

# Assessment of The Effect of Physical Factors on The Antimicrobial Activity of *Solanum surattense* (Burm. f.) Extract

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## Abstract:

*Solanum surattense* Burm. f., a medicinal plant widely used in traditional Indian medicine, exhibits diverse pharmacological properties, including antimicrobial activity. This study aimed to evaluate the effect of physical factors, temperature, pH, and light exposure, on the antimicrobial efficacy of its ethanolic plant extract (root, stem, and leaves). The extract was tested against four bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae* using the cup well method. The results indicated significant antibacterial activity, with *S. aureus* being the most susceptible ( $19.8 \pm 0.7$  mm ZOI). Temperature, extreme pH, and sunlight exposure significantly reduced activity, whereas low temperatures, neutral pH, and dark storage preserved efficacy. These findings highlight the sensitivity of bioactive compounds to environmental conditions and the need for optimal handling and storage. The study underscores the potential of *S. surattense* as a natural antimicrobial agent and provides a foundation for developing stable, plant-based therapeutic formulations.

**Keywords:** Antibacterial activity, Antifungal activity, *Solanum surattense*.

## 1. INTRODUCTION

*Solanum surattense* Burm. f., commonly known as the Indian nightshade, is a perennial herbaceous plant belonging to the Solanaceae family (Kirtikar et al., 2005; Rehman and Chaudhary, 1995). Indigenous to the Indian subcontinent, it thrives in arid and semi-arid regions, often growing along roadsides and wastelands (Hasan, 2024). Traditionally, various parts of this plant, roots, stems, leaves, fruits, and seeds, have been utilized in indigenous medicine to treat ailments such as asthma, cough, skin diseases, gastrointestinal disorders, and urinary problems (Chapra et al., 1986; Tekuri et al., 2019; Jain and Rao, 1977).

Phytochemical investigations have revealed that *S. surattense* contains a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and glycosides (Muruhan, 2013). These constituents are considered responsible for the plant's pharmacological activities, which include antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and antitumoral effects (Hasan, 2024; Gębarowska, 2022). Among these properties, antimicrobial activity has gained particular attention due to the increasing prevalence of antibiotic-resistant pathogens worldwide.

Previous studies have demonstrated that ethanolic plant extracts of *S. surattense* exhibit significant antibacterial activity against a range of bacterial pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae* (Tekuri et al., 2019). Similarly, seed extracts have been reported to inhibit bacteria involved in oral infections, such as *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* (Hasan, 2024). These findings underscore the potential of *S. surattense* as a source of natural antimicrobial agents for therapeutic use.

Despite the well-documented antimicrobial properties, the effect of physical factors, such as temperature, pH, and light exposure, on the bioactivity of *S. surattense* extracts remains poorly understood. Physical conditions can significantly influence the stability and efficacy of phytochemicals. For instance, elevated temperatures may degrade thermolabile compounds, while variations in pH can alter the solubility and ionization of active

constituents (Gębarowska, 2022). Additionally, exposure to light can induce photodegradation of sensitive compounds, reducing the potency of plant extracts (Murhan, 2013).

Understanding how these factors affect the antimicrobial activity of *S. surattense* is crucial for optimizing its therapeutic application. This knowledge can inform the development of standardized extraction, storage, and formulation protocols, ensuring that the plant's medicinal properties are preserved. Therefore, the present study aims to evaluate the impact of temperature, pH, and light exposure on the antimicrobial efficacy of *S. surattense* plant extract, providing insights into optimal conditions for their practical use.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material Collection and Authentication

Fresh and healthy whole plants of *Solanum surattense* Burm. f. was collected from Road sides and the adjoining area of Udaipur, Rajasthan, India during the flowering. The roadside plant specimens were carefully uprooted to include roots, stems, leaves, and fruits, ensuring no diseased or damaged parts were selected. The collected materials were initially washed with tap water to remove adhering soil and debris, followed by rinsing with distilled water to eliminate surface contaminants. The sample plant was identified at Herbarium of the Department of Botany, University of Rajasthan, Jaipur and was provided with the accession no. RUBL21956, *Solanum surattense*. The cleaned plant material was shade-dried at room temperature (25–28 °C) for 10–15 days until a constant weight was achieved. The dried material was then pulverized into a fine powder using a mechanical grinder and stored in airtight containers at room temperature for subsequent extraction and antimicrobial studies.

### 2.2. Preparation of plant extract

The collected whole plant were washed thoroughly under running tap water to remove dust and debris, followed by rinsing with distilled water. The plant (root, stem, leaf) were then air-dried at room temperature (25 ± 2°C) for 10 days and ground to a fine powder using a sterile electric grinder. 50 g of powdered plant material was extracted sequentially with chloroform, methanol, petroleum ether, benzene, acetone and distilled water using a soxhlet apparatus for 6–8 h each. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to yield a crude ethanolic extract. The extract was stored in amber-colored bottles at 4°C until further use.

### 2.3. Microbial Strains

The antimicrobial activity was evaluated against bacterial strains commonly implicated in human infections:

- *Staphylococcus aureus* (NCIM 2079)
- *Escherichia coli* (NCIM 2065)
- *Salmonella typhi* (NCIM 2501)
- *Shigella dysenteriae* (NCIM 2540)

All microbial cultures were obtained from the MICROBIQ Lab, Indore, India, and maintained on nutrient agar plate at 4°C (Mahalakshmi, 2019).

### 2.4. Preparation of Inoculum

Fresh bacterial cultures were grown overnight in nutrient broth at 37°C. The turbidity of the bacterial suspension was adjusted to match 0.5 McFarland standard (approximately  $1.5 \times 10^8$  CFU/mL) to ensure uniform inoculation (Gębarowska, 2022).

### 2.5. Assessment of Antimicrobial Activity

The antimicrobial activity of *Solanum surattense* extracts was evaluated using the cup well method. Sterile Mueller–Hinton agar plates were uniformly inoculated with the test pathogenic microorganisms. Wells of 8 mm diameter were aseptically bored into the agar, and a defined volume of the extract was added to each well. To assess the effect of physical factors, extracts were pre-treated under different conditions such as temperature (e.g., 40°C, 60°C, 80°C), pH (e.g., 4, 7, 9), and exposure to light or storage periods before application. Plates were incubated under optimal conditions for the respective microorganisms (usually 18–24 h at 37°C for bacteria). Antimicrobial efficacy was determined by measuring the zones of inhibition surrounding each well. Standard antibiotics and solvent controls were included for comparison. All experiments were performed in triplicate to ensure reproducibility.

## 2.6. Evaluation of Physical Factors

### 2.6.1. Temperature:

Aliquots of the extract were exposed to 4°C, 25°C, 37°C, and 60°C for 30 minutes. Post-treatment, antimicrobial activity was measured using the cup well method (Hasan, 2024).

### 2.6.2. pH:

The pH of the extract was adjusted to 4, 7, and 9 using 0.1 M HCl or 0.1 M NaOH. The effect of pH on antimicrobial activity was assessed against all test bacteria (Muruhan, 2013).

### 2.6.3 Light Exposure:

Extract aliquots were exposed to direct sunlight for 1 hour to evaluate photostability. Control samples were kept in dark conditions. Post-exposure, antimicrobial activity was measured (Gębarowska, 2022).

## 2.7. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean  $\pm$  standard deviation (SD). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences between treatments, with  $p < 0.05$  considered statistically significant.

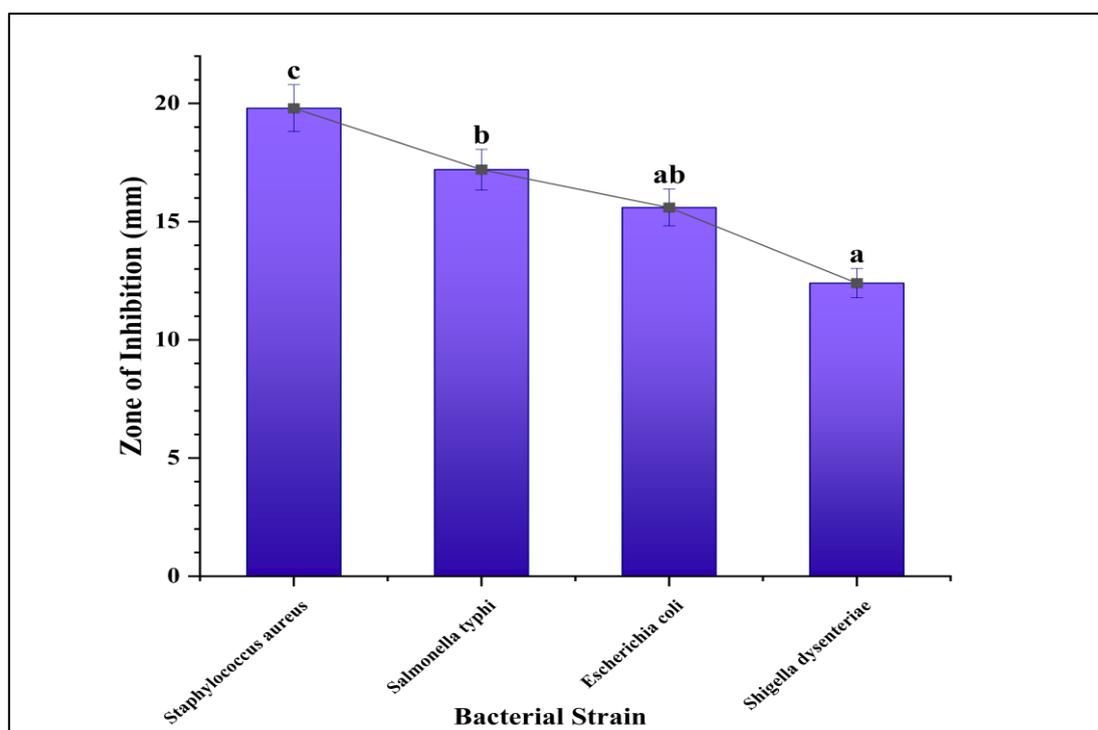
## 3. RESULT

### 3.1. Antimicrobial Activity of *Solanum surattense* Extract

The ethanolic plant extract of *Solanum surattense* exhibited significant antimicrobial activity against all tested bacterial strains. Zones of inhibition (ZOI) ranged from  $12.4 \pm 0.5$  mm to  $19.8 \pm 0.7$  mm. The highest activity was observed against *Staphylococcus aureus* ( $19.8 \pm 0.7$  mm), followed by *Salmonella typhi* ( $17.2 \pm 0.6$  mm), *Escherichia coli* ( $15.6 \pm 0.5$  mm), and *Shigella dysenteriae* ( $12.4 \pm 0.5$  mm) (Table 1).

**Table 1 Antimicrobial activity of *S. surattense* extract**

Bacterial Strain	Zone of Inhibition (mm)
<i>Staphylococcus aureus</i>	$19.8 \pm 0.7$
<i>Salmonella typhi</i>	$17.2 \pm 0.6$
<i>Escherichia coli</i>	$15.6 \pm 0.5$
<i>Shigella dysenteriae</i>	$12.4 \pm 0.5$



### 3.2. Effect of Temperature

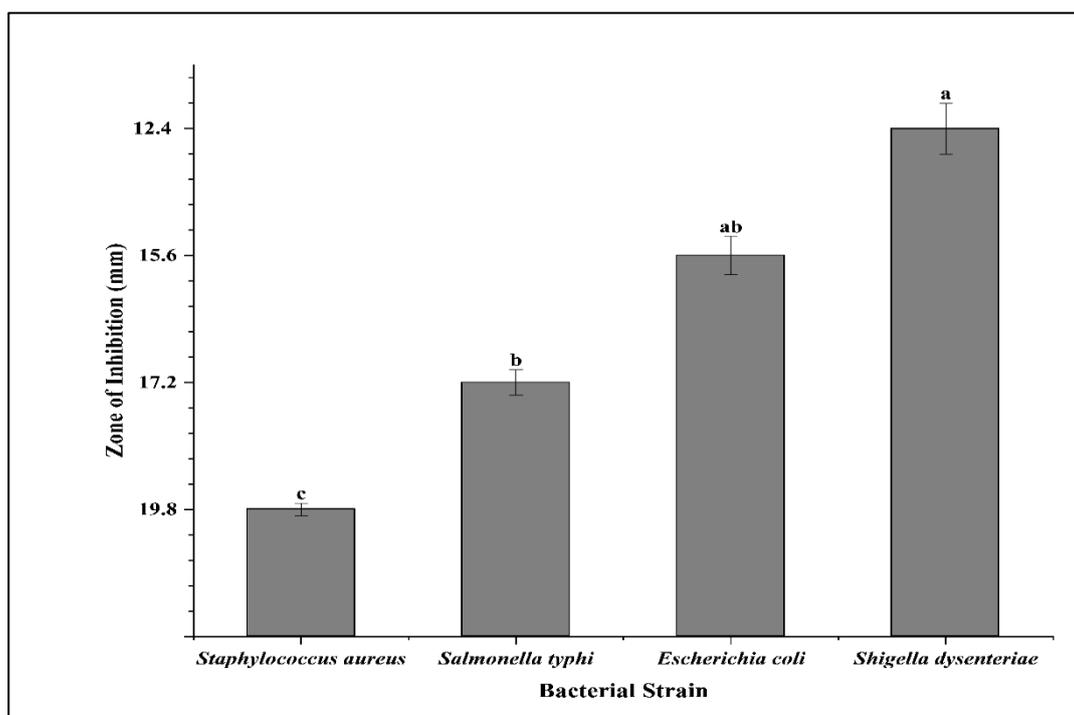
Temperature had a significant effect on antimicrobial activity (Figure 1). Exposure to 4°C and 25°C did not cause a substantial change in activity, indicating stability at low and ambient temperatures. However, higher temperatures (37°C and 60°C) led to a noticeable decrease in the zones of inhibition. The ZOI against *S. aureus* decreased from  $19.8 \pm 0.7$  mm at 25°C to  $16.3 \pm 0.5$  mm at 37°C and  $13.1 \pm 0.6$  mm at 60°C, suggesting partial thermal degradation of active compounds.

### 3.3. Effect of pH

The antimicrobial efficacy of the extract was influenced by pH (Figure 2). Maximum activity was observed at neutral pH (7.0), with ZOI values similar to those at ambient conditions. Acidic (pH 4) and alkaline (pH 9) conditions resulted in slightly reduced activity. For instance, the ZOI against *S. aureus* was  $18.6 \pm 0.6$  mm at pH 4 and  $17.9 \pm 0.5$  mm at pH 9, indicating that the active constituents are more stable under neutral conditions.

### 3.4. Effect of Light Exposure

Light exposure significantly decreased the antimicrobial activity of the extract. Direct sunlight for 1 hour reduced the ZOI against *S. aureus* from  $19.8 \pm 0.7$  mm to  $13.9 \pm 0.6$  mm (approximately 30% reduction). Similar trends were observed for other bacterial strains, suggesting that the bioactive compounds are susceptible to photodegradation (Figure 3).



## 4. DISCUSSION

The present study evaluated the antimicrobial activity of *Solanum surattense* plant extract and the effects of key physical factors, temperature, pH, and light exposure, on its efficacy. The findings revealed that the ethanolic extract exhibited significant inhibitory activity against all tested bacterial strains, with *Staphylococcus aureus* being the most susceptible. This observation is consistent with previous studies that demonstrated the strong antibacterial potential of *S. surattense* against Gram-positive bacteria, likely due to the presence of bioactive phytochemicals such as alkaloids, flavonoids, and saponins (Tekuri *et al.*, 2019; Hasan, 2024). Gram-negative bacteria such as *E. coli* and *Shigella dysenteriae* showed comparatively lower sensitivity, which may be attributed to the structural differences in their cell walls, specifically the presence of an outer lipopolysaccharide layer that provides additional protection against hydrophobic compounds (Gębarowska, 2022).

The influence of temperature on antimicrobial activity was evident in the gradual reduction of the zones of inhibition at higher temperatures (37°C and 60°C). This trend indicates that some bioactive constituents in *S.*

*surattense* are thermolabile and susceptible to denaturation or chemical degradation upon exposure to elevated temperatures. Similar findings have been reported for other plant extracts, where heat treatment resulted in decreased antimicrobial efficacy, likely due to structural alterations in phenolic compounds or alkaloids that are responsible for microbial inhibition (Muruhan, 2013; Mahalakshmi, 2019). This emphasizes the importance of controlling temperature during extraction, storage, and handling to maintain the therapeutic potential of plant-based formulations.

pH also played a significant role in modulating the antimicrobial activity of the extract. Maximum efficacy was observed at neutral pH (7.0), whereas acidic (pH 4) and alkaline (pH 9) conditions caused a slight reduction in activity. The reduction in activity under extreme pH conditions can be explained by changes in ionization states of active compounds, affecting their solubility and interaction with bacterial membranes. Flavonoids and alkaloids, for instance, often exhibit optimal stability and antimicrobial activity at neutral pH, while extreme pH conditions can lead to hydrolysis or oxidation, reducing their bioavailability (Hasan, 2024; Gębarowska, 2022). This observation underlines the need to maintain neutral pH during the preparation and storage of extracts for consistent antimicrobial performance.

Light exposure had a pronounced negative effect on antimicrobial activity, with a notable decrease in the zones of inhibition following one hour of sunlight exposure. This suggests that certain bioactive molecules in the extract, possibly flavonoids or phenolic compounds, are photosensitive and undergo photodegradation upon exposure to UV or visible light. Similar photolytic degradation has been reported in other medicinal plants, such as *Azadirachta indica* and *Curcuma longa*, where light-sensitive compounds lost efficacy when not protected from sunlight (Muruhan, 2013). Therefore, proper storage in amber-colored bottles or dark conditions is crucial to preserve the potency of *S. surattense* extracts.

The combined effects of temperature, pH, and light exposure highlight the importance of understanding physicochemical stability when developing herbal therapeutics. The results of this study suggest that *S. surattense* extracts are most effective when prepared under controlled conditions, stored at low temperatures, shielded from direct sunlight, and maintained at neutral pH. Such considerations are particularly relevant for pharmaceutical formulations, as inconsistent antimicrobial activity due to improper handling could compromise efficacy and therapeutic reliability.

Furthermore, the study emphasizes the potential of *S. surattense* as a natural antimicrobial agent in the context of rising antibiotic resistance. The observed activity against both Gram-positive and Gram-negative pathogens indicates that the plant could serve as a source for the development of novel phytopharmaceuticals or as a complementary therapy to conventional antibiotics. Future studies should focus on isolating and characterizing the specific compounds responsible for the observed antimicrobial activity. Techniques such as HPLC, LC-MS, and NMR can provide detailed insights into the chemical structure and stability of bioactive constituents under varying physical conditions. Additionally, evaluating the mechanism of action at a molecular level, such as membrane disruption, enzyme inhibition, or interference with bacterial quorum sensing, would enhance understanding of the antimicrobial potential of *S. surattense* (Tekuri et al., 2019; Hasan, 2024).

Finally, while the current study provides comprehensive insights into the effects of physical factors on antimicrobial activity, *in vivo* studies and clinical validations are essential to translate these findings into practical applications. Investigations into dosage optimization, cytotoxicity, and pharmacokinetics will be crucial for developing safe and effective herbal formulations.

Overall, this study demonstrates that *Solanum surattense* plant extract possesses robust antimicrobial activity, which is significantly influenced by temperature, pH, and light exposure. Maintaining optimal conditions enhances efficacy, ensuring the stability of active compounds and maximizing therapeutic potential. These findings support the use of *S. surattense* as a promising natural antimicrobial agent and provide a foundation for further research on its phytochemistry, pharmacology, and application in herbal medicine.

## 5. CONCLUSION

The present study demonstrated that *Solanum surattense* plant extract possesses significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. Among the tested strains, *Staphylococcus aureus* showed the highest sensitivity, highlighting the potential of the plant as a source of natural antibacterial agents. The study further revealed that physical factors, including temperature, pH, and light exposure, have a marked influence on the stability and efficacy of the extract. High temperatures, extreme pH levels, and

direct sunlight significantly reduced antimicrobial activity, whereas neutral pH, low temperatures, and dark storage conditions preserved the bioactivity of the extract. These findings emphasize the importance of optimizing extraction, storage, and handling conditions to maintain the therapeutic potential of *S. surattense*. The study underscores the plant's relevance as a natural alternative to conventional antibiotics and lays the foundation for future research aimed at isolating active compounds, elucidating mechanisms of action, and developing standardized phytopharmaceutical formulations. Overall, *S. surattense* demonstrates promising potential for incorporation into natural antimicrobial therapies, particularly in the context of rising antibiotic resistance.

**Ethical approval and Consent to participate:** Not applicable, and all authors participated in this work.

**Consent for publication:** All authors agree to publication.

**Availability of data and materials:** The data and materials that support the findings of the study are available from the corresponding author upon request.

**Competing Interests:** The author declare that they have no competing financial interest or personal relationship that could have appear to influence the work reported in this paper.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Authors contribution:** UPS conceived the idea; UPS and NS collect the plant material; UPS designed experiment; NS setup and performed experiment; NS data analysis and visualization, wrote the whole manuscript; UPS, revised and edited complete manuscript, supervised and finalized the manuscript. All authors read and approved the final manuscript.

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