



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**ASSESSMENT OF ANTIMICROBIAL AND ANTIFUNGAL EFFECTS
OF *TYPHA ELEPHANTINA* HERBAL SPRAY: EVIDENCE FROM
ZONE OF INHIBITION ASSESSMENT**

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Received 24th March 2024; Revised 30th April 2024; Accepted 27th Aug. 2024; Available online 1st July 2025

<https://doi.org/10.31032/IJBPAS/2025/14.7.8990>

ABSTRACT

Typha elephantina, commonly referred to as elephant grass, has historically been utilized for various medicinal purposes. This research aimed to evaluate the antimicrobial and antifungal attributes of extracts from *Typha elephantina* by measuring zones of inhibition. *Typha elephantina* is characterized as an herb, colonial; rhizomatous, perennial plant featuring large, slender, green stalks topped with brown, fluffy, sausage-shaped flowering heads. As a perennial herb, it typically grows up to 3 meters (9 feet) and frequently forms extensive colonies along the 'shores of shallow ponds, lakes, and marshes'. Standard microbiological techniques were employed in this study to examine the efficacy of *Typha elephantina* against a variety of bacterial and fungal strains. The findings discussed herein offer valuable insights into the potential antimicrobial and antifungal properties associated with *Typha elephantina*.

Keywords: *Typha elephantina*, Anti-bacterial, Anti-fungal, Herbal spray, Zone Inhibition

INTRODUCTION:

Natural products have long been a source of medicinal compounds, and plants, in particular, has played an important role in the discovery of antimicrobial agents. *Typha elephantina*, a plant with a rich history in traditional medicine, is known for its diverse pharmacological properties. *Typha elephantina*, commonly known as elephant grass, has been a subject of increasing scientific interest due to its rich traditional medicinal uses. With a history deeply rooted in ethnomedicine, *Typha elephantina* has been employed for various therapeutic purposes across different cultures [1]. The herb displays a variety of forms and is distributed across numerous countries worldwide. In Turkish traditional medicine; the flowers of the plant are employed for healing of wound, while the leaves possess analgesic, astringent, along with dehydrating, simultaneously as an antioxidant, diuretic, and hemostatic properties. They are utilized to address various conditions such as nosebleeds, hematemesis, hematuria, in uterine bleeding, post-partum abdominal pain, gastralgia, scrofula, and abscesses, although caution is advised in case of pregnant women. In Pakistan; pollen of *Typha elephantina* is consumed due to its antipyretic properties, ability to increase urine flow, and efficacy in treating injuries. In south-region of Iraq; Al Ahwar, pollen

dried powder is used to enhance male fertility, earning it the moniker "Viagra of Al Ahwar." Studies have indicated that *T. elephantina* demonstrates effectiveness in reducing bacterial contamination and decreasing enterobacteria levels in the human intestine, thus potentially mitigating the onset of various diseases. Recent attention has focused on its potential antimicrobial and antifungal properties, warranting systematic investigation to elucidate its efficacy and broaden our understanding of its pharmacological potential.

The rise of antimicrobial resistance has underscored the urgent need for novel therapeutic agents derived from natural sources [2]. *Typha elephantina*, with its diverse phytochemical composition, emerges as a promising candidate for such exploration. Preliminary studies suggest the presence of bioactive compounds in *Typha elephantina* that may exhibit inhibitory effects against bacteria and fungi [3]. This study seeks to contribute to the growing body of knowledge surrounding *Typha elephantina's* medicinal properties, specifically in the context of its antimicrobial and antifungal potential. The assessment will be conducted through the widely accepted zone of inhibition method [4], providing tangible evidence of the plant's efficacy against key microbial

strains. The outcomes of the research may open the way in the development of new therapeutic agents derived from *Typha elephantina*, addressing the pressing global challenge of antimicrobial resistance [5].

MATERIAL & METHODS:

Typha elephantina inflorescence were collected and subjected to extraction using appropriate solvents. The extracted substances were subsequently evaluated against a variety of bacterial strains, encompassing; both i.e. Gram-positive (like *Staphylococcus aureus*) and Gram-negative (such as *Escherichia coli*) bacteria. Furthermore, their anti-fungal efficacy was tested against prevalent fungal strains like *Candida albicans*. The “Agar well diffusion technique” was utilized to gauge the inhibition zones for each type of microorganism [6].

Evaluation of antibacterial activity utilizing the agar well diffusion technique:

In order of antibacterial effectiveness, of the test formulations was evaluated using the “Agar well diffusion” method. Very firstly, bacterial stock cultures, were revived by inoculating them into broth media and then incubated at 37°C for 24 hours. Nutrient Agar/Muller Hilton agar media plates were prepared for the antibacterial assay. Each separate plate was inoculated with 0.1 ml aliquot of the bacterial suspension (10⁵-10⁶ CFU/ml) containing *Staphylococcus aureus*

and *E. coli*, respectively, which was evenly spread on the plate under sterile conditions. The suspension on each plate was allowed to dry for 20-25 minutes. Subsequently, wells of 6 mms diameter, were created in the solid medium with the use of a sterile cork – borer and filled with different volumes/concentrations of test samples. All plates; were then incubated at 37°C for 24 hours. The antibacterial activity of each sample, was determined by measuring the diameter of the inhibition zone (in mm) around to the each well. Three replications were performed for each test formulation against each test organism. The results were compared with alcohol as control.

Procedure for In-vitro antifungal activity determination:

The” In-vitro”, anti-fungal activities of sample formulations were determined using either the cup-plate method or the agar well diffusion assay. ‘Fungal stock- cultures’ were kept, at 4°C on slants of the Sabouraud dextrose Agar [7]. For the experiment, the active- cultures were prepared by transferring a loopful of cells from the stock cultures to appropriate test -tubes containing nutrient broth, for the fungi or sterile distilled water, and then incubated for 24 hours at 37°C.

The assay, was attributed by cup-plate technique [8]

The necessary amount of the “Sabouraud dextrose agar”, was dissolved in 100 ml of

distilled water while continuously heating and stirring to maintain a pH of 5.5. The prepared medium was then autoclaved at 121°C for 15 minutes for sterilization. Sterile glass Petri plates were taken, and the media was poured into them under aseptic conditions. After the media solidified, the surface of the agar plate was pierced using a sterile cork borer to create cups with a diameter of 6 mm. Fungal species were inoculated by streaking them on the surface of the media. The prepared wells were then filled with the sample formulation and allowed to rest for at least 30 minutes before being incubated for 24 to 48 hours at a temperature of $25 \pm 1^\circ\text{C}$. Following incubation, the most consistent outer diameter, of the inhibition- zone was measured in millimeters. Each assay was performed in triplicate.

RESULTS:

The, zone of inhibition assessment revealed significant antimicrobial and antifungal activity of *Typha elephantina* extracts. The results demonstrated different degrees of the, inhibitory effects against; both “Gram-positive and Gram-negative bacteria”. *Staphylococcus aureus*, a common pathogenic bacterium, showed a notable zone of inhibition, indicating the effectiveness of *Typha elephantina* against this strain. The findings from the zone of inhibition assessments provide scientific justification for the antibacterial activity of

the test formulations against “*Staphylococcus aureus*”.

The anti-bacterial; activity of the test formulations, against “*Staphylococcus aureus*” was assessed through, zone of inhibition measurements, where the lotion with a volume of 50 μl and a concentration of [$\mu\text{g}/\text{ml}$] displayed an average diameter of 2.66 ± 0.20 cm. Similarly, the lotion with a volume of 100 μl and the same concentration exhibited an average diameter of 3.3 ± 0.1 cm, indicating a notable increase in inhibitory effect compared to the lower volume formulation. In contrast, the control group, consisting of sterile distilled water (50 μl), showed no discernible zone of inhibition against *Staphylococcus aureus*. The absence of a zone of inhibition in the control group, represented by sterile distilled water (50 μl), serves as a critical control, confirming that the observed inhibitory effects are attributable to the components within the test formulations rather than any artifacts introduced during the experiment (**Table 1**).

Similarly, The findings of the anti-bacterial activity against *E. coli*, as determined by the, zone of inhibition assessments, revealed that the lotion with a volume of 50 μl and a concentration of [$\mu\text{g}/\text{ml}$] displayed an average diameter of 2.8 ± 0.35 cm. Similarly, the lotion with a volume of 100 μl and the same concentration exhibited an average diameter of 3.06 ± 0.11 cm, indicating a

modest increase in inhibitory effect. In contrast, the control group (50 µl sterile distilled water) showed no observable zone of inhibition against *E. coli*. These results suggest a discernible but modest enhancement in inhibitory effect with higher formulation volume (**Table 2**).

Moreover, the antifungal activity of *Typha elephantina* was evident in the zones of inhibition observed against *Candida albicans*. The robust inhibitory effects on fungal growth suggest the potential application of *Typha elephantina* in combating fungal infections.

The antifungal activity of the test formulations against *Candida albicans* was

evidenced through zone of inhibition assessments, where the lotion with a volume of 50 µl and a concentration of [µg/ml] exhibited an average diameter of 4.13±0.11 cm. Similarly, the lotion with a volume of 100 µl and the same concentration displayed an increased average diameter of 4.46±0.50 cm, indicating a notable enhancement in inhibitory effect compared to the lower volume formulation. In contrast, the control group, consisting of sterile distilled water (50 µl), showed no observable zone of inhibition against *Candida albicans* (**Table 3**).

Table 1: Zone of Inhibition of Test formulations against *Staphylococcus aureus*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
Lotion	50 µl	2.9	2.5	2.6	2.66±0.20
Lotion	100 µl	3.4	3.2	3.3	3.3±0.1
Control (Sterile Distilled Water)	50 µl	No Results found			

Table 2: Zone of Inhibition of Test formulations against *E.coli*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
Lotion	50 µl	3.2	2.5	2.8	2.8± 0.35
Lotion	100 µl	3.0	3.2	3.0	3.06±0.11
Control (Sterile Distilled Water)	50 µl	No Results found			

Table 3: Zone of Inhibition of Test formulations against *Candida albicans*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
Lotion	50 µl	4.2	4	4.2	4.13±0.11
Lotion	100 µl	5	4.4	4	4.46±0.50
Control (Sterile Distilled Water)	50 µl	No result			

DISCUSSION:

In the in-vitro study of *Typha elephantina*, remarkable antibacterial and antifungal effects were observed. The observed zones of inhibition in this study provide compelling evidence of the antimicrobial

and antifungal properties of *Typha elephantina* extracts. The dose-dependent response, control group validation, and consistent replicability collectively strengthen the reliability and significance of these findings, contributing valuable

insights into the potential antimicrobial efficacy of the formulations. The variation in inhibitory effects across different microbial strains suggests a broad-spectrum activity, making *Typha elephantina* a promising candidate for further exploration in drug development. Assessing the potential of *Typha elephantina* extracts in agriculture for controlling plant pathogens could have dual benefits. If effective, these extracts might serve as eco-friendly alternatives to synthetic pesticides, contributing to sustainable agricultural practices. Synthesizing derivatives and analogs of the active compounds identified in *Typha elephantina* can lead to the development of compounds with improved efficacy and reduced side effects.

The potential bioactive compounds responsible for the observed effects need to be identified through further chemical analysis. Additionally, the mechanism of action underlying the antimicrobial and antifungal activities of *Typha elephantina* warrants in-depth investigation to enhance our understanding of its therapeutic potential.

CONCLUSION

This unique study contributes to new budding subject of evidence supporting the “anti-microbial and antifungal” properties, of *Typha elephantina*. The zones of inhibition observed against a range of microbial strains suggest that *Typha*

elephantina extracts may contain bioactive compounds with therapeutic potential. Further research is essential to isolate and characterize these compounds, paving the way for the sake of development of novel anti-microbial and anti-fungal agents derived from *Typha elephantina*.

The assessment of the antimicrobial and antifungal effects of *Typha elephantina* through zone of inhibition analysis provides a strong foundation for further research and development. The promising results presented in this study warrant continued exploration of *Typha elephantina* as a potential source of novel antimicrobial and antifungal agents with clinical applications. The comprehensive exploration of *Typha elephantina's* antimicrobial and antifungal properties should extend beyond basic efficacy assessments. Embracing a multidisciplinary approach, encompassing pharmacology, ethnobotany, chemistry, and ecology, will enhance our understanding of the full potential of *Typha elephantina* in addressing microbial infections and contribute to the development of sustainable and effective therapeutic strategies. These findings suggest the presence of bioactive compounds with notable therapeutic properties, showcasing the promising antimicrobial potential of *Typha elephantina* as a natural drug candidate.

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