as mg gallic acid equivalent (GAE) $g^{-1}$ of the extract. As can be seen from Table 1, the methanolic hot extract possessed the highest TPC value (53.55 ± 1.22 mg GAE $g^{-1}$), whereas the water extract had the lowest (38.19 ± 1.20 mg GAE $g^{-1}$).

### 3.2. Total flavonoid content

Having a wide range of phenolic structures, flavonoids are a significant family of secondary metabolites found in plants. They are essential components of numerous nutraceutical, therapeutic, pharmacological, and cosmetic goods due to their various biological properties like anti-inflammatory, antioxidant, anti-carcinogenic, and anti-mutagenic [38]. The value of total flavonoid content (TFC) of each extract was determined using the regression equation derived from the calibration curve ($y = 0.0017x + 0.0794$; $R^2 = 0.991$), which was obtained from the linear plot of various concentrations of rutin solution (2000, 1500, 1000, 500 and 250 μg $mL^{-1}$) against their corresponding absorbance. The TFC values of the extracts are presented as mg rutin equivalent $g^{-1}$ of the extract in Table 1. It was observed that water extract had the lowest TFC value (7.68 ± 0.48 mg rutin equivalent $g^{-1}$), whereas methanol hot extract had the highest value (21.01 ± 2.86 mg rutin equivalent $g^{-1}$).

### 3.3. Antioxidant properties

Antioxidant assays, like the DPPH assay, are frequently used to assess a sample's capacity to scavenge free radicals. The colour of the solution containing the stable free radical is deep violet. Molecules possessing electron-donating capacity react with this radical. The decrease in the radical's absorbance at 517 nm can be used to track the quenching of DPPH. As can be seen from Table 1 in the DPPH assay, CL-MC exhibited the highest and CL-W exhibited the lowest antioxidant potential with a half maximal inhibitory concentration ($IC_{50}$) of 127.83 ± 1.74 μg $mL^{-1}$ and 297.00 ± 0.98 μg $mL^{-1}$ respectively. At a fixed concentration of 500 μg $mL^{-1}$, each extract showed a different highest average percentage of inhibitions, viz., CL-MC (91.5%), CL-MH (89.8%), CL-EH (84.5%), CL-EC (74.6%), and CL-W (69.8%).

$ABTS^{\bullet+}$ assay, one of the most widely used electron transfer-based techniques for determining antioxidant capacity measures the potential of a substance to scavenge the $ABTS^{\bullet+}$ radical. This test is very useful for plant extracts as the wavelength absorption at 734 nm minimizes colour interference [39]. In this assay, potassium persulfate acts as an oxidant to oxidize ABTS, which is then reduced in the presence of an antioxidant. The $IC_{50}$ value, or the concentration of extract needed to capture 50% of the radical, is the parameter used in these assays. A smaller $IC_{50}$ value indicates a stronger radical trapping activity and, thus, higher antioxidant activity. In the $ABTS^{\bullet+}$ assay, the highest and lowest antioxidant potential was shown by CL-EH and CL-W respectively with an $IC_{50}$ value of 87.85 ± 0.85 and 206.50 ± 0.58 μg $mL^{-1}$. The greatest percentages of inhibition were seen for CL-EH (100%), CL-MC (88.5%), CL-MH (88.3%), CL-EC (85%) and CL-W (75.1%) at a concentration of 500 μg $mL^{-1}$. The FRAP method is a frequently used protocol to evaluate the reducing power and antioxidant content of samples. The process involves an antioxidant agent reducing the Fe–(III)–TPTZ complex to a blue-coloured Fe–(II)–TPTZ complex. The higher absorbance in this method indicates a higher reducing power. A FRAP test is considered to be a reliable indicator of total antioxidant power since it adds up the individual reducing capabilities of all the compounds in a sample. In the FRAP assay, CL-MH (452.66 ± 0.02) μM and CL-W (334.63 ± 0.01) μM respectively demonstrated the highest and lowest antioxidant power. The ability of the extracts to scavenge DPPH and $ABTS^{\bullet+}$ free radicals suggests that they might have electron donors, which leads to the scavenging of the free radicals.

In the antioxidant assays different extracts showed different antioxidant potential. The explanation for the variation in the radical scavenging capability of the extracts may be attributed to the stoichiometry of interactions between the antioxidant chemicals and the different radicals. The efficiency of these antioxidant compounds is contingent upon their molecular weight, the quantity of aromatic rings present and the kind of hydroxyl group and their substitution pattern [40]. The variability of radical scavenging seen in these experiments might potentially be explained by the radicals' stereo selectivity or their varied solubility, which would make sense in the

**Table 2**
Correlation of antioxidant activities, total phenolic and flavonoid contents.

| | TPC | TFC | DPPH | ABTS | FRAP |
|---|---|---|---|---|---|
| **TPC** | 1 | | | | |
| **TFC** | 0.956 | 1 | | | |
| **DPPH** | −0.655 | −0.619 | 1 | | |
| **ABTS** | −0.525 | −0.289 | 0.272 | 1 | |
| **FRAP** | 0.621 | 0.735 | −0.794 | −0.051 | 1 |

case of crude extracts as they include a range of antioxidants.

### 3.3.1. Correlation among TPC, TFC and antioxidant activities

The Pearson correlation coefficient (PCC), also known as Pearson's r, was used to express the direction and magnitude of the linear connection of correlation among the extracts' flavonoid and phenolic contents with the antioxidant capabilities measured by the various techniques [41]. The PCC of DPPH, ABTS, FRAP, TPC and TFC are given in Table 2. There was a clear negative correlation of DPPH assay with TPC and TFC, as evidenced by the PCC values −0.655 and −0.619, respectively. Similarly, there was a substantial negative correlation of ABTS assay with TPC and TFC with PCC values of −0.525 and −0.289 respectively. The PCC values 0.621 and 0.735 respectively indicate a highly positive correlation of FRAP assay with TPC and TFC. Between TPC and TFC, the PCC was 0.956, which indicates a significant positive correlation as expected given that both classes contribute to the antioxidant activity of plants. In addition, both the FRAP and ABTS assays exhibited significant correlations with the DPPH assay (PCC = −0.794 and 0.272, respectively). However, the correlation between FRAP and ABTS is extremely low (PCC = −0.051). Since we used the $IC_{50}$ values of the ABTS and DPPH assays for correlation, the results revealed a negative correlation with the other variables, indicating that a rise in one would cause a corresponding drop in the other. Moreover, the variation in correlation among TPC, TFC and antioxidant activities may be due to the different reaction mechanisms involved in each assay.

## 3.4. Antidiabetic study via α-glucosidase inhibitory activity

The enzyme α-glucosidase is essential for the conversion of complex carbohydrates into glucose and other less complex sugars. Inhibiting this enzyme slows down the digestion and absorption of carbohydrates and improves blood sugar management, thus, it can help control diseases like diabetes [42,43]. The α-glucosidase inhibitory activity study was conducted using the standard acarbose and the extracts in concentrations ranging from 2000 μg $mL^{-1}$ to 31.25 μg $mL^{-1}$. The positive control and CL-W demonstrated the greatest average percentage inhibition (99.61 ± 0.02 and 36.25 ± 1.45) at 500 μg $mL^{-1}$ and 2000 μg $mL^{-1}$ concentrations, respectively. Other extracts, CL-MC (91.29 ± 0.33), CL-MH (58.50 ± 1.01), CL-EC (64.03 ± 1.26), CL-EH (48.74 ± 1.42) had shown their highest average percentage inhibition at the concentration 250 μg $mL^{-1}$. Among all the extracts, CL-W had the lowest inhibitory potential whereas CL-MC had the maximum inhibitory potential (19.63 ± 1.19) which was much better than the positive control (67.27 ± 1.04) (Table 3). This study reveals that the plant fundamentally contains compounds that inhibit the activity of the α-glucosidase enzyme. Since the plant exhibited quite higher levels of enzyme inhibition than the standard drug acarbose, further studies on this might result in the applicability of the plant in the management of diabetes.

## 3.5. Antibacterial activity

The results of the antibacterial activity studies are presented in Table 4 and Table 5. The $IC_{50}$ and minimum inhibitory concentration (MIC) values indicate that extracts of *C. leptospadix* in different solvents have different efficacy against the bacterial strains used. $IC_{50}$ values were calculated using the linear regression plot of concentration versus percentage of inhibition of bacterial growth. The concentration at which the least turbidity of bacterial growth was observed was taken as MIC of the tested sample. The aqueous extract (CL-W) showed significant inhibition of the bacterial strain *C. freundii* at a concentration of 80 μg$mL^{-1}$ and *E. coli* at 100 μg $mL^{-1}$. The methanolic cold extract (CL-MC) was found to be effective against *E. coli* at 80 μg $mL^{-1}$ and 100 μg $mL^{-1}$ against *S. pneumoniae* and *B. subtilis*. The methanolic hot extract (CL-MH) best inhibited the *C. freundii* and *S. pneumoniae* at 100 μg $mL^{-1}$. The ethanolic hot extract (CL-EH) was found to be effective only against *B. subtilis* at 100 μg $mL^{-1}$. It was observed that the methanolic extracts were the most effective against all the tested pathogens.

This study reveals insightful information on the inhibitory efficacy of *C. leptospadix* extracts and the standard Cefpodoxime Proxetil against the four bacterial strains. These findings shed light on the possible potential applications of plants extracted in different solvents as alternative or complementary treatments for bacterial infections. It is noteworthy that, across all bacterial strains and dilutions, the standard in most cases exhibited lower $IC_{50}$ compared to the plant extracts. This highlights the effectiveness of Cefpodoxime Proxetil as a reference antibiotic in our study and reinforces its status as a powerful tool in combating bacterial infections. The difference in the efficacy of extracts prepared using different solvents suggests that there is a significant variation in the nature of the phytochemicals extracted depending on the solvent used. This variation paves the way for extracting specific phytochemicals for

**Table 3**
α-glucosidase inhibitory assay.

| Extract/standard | % Inhibition[a] | $IC_{50}$[b] |
|---|---|---|
| Acarbose | 99.61 ± 0.02 | 67.27 ± 1.04 |
| CL-W | 36.25 ± 1.45 | 34072.21 ± 2.87 |
| CL-MC | 91.29 ± 0.33 | 19.63 ± 1.19 |
| CL-MH | 58.50 ± 1.01 | 135.72 ± 1.70 |
| CL-EC | 64.03 ± 1.26 | 102.67 ± 1.73 |
| CL-EH | 48.74 ± 1.42 | 224.98 ± 1.86 |

[a,b]The values (mean ± SD) are the average of three independent experiments.
[a] Highest average percentage inhibition.
[b] Concentration required for 50 % inhibition in μg $mL^{-1}$.

**Table 4**
Antibacterial properties of tested samples against bacterial strains.[a].

| Tested samples | Concentration (μg mL$^{-1}$) | *E. coli* | *S. pneumoniae* | *B. subtilis* | *C. freundii* |
|---|---|---|---|---|---|
| Standard drug | 100 | – | – | – | – |
| | 80 | – | + | – | – |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |
| CL-W | 100 | – | + | + | – |
| | 80 | + | + | + | – |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |
| CL-EC | 100 | + | + | + | + |
| | 80 | + | + | + | + |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |
| CL-EH | 100 | + | + | – | + |
| | 80 | + | + | + | + |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |
| CL-MC | 100 | – | – | + | + |
| | 80 | – | + | + | + |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |
| CL-MH | 100 | + | – | + | – |
| | 80 | + | + | + | + |
| | 60 | + | + | + | + |
| | 40 | + | + | + | + |
| | 20 | + | + | + | + |

[a] (+) denotes bacterial growth and (−) denotes bacterial growth inhibition which is considered as MIC for the tested samples.

**Table 5**
Results of antibacterial studies.

| Bacterial strain | Test sample | $IC_{50}$ (μg mL$^{-1}$)[a] | MIC (μg mL$^{-1}$) |
|---|---|---|---|
| *C. freundii* | Standard | 5.83 ± 0.932 | 80 |
| | CL-W | 5.98 ± 0.984 | 80 |
| | CL-EC | 7.37 ± 0.931 | – |
| | CL-EH | 8.61 ± 0.412 | – |
| | CL-MC | 9.15 ± 0.683 | – |
| | CL-MH | 6.28 ± 0.793 | 100 |
| *E. coli* | Standard | 55.80 ± 1.240 | 80 |
| | CL-W | 99.30 ± 0.772 | 100 |
| | CL-EC | 257.72 ± 0.305 | – |
| | CL-EH | 111.48 ± 0.783 | – |
| | CL-MC | 98.20 ± 0.708 | 80 |
| | CL-MH | 113.06 ± 0.884 | – |
| *S. pneumoniae* | Standard | 67.17 ± 1.042 | 100 |
| | CL-W | 131.002 ± 0.624 | – |
| | CL-EC | 103.154 ± 0.709 | – |
| | CL-EH | 191.86 ± 0.447 | – |
| | CL-MC | 98.79 ± 0.819 | 100 |
| | CL-MH | 94.48 ± 1.160 | 100 |
| *B. subtilis* | Standard | 91.69 ± 1.316 | 80 |
| | CL-W | 145.79 ± 0.603 | – |
| | CL-EC | 117.14 ± 0.586 | – |
| | CL-EH | 112.97 ± 0.884 | 100 |
| | CL-MC | 102.44 ± 0.937 | 100 |
| | CL-MH | 120.62 ± 1.026 | – |

[a] The values of $IC_{50}$ are represented as $IC_{50}$ ± SEM (standard error of mean) which is the average of three independent experiments.

future treatment alternatives to infectious diseases, potentially reducing dependence on chemically synthesized drugs and consequently lowering the risk of antibiotic resistance.

This study has certain limitations, including the use of *in vitro* assays, which may not fully replicate the complex interactions that

occur within a living organism. Additionally, we tested only a limited number of bacterial strains, and the inhibitory efficacy of plant extracts may vary with different strains or under different conditions. Thus, it is essential to translate these promising laboratory results into clinical settings cautiously. Additional research, including *in vivo* studies and clinical trials, will be necessary to assess the effectiveness and safety of plant extracts for human use.

### 3.6. Characterization of the isolated compound

The compound isolated (yield 12 mg, 0.000013%) was identified as 4-(5,7-dimethoxychroman-2-yl)phenol (Fig. 1) from its single crystal XRD, $^{1}H$ and $^{13}C$ NMR, IR and HRMS studies.

4-(5,7-dimethoxychroman-2-yl)phenol**:** White crystals, m. p. 136.1 °C, IR (KBr) $\nu$($cm^{-1}$): 3388 (O–H stretching), 2936 (C–H stretching), 1610 (C═C stretching), 1445 (C–H bending), 1207 (C–O stretching), 1119 (C–O stretching), 828 (C═C bending). $^{1}H$ NMR (600 MHz, $CDCl_3$): $\delta = 7.30$ (d, $J = 8.3$ Hz, 2H), 6.84 (d, $J = 8.3$ Hz, 2H), 6.12 (s, 1H), 6.08 (s, 1H), 5.11 (s, 1H), 4.92 (d, $J = 10.4$ Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 2.74 (m, 1H), 2.63 (m, 1H), 2.16 (m, 1H), 2.01 (m, 1H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$): $\delta =$ 159.4, 158.7, 156.5, 155.4, 134.0, 127.8, 115.5, 103.5, 93.6, 91.5, 77.7, 55.6, 55.5, 29.5, 19.5 ppm. $[M+H]^{+}$ peak was observed for the compound at 287.12707 whose calculated mass was 287.12833.

#### 3.6.1. Crystallography data

SCXRD data were collected on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ = 0.71073 Å). Data integration was done using SAINT [44]. Intensities for absorption were corrected using SADABS. Bruker SHELXTL was used for the structure solution and refinement [45]. The hydrogen atoms were refined isotropically, and all the other atoms were refined anisotropically. O–H hydrogens were located from different electron density maps, and C–H hydrogens were fixed using the HFIX command in SHELXTL. The crystallographic parameters of the structure are given in Table 6.

## 4. Conclusion

To conclude, the effect of extracting solvents on the TPC and TFC values, antioxidant, antidiabetic and antibacterial properties on the tender shoots of *Calamus leptospadix* was studied in this work. The study revealed that methanol both in hot and cold conditions is the most effective solvent for extraction of the phenolics and flavonoids present in the plant biomass as well as compounds that possess α-glucosidase inhibitory and antibacterial properties. In the antioxidant assays, the methanolic extract was found to possess DPPH scavenging activity ($IC_{50}$) of 127.83 ± 1.74 μg $mL^{-1}$ in comparison to that of the control ascorbic acid 6.54 ± 2.31 μg $mL^{-1}$ and ferric reducing antioxidant power of 452.66 ± 0.02 μM ascorbic acid $g^{-1}$ of extract. In the antidiabetic assay, the extract exhibited a

**Table 6**
Crystal data and structure refinement of 4-(5,7-dimethoxychroman-2-yl)phenol.

| Formula Unit | $C_{17}H_{18}O_4$ |
|---|---|
| Formula weight | 286.31 |
| Crystal system | Monoclinic |
| Space group | $P2_1$ |
| T [K] | 296 |
| a [Å] | 11.723 (3) |
| b [Å] | 5.5323 (10) |
| c [Å] | 12.493 (4) |
| α [°] | 90 |
| β [°] | 117.86 (4) |
| F067 [] | 90 |
| Volume [Å]$^3$ | 716.3 (4) |
| Z | 2 |
| Radiation type | Mo Kα |
| μ ($mm^{-1}$) | 0.09 |
| D [g $cm^{-3}$] | 1.327 |
| Diffractometer | Bruker APEX-II CCD |
| Absorption correction | Multi-scan |
| No. of measured reflections | 8306 |
| No. of independent reflections | 3950 |
| No. of observed [I > 2σ(I)] reflections | 2013 |
| $R_{int}$ | 0.096 |
| R [F2 > 2σ(F2)] | 0.092 |
| w*R* (F2) | 0.203 |
| S | 1.08 |
| Δρmax, Δρmin (e $Å^{-3}$) | 0.28, −0.29 |
| CCDC Reference No. | 2289196 |

The compound 4-(5,7-dimethoxychroman-2-yl)phenol did not show any significant activity against the four bacterial strains or in the α-glucosidase and α-amylase inhibitory assays.

maximum inhibitory potential of 19.63 ± 1.19 μg $mL^{-1}$ which was much better than that of the positive control acarbose. The extract was found to be effective against both gram-positive and gram-negative strains. The hot aqueous extract also showed significant TPC and TFC values and bioactive potential. It suggests that the beneficial effects of the plant biomass are not destroyed by cooking. Isolation of a flavan 4-(5,7-dimethoxychroman-2-yl)phenol from the cold methanolic extracts confirms the presence of flavonoids in the plant. Since the plant part used is edible, further studies may provide insight into the discovery of potential leads with antidiabetic and/or antibacterial potential which will be safe for healthcare applications. Although the antioxidant, antidiabetic and antibacterial properties of the plant were studied in this work, the endeavour does not cover all the possible pharmacological properties such as toxicity, pharmacokinetics, or mechanism of action, which may be required for the exploration of its full medicinal potential.

## Data availability statement

The supporting information contains the data related to this work.

## CRediT authorship contribution statement

**Utpal Dutta:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Manab Jyoti Goswami:** Formal analysis, Data curation. **Tage Seema:** Data curation. **Temin Payum:** Investigation, Data curation. **Tanzim Nishad Ullah:** Investigation, Data curation. **Pallabi Kalita Hui:** Investigation, Data curation. **Dwipen Kakati:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34638.

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