Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

https://doi.org/10.36673/AJRPSB.2022.v10.i04.A11

ANTIOXIDANT, ANTIBACTERIAL AND WOUND HEALING PROPERTIES OF *CROSSANDRA INFUNDIBULIFORMIS* (L.): A COMPREHENSIVE STUDY

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ABSTRACT

This study focused on the acquisition, authentication and comprehensive evaluation of *Crossandra infundibuliformis* (L.), a medicinal plant collected from the Jamia Salafiya Pharmacy College Campus in Kerala, South India. Thorough authentication by experts from the Institute of Forest Genetics and Tree Breeding, Coimbatore, ensured the reliability of the plant material, which was deposited in the Fischer Herbarium under accession number 25233.An ethanolic Soxhlet extraction method was employed to extract bioactive compounds from finely ground and dried leaves of *Crossandra infundibuliformis* (L.). This technique allows for the efficient extraction of phytochemicals and secondary metabolites. The resulting extract exhibited a semi-solid consistency with a distinctive brown colour, indicating the presence of specific compounds or pigments. *In vitro* antioxidant assays using the DPPH radical scavenging method revealed the significant antioxidant activity of the ethanolic extract. Ascorbic acid, a standard antioxidant, exhibited an IC_{50} of 1.562µg/mL, whereas the extract demonstrated a slightly higher IC_{50} of 12.5µg/mL, emphasising its substantial antioxidant potential. The antibacterial activity of the ethanolic extract was evaluated using the well-diffusion method, which showed notable inhibition zones, particularly against *Staphylococcus aureus* (17mm). The efficacy of this extract is comparable to that of tetracycline. Electron microscopic examination confirmed bacterial cell lysis and shrinkage, supporting the antibacterial activity of the ethanolic extract. The inhibitory concentration was determined using a minimal inhibitory concentration (MIC) assay, further highlighting the antimicrobial potential of the extract. Additionally, the ethanolic extract exhibited in vitro inflammatory activity by promoting the migration rate of fibroblasts in a scratch assay, suggesting its potential wound healing properties. In conclusion, *Crossandra infundibuliformis* (L.) showcases promising bioactivity, including antioxidant, antibacterial and wound healing properties, making it a valuable candidate for further exploration in pharmaceutical and healthcare applications. This study provides a comprehensive understanding of the medicinal potential of this plant and sets the stage for future research and development.

KEYWORDS

Crossandra infundibuliformis (L.), Antioxidant, Antibacterial and Wound healing properties.

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INTRODUCTION

Nature is a testament to the fascinating phenomenon of symbiosis, historically providing an abundance of medicinal plants that have traced the evolution of traditional medicine[1,2]. The pursuit of innovative remedies for ailments has been

seamlessly integrated with modern medicine throughout human history. India, with its rich heritage in traditional medicine such as Ayurveda, Siddha and Unani, has witnessed a resurgence in interest in alternative medicinal systems, particularly those derived from plants[3]. Plants, which have been fundamental in medicine since ancient times, play a pivotal role. The World Health Organization (WHO) notes that approximately 80% of the population in developed countries relies on traditional or alternative medicine for primary health care, with a substantial reliance on plant extracts and their active compounds. Herbal medicine, also known as phytotherapy or botanical medicine, is an ancient healing practice rooted in the therapeutic properties of plants. This traditional form of medicine, integral to human culture for millennia, integrates wisdom from indigenous cultures, historical texts and modern scientific research to tap into the healing potential of nature. Herbal remedies encompass a diverse range of plants, herbs, roots, flowers and fungi, each of which contains unique chemical compounds that contribute to their medicinal properties. These natural compounds address a spectrum of health concerns, from common ailments, such as colds and digestive issues, to chronic conditions, such as antibacterial and wound healing. Herbal medicine transcends cultural boundaries with diverse traditions that have evolved over centuries[4,5]. Examples include Traditional Chinese Medicine, Ayurveda, Native American herbalism, and European herbalism. Phytochemical compounds derived from plants are gaining attention for their potential to combat cancer. These naturally occurring substances contain a diverse array of chemical compounds, each with unique properties that contribute to their therapeutic potential against cancer. The exploration of phytochemicals as anticancer agents is rooted in the rich chemical diversity found in plant sources, which offers a vast repertoire of compounds with varying mechanisms of action[6].

Free radicals, generated through normal bodily processes and external factors, such as pollution, can wreak havoc by causing oxidative stress, which is linked to various diseases and aging. Antioxidants neutralise these free radicals and prevent them from causing damage to cells. They are like the body's clean-up crew, ensuring cellular health and reducing the risk of chronic conditions[7]. These powerful defenders are found in various foods, particularly fruits and vegetables. Vitamins such as C and E as well as minerals such as selenium act as antioxidants. In addition, many phytochemicals in plants, such as flavonoids and polyphenols, have antioxidant properties. The importance of antioxidants extends beyond cellular protection; they play a role in supporting the immune system, promoting heart health, and contributing to radiant skin.

Bacterial resistance is a growing challenge that requires urgent attention in the field of public health. Bacteria have evolved mechanisms to withstand the effects of antibiotics, rendering once-effective treatments ineffective[8]. The misuse and overuse of antibiotics in medicine and agriculture has accelerated this resistance, creating a pressing global health crisis. Resistant bacteria not only complicate the management of infectious diseases but also pose a threat to routine medical procedures such as surgery and chemotherapy[9]. Addressing bacterial resistance requires a multifaceted approach, emphasising judicious antibiotic use, development of novel antimicrobial agents, and international collaboration to implement effective strategies. Without concerted efforts to curb bacterial resistance, we risk entering a future in which common infections become life-threatening, underscoring the critical importance of a united front in the battle against antibiotic resistance. The present study evaluated the antioxidant, anti-inflammatory and antibacterial activities of *Crossandra infundibuliformis* (L.) ethanolic extract of *Crossandra infundibuliformis*.

MATERIAL AND METHODS

The acquisition and authentication of *Crossandra infundibuliformis* (L)

Crossandra infundibuliformis (L.) plants were collected at the Jamia Salafiya Pharmacy College Campus, Pulikkal, Malappuram District, Kerala, South India, and subjected to thorough authentication by a plant authentication expert from the Institute of Forest Genetics and Tree Breeding, Coimbatore. A sample specimen was deposited in the Fischer Herbarium under the accession number 25233. Freshly harvested leaves were air-dried in the shade. The dried leaves were finely ground using a mechanical grinding machine and the resulting powder was sifted through a mesh of size 60μm to achieve the desired particle size. The reduced-size plant material was carefully stored in an airtight container for the duration of this study[10].

Preparation of the ethanolic extraction

Crossandra infundibuliformis (L.) Ethanolic Soxhlet extraction is a specific method employed to extract bioactive compounds from *Crossandra infundibuliformis* (L.) plant material using ethanol (ethyl alcohol) as the solvent and the Soxhlet extraction technique. This process allows for efficient extraction of various phytochemicals, secondary metabolites, and potentially medicinal compounds from plants. The plant material, often the leaves or other relevant parts, is first finely ground or powdered to increase the surface area and facilitate extraction. Finely ground plant material is loaded into a porous thimble, which is typically composed of cellulose or glass microfibres. The Soxhlet extraction apparatus consisted of a Soxhlet extractor (containing the sample thimble), flask to hold the ethanol solvent and condenser. The thimble containing the sample was placed above the flask. In this process, ethanol is heated in the flask, causing it to vaporise and rise through a vertical reflux system. As it condenses in the condenser, ethanol drips back into the thimble containing plant material. This cycle continues, allowing the solvent to continuously extract various compounds from the plant material. The condensed extract, which contained dissolved phytochemicals and bioactive compounds from *Crossandra infundibuliformis* (L.), accumulated in flasks. The extraction process was repeated until the desired level of extraction was achieved, which varied depending on the specific target compound. Ethanolic Soxhlet extraction was chosen when ethanol was suitable for extracting a range of compounds from *Crossandra infundibuliformis* (L.) for various purposes, such as pharmaceutical or herbal applications. Ethanol is known for its ability to dissolve both polar and nonpolar compounds, making it versatile for the extraction of a wide spectrum of phytoconstituents. The solvent was selected based on the specific compounds of interest and their solubility. Additionally, safety precautions, including proper ventilation and adherence to flammability guidelines, were followed when working with ethanol owing to its flammable nature[11].

In vitro antioxidant assays - DPPH (Diphenyl-2-picrylhydrazyl) radical scavenging activity

The free radical scavenging activity of the ethanolic extract was measured in terms of its hydrogen-donating or radical-scavenging ability using the stable DPPH radical method. DPPH solution (0.1mM) in ethanol was prepared and 1.0ml of this solution was added to 3.0ml of the extract solution in water at different concentrations. After 30 min, the absorbance was measured at 517nm. A lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging ability was calculated using the following equation:

% Of DPPH of radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

Where control is the absorbance of the control reaction and the test is the absorbance in the presence of extracts. The mean values were obtained from triplicate experiments.

Antibacterial activity of ethanolic extract

Culture media and clinical microorganisms

Crossandra infundibuliformis ethanolic was used to examine the antibacterial activity against clinically important microorganisms. Clinical microbial cultures of *Staphylococcus aureus, Proteus*

mirabilis, Enterococcus faecalis, Klebsiella pneumoniae, and Pseudomonas aeruginosa were procured from the Clinical Microbiology Lab Coimbatore, India. Clinical microorganisms were subcultured independently in 5mL of sterile nutrient broth for 24h at 37°C and adjusted to match the turbidity of the MacFarland standard.

Kirby-Bauer method (well-diffusion method)

The potential antibacterial properties of various compounds, including *Crossandra infundibuliformis* ethanolic, were investigated using the well diffusion method. Fresh agar plates were prepared using double-strength Mueller-Hinton Agar (MHA) medium (7.6g in 100mL) from Himedia, which had been previously sterilised by autoclaving at 121°C for 15 min. Clinical microbial cultures were then applied to the surfaces of MHA plates using sterile cotton swabs.

Wells were created within the agar using a sterile borer and 100µL of the DMSO-diluted lyophilised *Crossandra infundibuliformis* ethanolic extract (n=2 replicates) was aseptically added to these wells. As a control, ciprofloxacin (5µg/mL) was introduced into separate agar wells. The culture plates were refrigerated for 30 min to allow phytoconstituents to diffuse into the agar. The plates were then incubated at 37°C for 24h. To assess the antibacterial activity, the diameters of the inhibition zones were measured using a zone reader from Himedia.

Determination of minimal inhibitory concentration (MIC)

The assay was carried out as described by Muddukrishnaiah *et al*[12], with modifications. Briefly, a 1% solution of 2, 3, 5-triphenyl-tetrazolium salt (TTC) was prepared by dissolving TTC in sterile water. Different concentrations of lyophilised *Crossandra infundibuliformis* ethanolic extract (250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90625µg/mL) and the respective controls were added to 1.0mL cell culture plates. One hundred microlitres of the test microorganism (*S. aureus*) was added to each well. The plates were then incubated overnight at room temperature for 24 h. 100µL TTC solution (0.5% w/v) solution was added in 1mL from each well containing the treated and the control cultures. The plates were then incubated at room temperature for 20 min. After 20 min, colour changes were observed in the MIC wells.

Scanning and Transmission Electronic Microscopy (FESEM and HRTEM) to examine antimicrobial effects of ethanolic extract

S. aureus that had been treated with the ethanolic extract of *Crossandra infundibuliformis* was centrifuged, washed twice and resuspended in PBS. The cells were fixed for 2h at room temperature in 2.5% glutaraldehyde, dehydrated with alcohol, dried with hexamethyldisilazane (HMDS) and coated with gold. FESEM and HRTEM were used to investigate the effect of ortho nitro aniline and ortho-anisidine on the bacterial cells (FESEM, TESCAN). From harvest to centrifugation, the sample preparation for transmission electron microscopy (TEM) was the same as that for SEM. A drop of the suspension was deposited on a copper grid and dried. The grids were run at 200 kV using a JEM-2100PLUS TEM (JEOL Ltd., Japan)[11].

***In vitro* inflammatory activity-scratch assay of *Crossandra infundibuliformis* ethanolic extract**

Fibroblast cells (L6 cell line) were grown in sterile six-well plates to form a confluent monolayer. Healthy monolayer cells were scraped straight line with sterile pipet tip. Detached cells were removed by rinsing with sterile phosphate buffer solution. *Crossandra infundibuliformis* ethanolic extract (50µg) and 0.2% FBS as control were added aseptically in the tested cells and incubated for 48hrs at 37°C cell culture incubator. After 48hr the 6 well plates were observed under a phase-contrast microscope, and the scratched cell growth pattern was observed.

RESULTS AND DISCUSSION

Plant collection and Authentication

Crossandra infundibuliformis (L.) plants were collected at the Jamia Salafiya Pharmacy College Campus, Pulikkal, Malappuram District, Kerala, South India and subjected to thorough authentication by a plant authentication expert from

the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. A sample specimen was deposited in the Fischer Herbarium under accession number 25233 (Figure No1).

Crossandra infundibuliformis (L.) selected medicinal plant collection and extraction

In the investigation of the extractive values of ethanolic solvents from *Crossandra infundibuliformis* (L.), various physical and chemical characteristics were meticulously recorded to unveil the intricate properties of the extracted substance. The sample, identified by the *Crossandra infundibuliformis* (L.) code, was extracted using ethanol as the solvent. The resulting consistency of the extract was noted as semi-solid, indicative of a substance possessing qualities between those of the liquid and solid states. The extract exhibited a distinctive brown colour, suggesting the presence of specific compounds or pigments within the extractive material. The extraction process yielded a percentage of 8.67, reflecting the efficiency of the extraction method in retrieving the desired components from *Crossandra infundibuliformis* (L.). These recorded parameters not only provide a comprehensive understanding of the physical nature of the extract but also contribute valuable insights into the potential bioactive compounds present in *Crossandra infundibuliformis* (L) which may hold significance in various applications.

Antioxidant activity of _Crossandra infundibuliformis_ (L.) ethanolic extract

Antioxidant evaluation through the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method, the quantification of inhibitory concentration 50% (IC_{50}) values serves as a pivotal metric. Ascorbic acid, a well-known and potent antioxidant, exhibits an impressive IC_{50} of 1.562µg/mL, emphasising its robust ability to effectively neutralise free radicals. In contrast, the ethanolic extract derived from *Crossandra infundibuliformis* (L.), a plant with diverse bioactive compounds, demonstrated a slightly higher IC_{50} of 12.5µg/mL. This discrepancy in IC_{50} values signifies varying antioxidant potency between the standard ascorbic acid and the natural extract. While ascorbic acid showed exceptional efficacy in scavenging DPPH radicals, the ethanolic extract, with an IC_{50} of 12.5µg/mL, underscored its substantial antioxidant activity, albeit at a slightly higher concentration. The DPPH method, renowned for its simplicity and reliability, reveals the antioxidant potential of compounds and provides valuable insights into their free radical-scavenging capabilities. Comparative analysis of IC_{50} values illuminates the nuanced landscape of antioxidant activities, paving the way for further exploration of natural sources in the quest for potent antioxidants (Figure No.2).

Antibacterial activity (well-diffusion method)

Crossandra infundibuliformis ethanolic extract studied antibacterial activity. *Crossandra infundibuliformis* showing antibacterial activity against *S. aureus* with 17mm (Table No.1).

Electron microscopy (FE-SEM and HR TEM) examination of _Crossandra infundibuliformis_ (L.) ethanolic extract-treated bacterial cells

The morphology of *Crossandra infundibuliformis* (L.) ethanolic extract-treated bacterial cells was observed using an electron microscope. From the electronic microscopic observations (FE-SEM and HR TEM), bacterial cell lysis and shrinking confirmed that *Crossandra infundibuliformis* (L.) ethanolic extract (62.5µg/ml) inhibited the growth of the clinical bacteria *S. aureus* (Figure No.3 and Figure No.4).

***In vitro* inflammatory activity-scratch assay**

To characterise the potential influence of the molecules on skin fibroblasts, the cells were cultured. The extent of regrowth to close the scratch wound was measured after 0, 24 and 48 h of incubation in a medium containing *Crossandra infundibuliformis* (L.) ethanolic extract (50µg). The restoration of the full cellular density of the mesothelium in fibroblasts was faster in the molecular group than in the control group. In other words, *Crossandra infundibuliformis* (L.) ethanolic extract promoted the migration of fibroblasts at each time point (Figure No.5).

Table No.1: Antibacterial activity of *Crossandra infundibuliformis* ethanolic extract and tetracycline against clinical bacteria

S.No	Drug	*S. aureus*	*K.pneumoniae*
1	*Crossandra infundibuliformis* (L.) ethanolic extract	17	R
2	Tetracycline	16	R

Figure No.1: *Crossandra infundibuliformis* (L.)

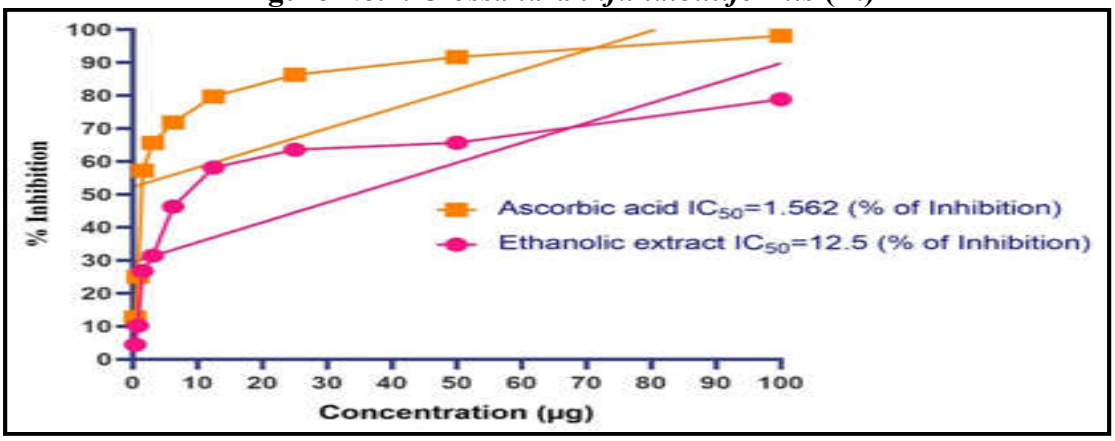

Figure No.2: Antioxidant activity of *Crossandra infundibuliformis* (L.) ethanolic extract-DPPH

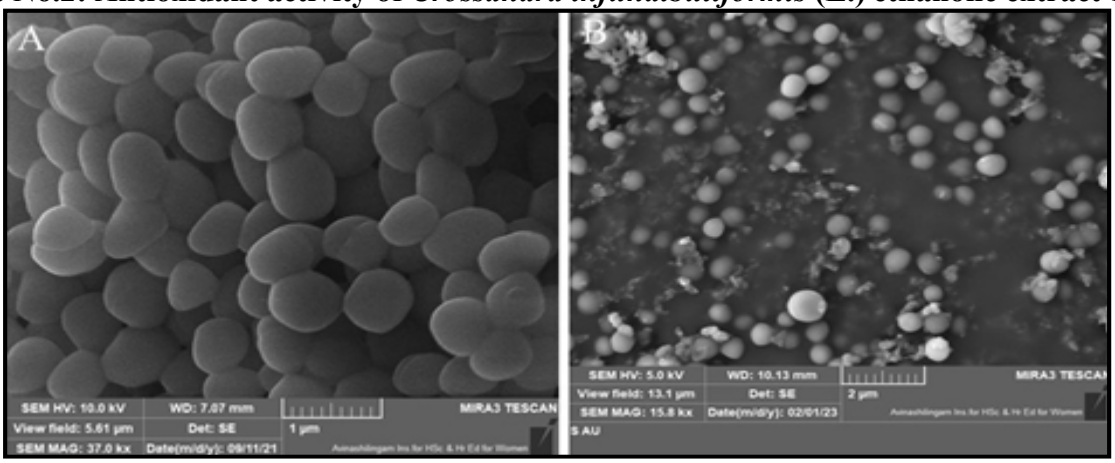

Figure No.3: A: FE-SEM observation of *S. aureus*, B: FE-SEM observation of *S. aureus treated with Crossandra infundibuliformis (L.) ethanolic extract* (bacterial cell damage)

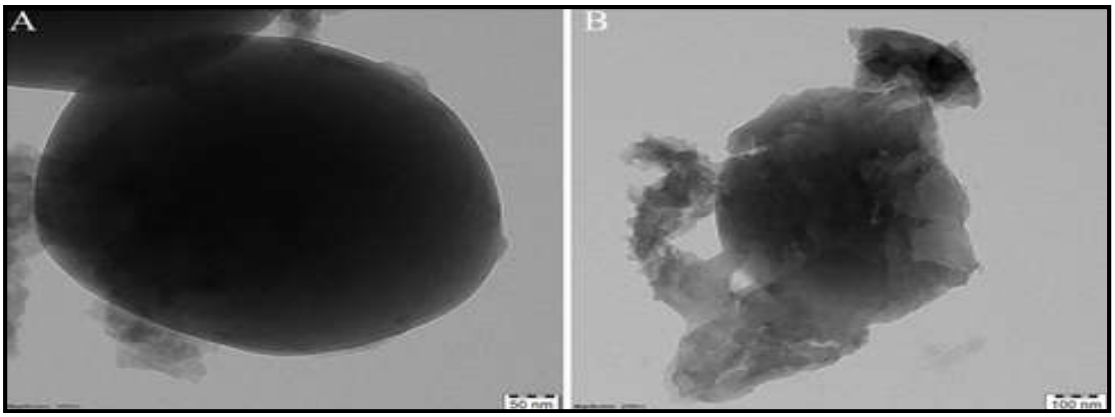

Figure No.4: A: HR-TEM observation of *S. aureus*; B: HR-TEM observation of *Crossandra infundibuliformis* (L.) ethanolic extract treated *S. aureus* (bacterial cell damage)

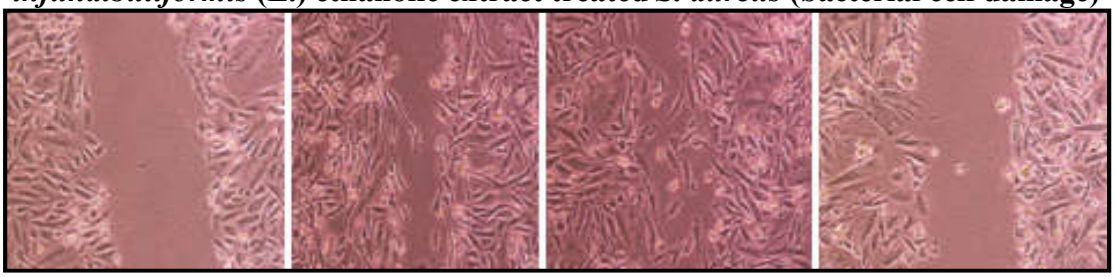

Figure No.5: *Crossandra infundibuliformis* (L.) ethanolic extract accelerated migration of fibroblasts in the scratch assay. (A) Scratch assay of fibroblasts treated with *Crossandra infundibuliformis* (L.) ethanolic extract for 0, 12 and 24 h and positive control (24 h)

CONCLUSION

A comprehensive study of *Crossandra infundibuliformis* (L.) conducted at the Jamia Salafiya Pharmacy College Campus in Kerala, South India and authenticated by experts from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, has provided valuable insights into the medicinal potential of this plant. The extraction process utilising ethanol as the solvent yielded a semi-solid extract with a distinctive brown colour, indicative of specific compounds within the extract. The recorded parameters shed light on the physical nature of the extract, contributing to our understanding of the bioactive compounds present in *Crossandra infundibuliformis* (L.). The antioxidant activity assessment, conducted using the DPPH method, revealed that the ethanolic extract possesses significant antioxidant capabilities, albeit at a slightly higher concentration than the standard ascorbic acid. This emphasises the potential of plants to scavenge free radicals, highlighting their role as a natural source of antioxidants. Furthermore, antibacterial activity against *S. aureus* demonstrated the effectiveness of the ethanolic extract, with a notable inhibition zone of 17mm. Electronic microscopic examination provided visual confirmation of bacterial cell lysis and shrinking, further supporting the antibacterial properties of the plant. The *in vitro* inflammatory activity assessment, employing a scratch assay on skin fibroblasts, highlighted the ability of the extract to accelerate fibroblast migration, indicating its potential wound healing properties.

ACKNOWLEDGMENT

I would like to thank the Department of Pharmacy, JJT University, Rajasthan, and the Centre for Biotechnology and Phyto Pharmacognosy Research, Kundampalyam, Coimbatore for providing facilities for conducting research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Remya S B *et al*. Antioxidant, antibacterial and wound healing properties of *Crossandra Infundibuliformis* (L.): A comprehensive study, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 10(4), 2022, 87-94.