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Evaluation of *Combretum indicum* Flower Extract for the Management of Ocular Pathogens: Pharmacognostic and Antimicrobial Perspectives

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Abstract: This study presents a comprehensive pharmacognostic and antimicrobial assessment of *Combretum indicum* flower extracts. Macroscopic analysis, along with physico-chemical profiling was performed to ensure quality and standardization. Moisture content, ash values, crude protein, fat, fiber, calcium, and phosphorus levels were determined using standard pharmacognostic methods. The extract was also tested for antimicrobial activity against gram-positive, gram-negative bacteria, and fungi using well diffusion and MIC methods. The results indicate significant antimicrobial efficacy and a strong phytochemical profile, supporting its traditional therapeutic uses.

Keywords: *Combretum indicum*, Pharmacognosy, Antimicrobial activity, Minimum inhibitory concentration, Crude extract, Flower analysis

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Introduction

Ocular infections encompass a wide spectrum of diseases affecting structures of and around the eye, from superficial conditions like conjunctivitis and keratitis to serious internal infections such as endophthalmitis. These infections may be caused by bacteria, fungi, viruses, or parasites, and often result from risk factors including contact lens wear, ocular trauma, surgery, or compromised immunity. A leading Gram-negative pathogen associated with contact lens-related bacterial keratitis, *P. aeruginosa* is notorious for its aggressive virulence factors and rapid corneal tissue destruction. Its secretion of proteases and exotoxins can cause liquefactive necrosis, leading to corneal weakening and perforation (Mehta *et al.*, 2022). Recent studies on Indian ocular isolates have reported frequent presence of Exo U, Exo Y, phospholipase C, and LasB genes, all contributing to biofilm formation, immune evasion, and drug resistance (Sharma *et al.*, 2024). Gram-positive diplococcus, *Streptococcus pneumoniae* is frequently implicated in ocular infections such as keratitis, conjunctivitis, and endophthalmitis. Its virulence arsenal includes the polysaccharide capsule, pneumolysin, neuraminidase, and zinc-metalloproteinases, facilitating immune evasion and causing rapid liquefactive necrosis of the cornea (Das *et al.*, 2024). In developing regions, *Streptococcus pneumoniae* may be one of the most prevalent causes of corneal ulcers, and even with prompt treatment, visual prognosis is often guarded with high rates of perforation and poor final acuity (Kulkarni *et al.*, 2023). *Staphylococcus aureus* becomes pathogenic when barrier defects occur. It is a key contributor to bacterial keratitis and conjunctivitis, especially in environments with rising antimicrobial resistance. Staphylococcal toxins and biofilm-forming capacity complicate treatment, particularly in implant-associated or contact lens-related infections (Verma *et al.*, 2023). *Candida albicans* and non-albicans species like *Candida tropicalis*, are emerging causes of fungal keratitis, particularly in tropical and agricultural regions where ocular trauma with vegetative matter is common (Roy *et al.*, 2025). *C. albicans* forms biofilms and can colonize ocular surfaces or contact lens cases, enabling persistence and resistance to antifungal treatments (Panda *et al.*, 2023). Non-albicans species such as *C. tropicalis* are increasingly isolated and associated with severe corneal ulcers and longer healing times (Raghunathan *et al.*, 2023).

Pharmacognosy, the scientific study of bioactive natural products derived from plants, animals, and minerals, plays a pivotal role in both clinical and nutritional sciences. Clinically, it forms the basis for the discovery and development of plant-based therapeutic agents, including antibiotics, anticancer agents, and immuno-modulators (Singh *et al.*, 2025). Several modern drugs such as paclitaxel, artemisinin, and morphine are derived directly from natural sources, highlighting the critical translational bridge pharmacognosy provides between traditional medicine and modern pharmaco-therapy (Das *et al.*, 2024).

From a nutritional standpoint, pharmacognosy contributes to understanding the health-promoting effects of phytochemicals such as flavonoids, tannins, alkaloids, and terpenoids. These bioactives not only offer antioxidant and anti-inflammatory benefits but also help in managing metabolic disorders, cardiovascular diseases, and gut health (Patel *et al.*, 2023).

Combretum indicum (L.) DeFilipps, commonly known as Rangoon Creeper, belongs to the Combretaceae family. Literature suggests the hardy nature and predominant presence of medically important phytometabolites within these plants (Lima *et al.*, 2012; Alfei *et al.*, 2022). *C. indicum* is traditionally used in Asian medicinal systems for its antimicrobial, anti-inflammatory, and antiparasitic properties. Despite its widespread traditional usage, standardized pharmacognostic and antimicrobial validation remains limited. This study aims to assess key pharmacognostic parameters *Combretum indicum* flower extract and evaluate its antimicrobial activity against selected ocular pathogens.

Materials and Methods

Collection and Preparation of Samples

Flowers that appeared healthy were collected from different branches of *Combretum indicum* from Ottapalam, Palakkad, Kerala, India. The plant authentication was done at Botanical Survey of India, Southern Regional Centre, Coimbatore with reference no: BSI/SRC/5/23/2023/Tech-638. In the laboratory, the flower samples were washed 2-3 times with running fresh water. The flower material was then air-dried in the shade. After complete drying, the plant material (500 g) was ground using a mechanical grinder, and the powder was stored in small labelled plastic bags.

C. indicum flower was extracted using distilled water, ethanol, acetone and n-Hexane by using Soxhlet's apparatus. The solvents were evaporated using a rotating vacuum evaporator, and the filtrate was stored in a desiccator for future use.

Phytochemical Analysis

The ethanolic extracts of *C. indicum* flowers went through preliminary phytochemical screening according to the standard procedures established by Gupta *et al.* (2010), which confirmed the presence of saponins, flavonoids, steroids, alkaloids, terpenoids, quinones, cardiac glycosides, phenols, fats and oils, proteins, and carbohydrates.

Pharmacognostic Evaluation

Macroscopic examination was conducted to observe key physical characteristics of *Combretum indicum* flowers, including colour, texture, shape, and odour (Khandelwal *et al.*, 2015). Moisture content was determined using the hot air oven drying method at 105°C following the Indian Pharmacopoeia (2014) guidelines. Total ash and acid-insoluble ash values were determined via muffle furnace incineration at 500–600°C (Khandelwal *et al.*, 2015; Kokate *et al.*, 2021; Siddiqui *et al.*, 2021). Crude fat was estimated using Soxhlet extraction with petroleum ether as the solvent (Khandelwal *et al.*, 2015; Kokate *et al.*, 2021). Crude fibre was analyzed using acid-alkali digestion techniques ((Khandelwal *et al.*, 2015; Kokate *et al.*, 2021). The Kjeldahl method was employed to determine crude protein levels (Pandey *et al.*, 2023). Calcium and phosphorus levels were assessed through mineral analysis techniques described in standard protocols (Pandey *et al.*, 2023).

Antimicrobial Studies

The antimicrobial efficacy of the ethanolic flower extract was evaluated against selected bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*) and fungi (*Candida albicans*, *Candida tropicalis*). The minimum inhibitory concentration (MIC) was determined using Mueller-Hinton Agar plates with extract concentrations of 100 µg/ml to 400 µg/ml (Balouiri *et al.*, 2016). Zones of inhibition were measured post-incubation at 37°C for 24-48 h. The well diffusion method was also employed to confirm antimicrobial activity using standard positive (Streptomycin/Amphotericin) and negative (DMSO) controls (Agrawal *et al.*, 2023; Shinde *et al.*, 2024).

Results and Discussion

Phytochemical Analysis

Preliminary phytochemical screening revealed that the ethanolic extract of *Combretum indicum* flowers contained a wide range of bioactive constituents compared to other solvent extracts (Table 1). On this basis, the ethanolic extract was chosen for subsequent pharmacological and biological investigations.

Pharmacognostic Evaluation

The flowers are typically tubular in shape and are borne in drooping clusters. They undergo a noticeable colour change as they mature, starting from white, transitioning to pink, and eventually turning deep red. Each flower consists of five spreading petals that are soft and delicate in texture. The flowers are medium-sized with a slender floral tube.

Moisture content of *Combretum indicum* flower was found to be 10.06%, which is within an acceptable range for dried plant materials. In pharmacognosy, this value is significant as it reflects the stability and shelf life of the extract and ensuring the preservation of bioactive compounds such as flavonoids, alkaloids, Terpenoids and glycosides. Lower moisture means a higher concentration of nutrients per gram, including proteins, fibres, and phytochemicals, making the extract more potent and efficient for therapeutic or dietary applications.

The total ash content of the flower powder was found to be 7.63% which reflects a good mineral profile, indicating that *Combretum indicum* flowers may serve as a natural source of essential minerals, contributing to various physiological functions such as bone health, nerve function, and enzyme activity. The acid-insoluble

Table 1: Qualitative analysis of phytochemicals from *Combretum indicum* (L.) flower extracts

S. No.	Phytochemical	Analysing method	Water	Ethanol	n-Hexane	Ethyl acetate
1	Alkaloids	Mayer's test	P	HP	N	P
2	Terpenoids	Copper acetate test	P	HP	N	P
3	Phenolic	Ferric chloride test	P	HP	N	P
4	Saponins	Foam test	HP	P	N	N
5	Flavonoids	Lead acetate test	P	HP	P	N
6	Quinines	NaOH test	HP	HP	P	N
7	Cardiac Glycosides	Keller Killani's test	P	P	N	N
8	Steroids	H_2SO_4 Test	N	P	P	N

P-Present; HP- Highly present; N- Not present

ash content of flower powder was found to be 0.34%. A low level like 0.34% is desirable, as it means the plant material does not contain significant amounts of indigestible inorganic matter, which could otherwise reduce digestibility or bioavailability of nutrients. This supports the safe use of the flower powder in nutraceuticals or herbal formulations.The calcium and phosphorus content were found to be 1.49% and 0.67%, respectively.

The total crude protein content of the flower powder was found to be 18.59%, indicating a substantial presence of nitrogenous compounds. High protein content suggests that the flower contains significant amounts of amino acids and related nitrogenous constituents, which may contribute to its therapeutic and nutritional properties.

The total crude fibre content of the *Combretum indicum* flower was found to be 9.41%, indicating a notable presence of indigestible plant components such as cellulose, lignin, and hemicellulose.This suggests that the flower may aid in gastrointestinal health, acting as a natural laxative by promoting bowel movement and improving digestion.

The Crude fat content of the flower powder was found to be 5.8%. Quantifying ether-soluble constituents provides insight into the presence of non-polar bioactive compounds, such as essential oils, waxes, sterols, and lipid-soluble secondary metabolites. From a nutritional perspective, fats are essential macronutrients that serve as a dense source of energy and are vital for the absorption of fat-soluble vitamins (A, D, E, and K).

Antimicrobial Studies

The antimicrobial activity of *Combretum indicum* flower extract was evaluated against a panel of clinically relevant ocular pathogens. These include: *Pseudomonas aeruginosa*: A frequent cause of microbial keratitis, particularly in contact lens users; *Staphylococcus aureus* and *Streptococcus pneumoniae*: implicated in conjunctivitis, blepharitis and endophthalmitis; and *Candida albicans* and *Candida tropicalis*: Known to cause fungal keratitis and ocular candidiasis, especially in immuno-compromised patients or post-trauma. The extract demonstrated clear inhibitory activity against all organisms

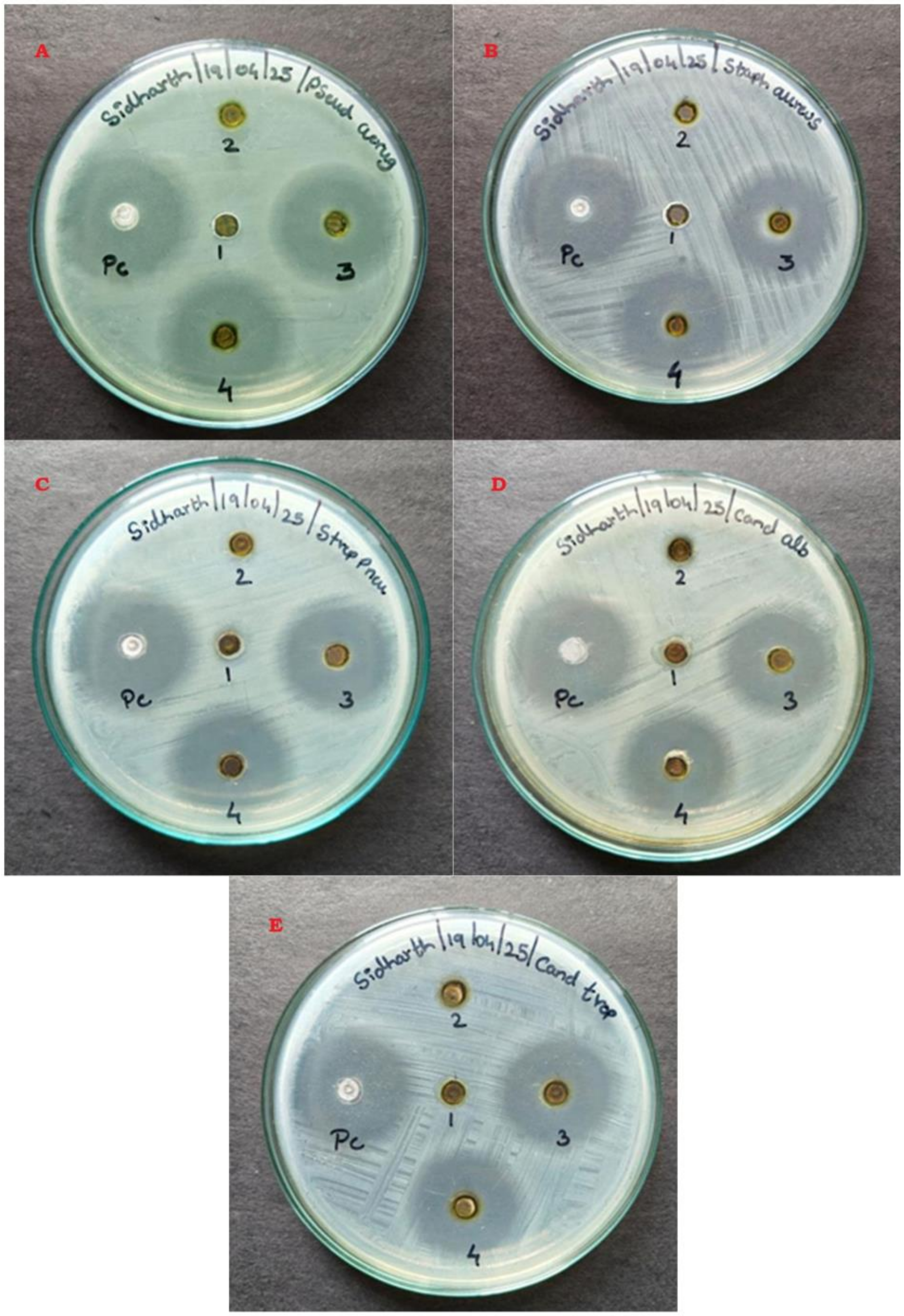

Fig. 1: MIC of flower extract against: (A) *Pseudomonas aeruginosa*; (B) *Staphylococcus aureus*; (C) *Streptococcus pneumoniae*; (D) *Candida albicans*; and (E) *Candida tropicalis.*

tested. The minimum inhibitory concentration (MIC) for all pathogens was recorded at 15-18 mmwith comparable results to the standard antibiotics and antifungal agents (streptomycin and amphotericin B). These results underscore the potential role of *Combretum indicum* extract in developing ocular formulations to manage superficial and deep eye infections, especially considering the increasing resistance of these pathogens to conventional antimicrobials. The MIC of *Combretum indicum* flower extract along with the standard drug againstocular pathogens are illustrated in Figure 1.

The extract exhibited inhibitory effects against

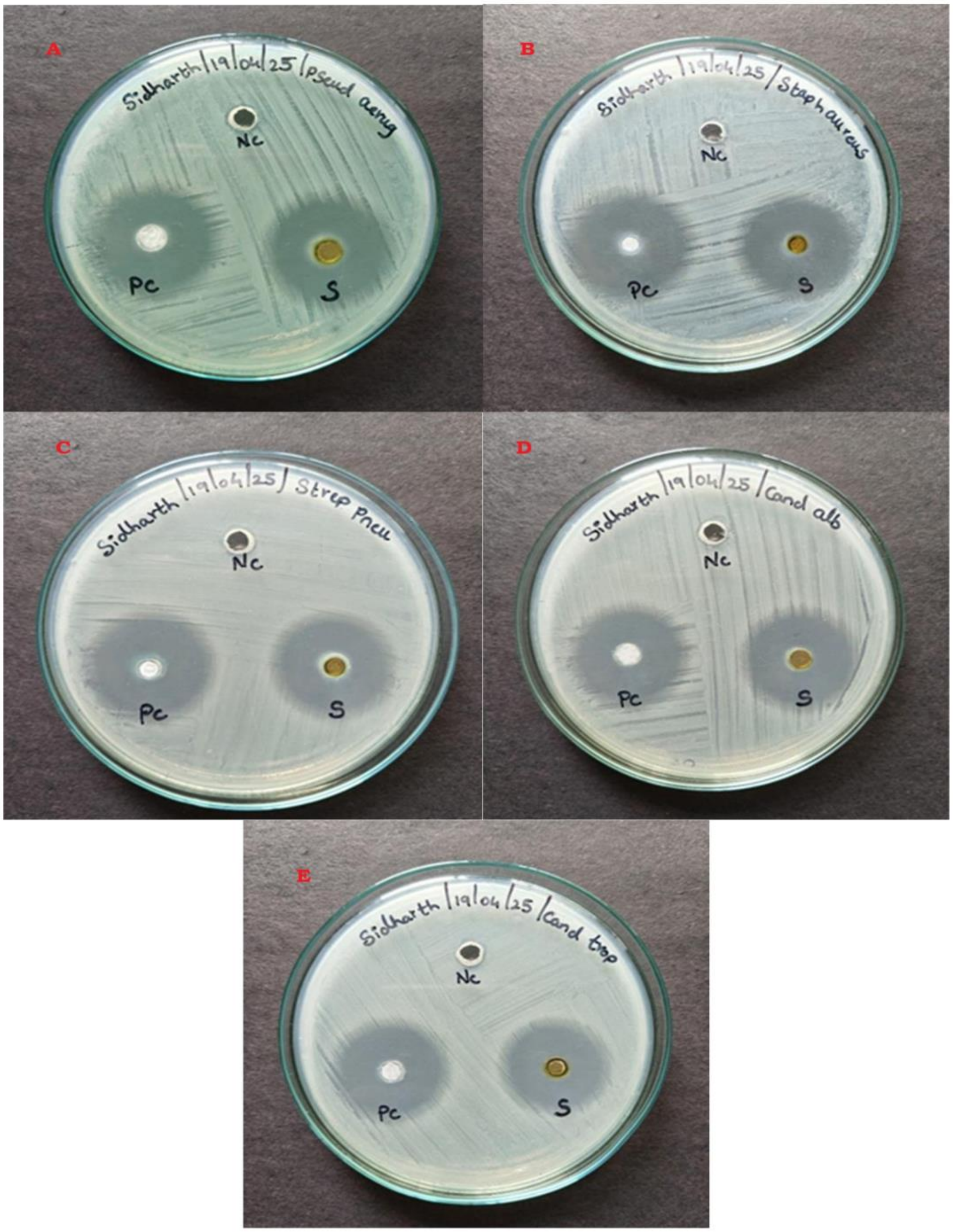

Fig. 2: Well diffusion assay of flower extract against: (A) *Pseudomonas aeruginosa*; (B) *Staphylococcus aureus*; (C) *Streptococcus pneumoniae*; (D) *Candida albicans*; and (E) *Candida tropicalis.*

all test organisms. MIC for each organism was found to be 300 µg/ml, with inhibition zones ranging from 16–18 mm. In well diffusion assays, zone diameters closely matched those of the standard positive controls. Specifically, *Pseudomonas aeruginosa* exhibited 18 mm (control: 18 mm), *Staphylococcus aureus* 17 mm (control: 18 mm), *Streptococcus pneumoniae* 17 mm (control: 18 mm), *Candida albicans* 16 mm (control: 16 mm), and *Candida tropicalis* 15 mm (control: 16 mm). The well diffusion assay of *Combretum indicum* flower extract along with the standard drug against ocular pathogens are illustrated in Figure 2.

Pharmacognostic parameters provide fundamental quality control standards for the identification and authentication of plant material. The low moisture content ensures minimal microbial contamination and enhanced stability. Acid-insoluble ash values confirm the absence of external contamination such as silica. The high protein and fibre contents suggest potential nutritional and therapeutic applications. The observed antimicrobial activity supports the traditional use of *Combretum indicum* and highlights the presence of bioactive constituents such as alkaloids, flavonoids, and terpenoids known for antimicrobial properties.

Conclusion

Combretum indicum has significant medicinal potential due to its wide range of bioactive compounds with varying pharmacological activities. The current study establishes pharmacognostic standards for *Combretum indicum* flower extracts and confirms its antimicrobial efficacy against a wide range of ocular pathogens. These findings reinforce its traditional uses and provide a scientific basis for further development as a phytotherapeutic agent.

Ethical Statement

This study did not involve the use of animals, and therefore ethical approval for animal experimentation is not needed.

Author Contributions

Sidharth K.: Conceptualization, methodology, interpretation and manuscript preparation; *Narayanasamy K.*: Conceptualization, editing and supervision; *Nirmala Devi N.*, *Mohan Sruthy*, *Menon Parvathy*, *Reshma B.P.*: Interpretation of results and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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