International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **15(4),** 19-25, November **(2026)** *Int. Res. J. Biological Sci.*

Evaluation of the Phytochemical composition and Biological activities of Ethanolic leaf extract of *Crossandra infundibuliformis* (L.) Nees

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Available online at: www.isca.in, www.isca.me
Received 28th August 2026, revised 13th September 2026, accepted 2nd October 2026

Abstract

Crossandra infundibuliformis (L.) Nees is a medicinal plant that has been used traditionally as a wound healing, skin condition, fever, and inflammatory condition. The scientific assessment of its phytochemical composition and biological activities, however, is still limited. The present study was designed to study the phytochemical profile and determine the antibacterial, antioxidant and anti-inflammatory properties of ethanolic leaf extract of C. infundibuliformis. The leaves were extracted with 70% ethanol in a Soxhlet extractor to recover 18.00% (w/w) of the extract. Preliminary phytochemical screening was done and found to be positive for the presence of phenolic compounds, tannins, cardiac glycosides and triterpenoids, which was later confirmed by thin layer chromatography (TLC). The results of quantitative analysis showed that there were 101.97mg GAE/g of total phenolic content, 79.80mg GAE/g of total tannin content and 118.25mg DE/g of total cardiac glycoside content. The antibacterial activity was concentration dependent, with the inhibition zones of the extract against S. mutans, S. aureus, E. coli, and P. aeruginosa being 13–16 mm. The extract displayed antioxidant activity in both DPPH and ABTS radical scavenging assays with IC_{50} values of 87.49µg/mL and 149.08µg/mL, respectively. The extract also exhibited moderate anti-inflammatory activity with the percentage inhibition of egg albumin denaturation being 18.57%. In conclusion, the results presented here suggest that C. infundibuliformis is a valuable source of bioactive phytochemicals possessing antibacterial, antioxidant, and anti-inflammatory activities, thereby corroborating its traditional medicinal applications, and underscoring its potential for further pharmacological studies.

Keywords: *Crossandra infundibuliformis*; phytochemical profiling; antibacterial; antioxidant; anti-inflammatory; thin-layer chromatography.

Introduction

Medicinal plants have been an important part of traditional healthcare systems for centuries and continue to play a crucial role role in modern drug discovery. Owing to their remarkable chemical diversity, natural products have contributed substantially to the development of therapeutics, particularly for infectious diseases and cancer[1]. The use of new analytical methods, metabolomics and phytochemistry also has enabled the identification of a wider range of bioactive phytochemicals for natural products with pharmacological potential[2].

Anti-microbial resistance (AMR) is a growing worldwide health problem because the use of traditional antibiotics is becoming more ineffective and the challenge to find alternative therapeutic agents is more urgent[3]. Besides, oxidative stress and chronic inflammation have a close relationship with the pathogenesis of several chronic non-communicable diseases such as diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, and cancer. The overproduction of reactive oxygen species (ROS) can disrupt cellular homeostasis by damaging proteins, lipids, and nucleic acids, and a sustained inflammatory response can cause the further development of tissue injury and disease[4]. Medicinal plants with antimicrobial, antioxidant and anti-inflammatory properties therefore have received great interest as a source of new therapeutic products.

Medicinal plant therapeutic effects are majorly attributed to phytochemicals such as phenolic compounds, flavonoids, tannins, terpenoids, alkaloids, glycosides and saponins[5]. Secondary metabolites have various biological activities, including free radical scavenging, inhibition of microbial growth, modulation of inflammatory mediators, and regulation of cellular signalling pathways[6]. Thus, phytochemical profiling coupled with biological activity assessment has emerged as an integral tool for the validation of medicinal potential of plant species and identification of bioactive molecules for medicinal applications.

C. infundibuliformis (also referred to as Kanakambaram and Firecracker Flower) is an evergreen ornamental shrub belongs to the family Acanthaceae. The plant, apart from its ornamental use has been long used in folk medicine for the treatment of wounds, skin diseases, inflammation and fever[7]. The phytochemical studies conducted previously revealed the presence of flavonoids, phenolics, tannins, terpenoids, cardiac glycosides and other secondary metabolites in various extracts of *C. infundibuliformis*, thus emphasizing its potentiality as a

valuable medicinal plant[8]. In addition, preliminary pharmacological studies have revealed antimicrobial and antioxidant properties, suggesting the presence of bioactive compounds that could be of therapeutic interest.

Although these significant results have been obtained, the scientific study of *C. infundibuliformis* is still incomplete. Most of the studies that are available have been conducted on the initial phytochemical screening or on the single pharmacological activity and there are few studies that correlate the phytochemical composition with antibacterial, antioxidant and anti-inflammatory activity. So, systematic investigation of its phytochemical profile and biological activities is required to have scientific evidence for its traditional medicinal use and to explore its therapeutic potential as a source of plant-derived therapeutic agents.

The present study was undertaken to evaluate the phytochemical profile and biological activities of the ethanolic leaf extract of *C. infundibuliformis*. The preliminary phytochemical determination, quantitative estimation of selected phytochemical constituents, TLC profiling, antibacterial evaluation against selected bacterial pathogens, antioxidant evaluation based on DPPH and ABTS radical scavenging assays and anti-inflammatory activity based on the egg albumin denaturation assay were part of the investigation. The results of this study will serve as scientific support for the use of *C. infundibuliformis* in medicinal products and will be useful in the development of plant-based therapeutic drugs in the future.

Materials and Methods

Collection and authentication of plant materials: Fresh and healthy leaves of *C. infundibuliformis* the specimens of Nees (Family: Acanthaceae) were collected from Kondasandra in Kolar district, Karnataka state, India in May 2026. The authentication of the plant material was done by Dr. Suresh Kumar, Professor, Department of Botany, Maharani Cluster University, Bengaluru, Karnataka, India and the authentication reference number MCU/MSCWB/BOT-37/2026-27 was assigned. The leaves were collected and washed with running tap water followed by distilled water to wash away the dust and impurities presenton the leaves. Shade dried at room temperature until constant weight was achieved and pulverised to coarse powder in a mechanical grinder. The powdered sample was kept at room temperature in an airtight container for further extraction and phytochemical analysis.



Figure-1: *Crossandra infundibuliformis* (L.) Nees.

Preparation of ethanolic extract: About 3g of the powdered leaf material of *C. infundibuliformis* was placed in a Soxhlet extraction apparatus and extracted using 100 mL of 70% (v/v) ethanol for 12–13 cycles[9] with modifications. The extract was concentrated at 5-6 rpm and 68°C under reduced pressure with a rotary evaporator. The concentrated extract was then dried in sterile petri dishes, rehydrated in 2 mL of 70% (v/v) ethanol and stored in an air-tight container until subjected to further phytochemical and biological characterization.

Preliminary phytochemical analysis: Qualitative phytochemical analysis for the major secondary metabolites was carried out on ethanolic leaf extract of *C. infundibuliformis* using standard methods. Alkaloids, saponins, tannins, flavonoids, cardiac glycosides, triterpenoids and phenolic compounds were qualitatively determined in the extract according to standard procedures[10] with minor modifications. The presence or absence of each phytochemical was confirmed according to the colour change or precipitate formation they caused in the various qualitative test. Alkaloids were tested by Wagner's test, saponins by foam test, tannins by ferric chloride test, phenolic compounds by ferric chloride test, cardiac glycosides by Keller–Killiani test and triterpenoids by alkaline reagent test.

Quantitative phytochemical analysis: Determination of total phenolic content (TPC): *C. infundibuliformis* leaves ethanol extract was used to determine TPC following the procedure outlined by Folin–Ciocalteu colorimetric method. The calibration curve was made using gallic acid as the reference standard (20-100µg/mL). Aliquot (0.1mL) of the plant extract or gallic acid standard was added to the Folin–Ciocalteu reagent, sodium carbonate solution and distilled water and incubated at 40°C for 30 min. The absorbance was measured at 765 nm wavelength using a UV–Visible spectrophotometer. Each sample was analysed three times and results were reported as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Determination of total tannin content (TTC): TTC was measured according to the modified Folin–Ciocalteu method[11]. Gallic acid (20-100µg/mL) was used as the reference standard. Aliquot (0.1 mL) of the plant extract or gallic acid standard was added to the reaction reagents and allowed to stand at room temperature for 35-40 min in darkness. The absorbance was measured at 700 nm wavelength with UV-Vis spectrophotometer. Each sample was analysed three times, and tannin content was calculated as the amount of gallic acid equivalents (mg GAE/g).

Determination of total cardiac glycoside content (TCGC): TCGC was estimated based on Baljet colorimetric method. Digoxin (12.5–200 µg/mL) was used as the reference standard. A 0.1 mL of the plant extract or digoxin standard was mixed with Baljet reagent and incubated 60 mins at room temperature. The absorbance was measured at 495 nm wavelength using a

UV–Visible spectrophotometer. Each sample was analysed three times and results were reported in terms of milligrams of digoxin equivalents per gram of dry extract (mg DE/g).

Thin-layer chromatography (TLC) analysis: The selected phytochemical constituents of the ethanolic extract of *C. infundibuliformis* leaves were qualitatively separated and identified by TLC[12]. Pre-coated silica gel 60 F_{254} aluminium-backed TLC plates were used as the stationary phase. The ethanolic extract was dissolved in ethanol, and the sample was spotted with a capillary tube on the TLC plate 1 cm above the lower edge of the plate. The appropriate mobile-phase compositions used for the separation of the selected phytochemical constituents are presented in Table-1. The plates were developed in a saturated chromatographic chamber to the level of the solvent front, which was about 8 cm. The development was carried out, and the solvent front was marked, and the plates were air-dried before being subjected to UV visualization at 254 nm and 365 nm. The retention factor (Rf) values were calculated using the following equation:

$$\mathrm{Rf} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solent front}}$$

Table-1: Mobile phase composition used for TLC analysis.

Phytochemical	Mobile phase composition (v/v)
Phenolic compounds	Formic acid: Ethyl acetate: Glacial acetic acid: Water (0.8: 6.7: 0.8: 1.7)
Tannins	Chloroform: Methanol: Water (5.9: 3.2: 0.9)
Cardiac glycosides	Chloroform: Methanol: Water (6.6: 2.3: 0.9)
Triterpenoids	n-Hexane: Diethyl ether: Glacial acetic acid (8.0: 1.9: 0.1)

Antibacterial activity: The antibacterial activity of the ethanolic leaf extract of *C. infundibuliformis* was determined by the agar well diffusion method[13] against two Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus mutans*, and two Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Fresh inoculums of bacteria were prepared, and 0.1 mL of each bacterial culture was evenly spread on the respective sterile agars with sterile L-shaped spreader[14]. *E. coli* and *P. aeruginosa* were grown on Luria–Bertani agar (LBA), whereas *S. aureus* was grown on Nutrient Agar (NA) and *S. mutans*are grown on Brain Heart Infusion (BHI) agar. Sterile 6 mm diameter wells were cut out with a cork borer and 25 and 50 µL of the crude ethanolic extract were added to the corresponding wells. The positive control was nitrofurantoin, and the negative control was distilled water (DW). The plates were incubated for 24 hours at 37°C and the antibacterial activity was measured in terms of the diameter of the zone of inhibition around each well in millimetres (mm). Each experiment was carried out at least three times, and the results are mean ± standard deviation (SD).

Antioxidant activity: The antioxidant activity of the ethanolic leaf extract of *C. infundibuliformis* was determined using two radical scavenging assays; 2,2-diphenyl-1-picrylhydrazyl (DPPH)[15] and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)[16]. The standard used was ascorbic acid[17]. Each experiment was carried out three times and the data presented as mean ± standard deviation (SD).

DPPH radical scavenging assay: The ethanolic leaf extract of *C. infundibuliformis* was tested for its free radical scavenging activity by a DPPH radical scavenging assay[17]. The ascorbic acid was used as the reference standard and ethanolic leaf extract was tested as 12.5, 25, 50, 100 and 200µg/mL. A 0.1 mM DPPH solution is freshly preparedusing methanol was used for the assay.

Equal volumes (1 mL) of the plant extract or standard solution and DPPH solution were mixed and incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 517 nm by a UV–Visible spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

ABTS radical cation scavenging assay: The radical-scavenging activity was measured by ABTS[16]. Ascorbic acid served as the reference standard. Solutions of plant extracts were made at concentrations of 12.5, 25, 50, 100 and 200 µg/mL. To 0.1 mL of plant extract or standard solution 1.9 mL of methanol and 2 mL of ABTS working solution was added. The reaction mixture was allowed to react in dark room at room temperature for 6 min and absorbance was measured at 734 nm on a UV-Visible spectrophotometer[18]. The percentage of ABTS radical scavenging activity was determined by the following equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Anti-inflammatory activity: The in vitro anti-inflammatory activity of the ethanolic leaf extract of *C. infundibuliformis* was evaluated by the inhibition of protein denaturation using the egg albumin method[19]. The standard used was diclofenac sodium. Each experiment was repeated three times and data is presented as mean ± SD.

Inhibition of protein denaturation by egg albumin method: The anti-inflammatory activity of ethanolic leaf extract was evaluated by egg albumin denaturation assay. Five concentrations - 50, 100, 200, 400, and 800µg/mL were used for the reference standard, Diclofenac sodium, and 200 µL was used for the ethanolic leaf extract. 0.1 mL of the plant extract or

standard solution was added to 0.2 mL of fresh egg albumin and phosphate buffered saline (PBS, pH 6.4) and the total volume made up to 5 mL by distilled water. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min to denature the protein. The absorbance was measured after cooling to room temperature at 660 nm using a UV–Visible spectrophotometer. Protein denaturation percentage inhibition was determined by the following equation:

$$\% \text{ Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Results and Discussion

Extraction yield: 0.54 g crude extract of *C. infundibuliformis* leaves was obtained from 3g of dried leaves powder, with an extraction yield of 18.00% (w/w). A satisfactory extraction yield from the leaves shows that 70% ethanol was able to extract bioactive phytoconstituents effectively. The observation is also reported for hydroethanolic extract of medicinal plants which are effective in recovering phenolic compounds, tannins, glycosides and other secondary metabolites[20].

Preliminary phytochemical screening: The ethanolic leaf extract of *C. infundibuliformis* showed the presence of tannins, cardiac glycosides, triterpenoids and phenolic compounds. The presence of these secondary metabolites indicates that the extract contains a variety of bioactive compounds that could be responsible for its antibacterial, antioxidant, and anti-inflammatory properties[21]. High amounts of phenolic compounds, tannins and triterpenoids have been reported in Crossandra species and other medicinal plants which have been correlated with different pharmacological properties[22].

Quantitative phytochemical analysis: Total phenolic content: TPC of the ethanolic leaf extract of *C. infundibuliformis* was determined using the Folin–Ciocalteu method and was found to be 101.97 mg GAE/g extract with absorbance value of 0.546 ± 0.003 (Figure-2). The result suggests that the extract contains a significant amount of phenolic compounds, known for their antioxidant properties and role in biological activities[22]. These antioxidant and antibacterial properties observed in the present study may be attributed to the relatively high contents of phenolics[23].

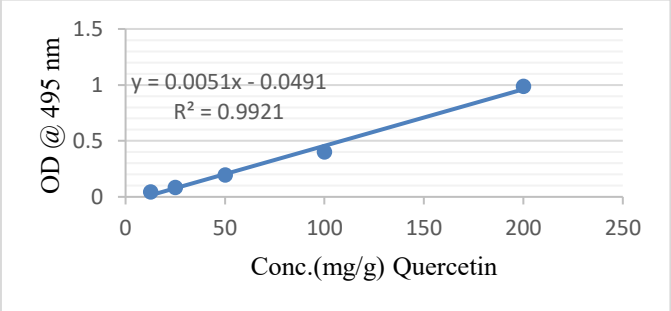

Figure-2: Gallic acid standard calibration curve for total phenolic content.

Total tannin content: TTC of *C. infundibuliformis* leaf extract in ethanol was estimated to be 79.80mgGAE/g extract with absorbance value of 0.213 ± 0.002 (Figure-3). The qualitative phytochemical results were confirmed by the presence of high amount of tannins which shows the effective extraction of polyphenolic compound using 70% ethanol. Tannins have been reported to show the antioxidant, antimicrobial and anti-inflammatory activities by free radical scavenging, metal ion chelation and interaction with microbial proteins. The same has been noted with medicinal plants with tannin-rich extracts[24].

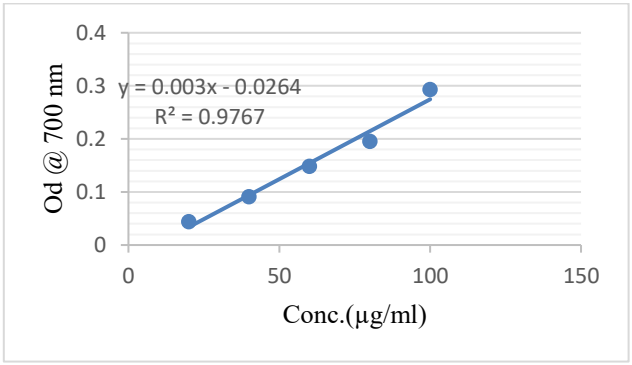

Figure-3: Gallic acid standard calibration curve for total tannin content.

Total Cardiac Glycoside Content: TCGC of *C. infundibuliformis* ethanolic leaf extract was found to be 118.25 mg DE/g extract and absorbance was 0.553 ± 0.001 (Figure-4). The significant amount of glycosides indicates that the extract possesses biological activity which could be responsible for its therapeutic actions. Cardiac glycosides have been claimed to have additional antimicrobial and anti-inflammatory properties as well as cardiac tonic properties[25]. These compounds could therefore have been responsible for the biological activities noted during the present investigation and accounted for the medicinal activities of *C. infundibuliformis*[25].

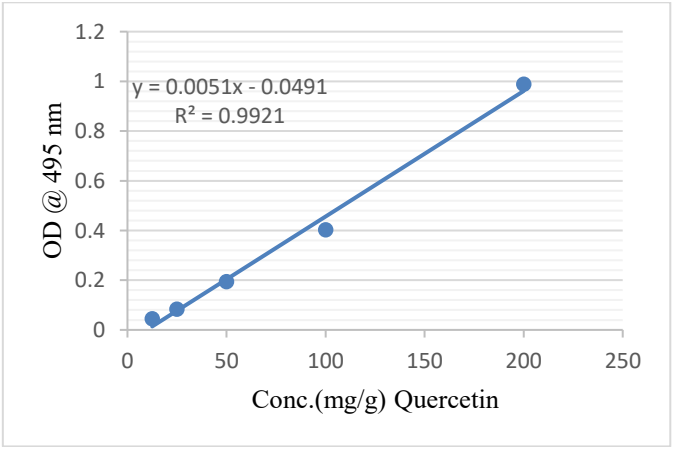

Figure-4: Digoxin standard calibration curve for total cardiac glycoside content.

Thin-Layer Chromatography (TLC) Analysis: The major phytochemical constituents of the ethanolic extract of the leaf of *C. infundibuliformis* were confirmed using the TLC. The UV light detection showed four distinct bands corresponding to phenolic compounds (Rf = 0.833), tannins (Rf = 0.811), cardiac glycosides (Rf = 0.870) and triterpenoids (Rf = 0.340), which was a confirmation of the preliminary phytochemical screening (Figure-5). The difference in Rf values is attributed to the difference in polarity of the phytochemicals and its interactions with stationary and mobile phase[10]. The results showed TLC as a quick tool for the qualitative identification of constituents with medicinal properties in medicinal plant extracts and will be helpful for the subsequent quantitative phytochemical and biological activity analysis[26].

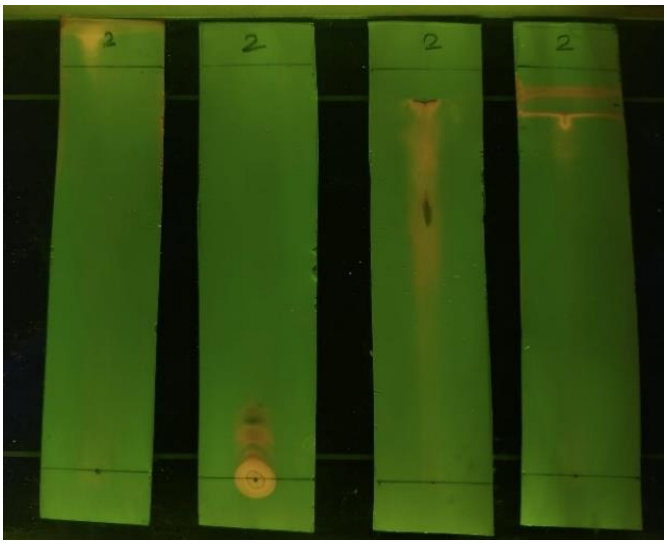

Figure-5: TLC chromatogram of Glycosides, Terpenoids, Phenols and Tannins respectively.

Antibacterial Activity: Ethanolic leaf extract of *C. infundibuliformis* has been tested for antibacterial activity against *S. mutans, S. aureus, E. coli* and *P. aeruginosa* by agar well diffusion method. The extract exhibited inhibitory activity against all four bacterial strains at both 25 µL and 50 µL concentrations (Figure-6 to 9). At 25 µL, inhibition zones of 13 mm, 13 mm, 14 mm, and 15 mm were observed against *S. mutans*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. The antibacterial activity was slightly increased with increase of extract volume (50µL) with inhibition zone of 15mm, 16mm, 16mm, 16mm respectively. A concentration-dependent response is determined by the increased effect of the extracts on the bacteria; the larger the volume, the greater the effect. The observed activity could be due to the presence of phenolic compounds, tannins, cardiac glycosides and triterpenoids, which are known to cause disruption of bacterial cell membranes, inhibition of essential enzymes and interference with cellular metabolism[27]. The result obtained here shows that *C. infundibuliformis* has wide range of antibacterial activity against bothgram-positive and gram-negative tested bacteria.

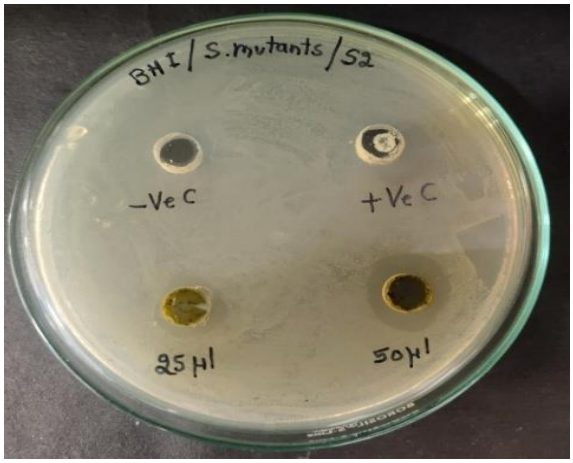

Figure-6: Antibacterial activity of the ethanolic leaf extract against *S. mutans.*

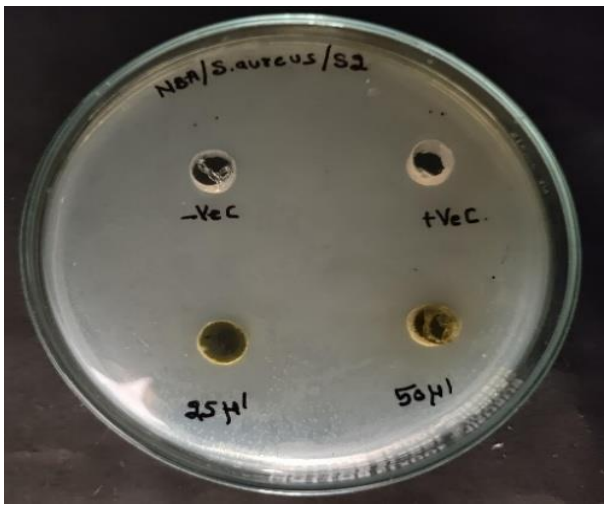

Figure-7: Antibacterial activity of the ethanolic leaf extract against *S. aures.*



Figure-8: Antibacterial activity of the ethanolic leaf extract against *E. coli.*

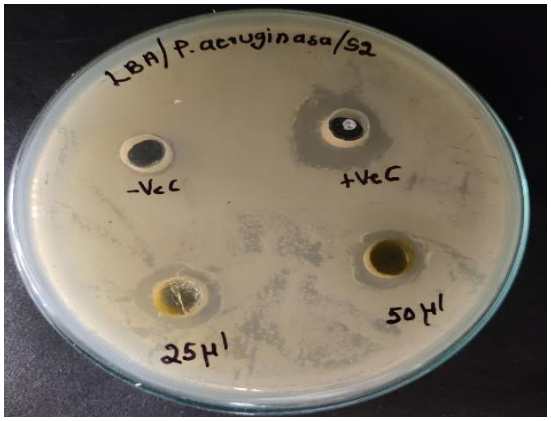

Figure-9: Antibacterial activity of the ethanolic leaf extract against *P. aeruginosa.*

Antioxidant activity: DPPH radical scavenging activity: The ethanolic leaf extract of *C. infundibuliformis* was tested for its antioxidant activity through DPPH radical scavenging assay. The percentage inhibition of the extract increased with concentration, from 17.86% at 12.5µg/mL to 61.67% at 200 µg/mL (Figure-10). The calculated IC_{50} value was 87.49µg/mL, indicating good free radical scavenging potential. This antioxidant activity could be due to the presence of phenolic compounds and tannins that can provide hydrogen atoms or electrons to neutralise free radicals[15]. The same observation has been reported for the extracts of the medicinal plants with a high phenolic content[23].

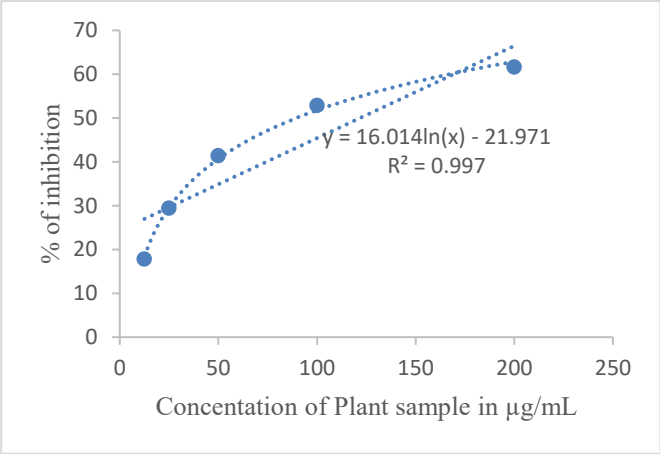

Figure-10: DPPH radical scavenging activity of the ethanolic leaf extract of *C. infundibuliformis*.

ABTS radical scavenging activity: The ABTS radical scavenging assay also showed the antioxidant property of the ethanolic leaf extract of *C. infundibuliformis*. The radical scavenging ability increased in a concentration dependent manner from 7.92% (12.5µg/mL) to 58.06% (200µg/mL) (Figure-11). The IC_{50} value was 149.08µg/mL, demonstrating effective ABTS radical scavenging activity. The antioxidant activity was observed, both in the DPPH and ABTS assays, agrees with the relatively high contents of phenolics and tannins in the extract, indicating that these phytoconstituents play an important role in the ability of the extract to inhibit free radicals[16]. These correlation between phenolic content and antioxidant activity has been reported earlier[22].

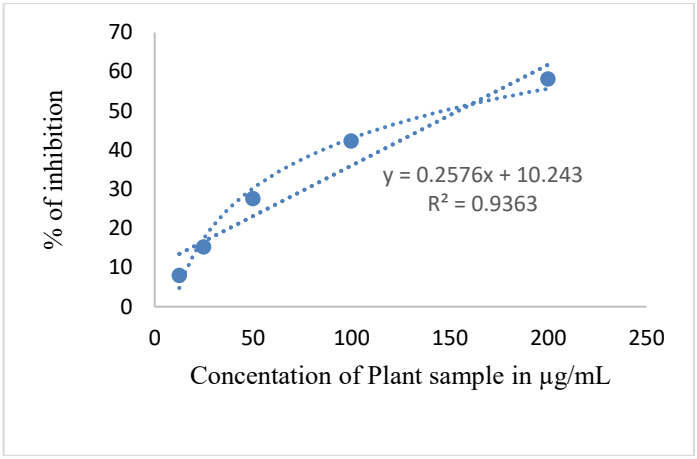

Figure-11: ABTS radical scavenging activity of the ethanolic leaf extract of *C. infundibuliformis*.

Anti-inflammatory activity: Anti-inflammatory activity of *C. infundibuliformis* leaves extract (EIE) was determined in vitro using the egg albumin denaturation assay with diclofenac sodium as a standard. The calibration curve of diclofenac sodium is shown in the Figure-12. The ethanolic extract showed moderate anti-inflammatory activity (Figure-10) with protein denaturation inhibition of 18.57%, having a mean absorbance of 0.709 ± 0.001. It is thought that the activity observed is due to the presence of phenolic compounds, tannins, cardiac glycosides and triterpenoids, all known for their ability to inhibit protein denaturation and inflammation[28]. The results indicated that *C. infundibuliformis* has potential natural anti-inflammatory activity and can be a source of bioactive compounds for further pharmacological studies, though the activity was not as much as that of diclofenac sodium[29].

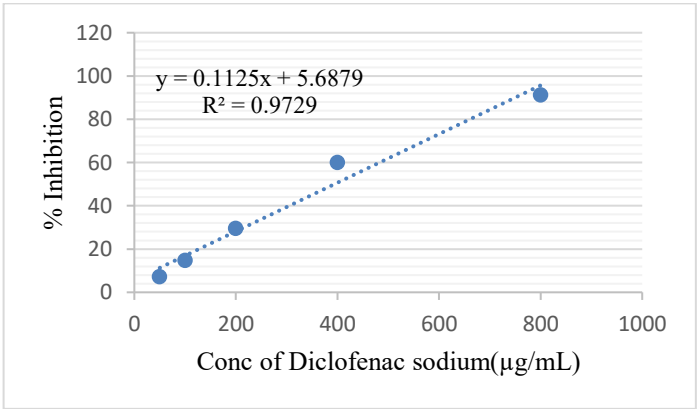

Figure-12: Standard calibration curve of diclofenac sodium for the egg albumin denaturation assay.

Conclusion

In the current study, the ethanolic extract of *C. infundibuliformis* leaves was found to have significant biological activities and rich phytochemical profile. The qualitative and quantitative phytochemical analysis along with TLC profiling supported the presence of phenolics, tannins, cardiac glycosides and triterpenoids. The extract showed broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria, good antioxidant activity in both DPPH and ABTS radical scavenging assays and moderate anti-inflammatory activity in the egg albumin denaturation assay. The results presented in this study corroborate with the traditional medicinal applications of *C. infundibuliformis*, suggesting that it is a good source of bioactive compounds in nature. The extract can be used to develop formulations for pharmaceutical, nutraceutical and herbal health care products with plant-based antimicrobial, antioxidant and anti-inflammatory benefits. A detailed phytoconstituent isolation and characterization study as well as in vivo/in vivo clinical studies is recommended to support its therapeutic efficacy and safety**.**

Acknowledgement

The authors are grateful to Mr. Aravind Ganessin, Managing Director, Dextrose Technologies Private Limited, for providing the laboratory facilities, technical support, and necessary resources to successfully carry out the experimental work. Special thanks are extended to Dr. Suresh Kumar, Professor, Department of Botany, Maharani Cluster University, Bengaluru, for authenticating the plant material used in this study. The authors also acknowledge the support of the faculty members and technical staff of the Department of PG Studies in Biotechnology, Nrupathunga University, Bengaluru, for their assistance during the research.

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