IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS

K.Nagaraju* and Dr. Madan Mohan Gunda

Department of Microbiology, Government Degree and P.G. College(A), Siddipet, Telangana, India

ABSTRACT

The rise of antimicrobial resistance has intensified the search for alternative therapeutic agents from natural sources. The present study was conducted to evaluate the antimicrobial activity of crude extracts from three widely used medicinal plants: Tulsi (*Ocimum sanctum*), Aloe vera (*Aloe barbadensis*), and Neem (*Azadirachta indica)*. Using the agar well diffusion assay, the extracts were tested against two representative bacterial strains: *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive). The plant tissues were processed to obtain aqueous/ethanolic extracts and inoculated into wells made on Nutrient Agar (NA) plates seeded with the respective bacterial suspensions. After incubation at 37°C for 24 hours, distinct zones of inhibition were observed and measured. Neem and Tulsi extracts exhibited prominent antibacterial activity, characterized by clear zones of inhibition, particularly against the Gram-positive organism. Aloe vera demonstrated moderate inhibitory effects. The study validates the traditional use of these medicinal plants and highlights their potential as natural antimicrobial agents in pharmacology and disease management.

KEYWORDS

Antimicrobial activity, Tulsi, Aloe vera, Neem, Agar well diffusion

INTRODUCTION

Medicinal plants have been used for centuries in traditional medicine systems to treat various infectious diseases. Unlike synthetic antibiotics, plant-derived bioactive compounds often exhibit fewer side effects and may reduce the development of antimicrobial resistance. Among the numerous medicinal plants studied for their therapeutic properties, Tulsi (*Ocimumtenuiflorum L., syn. Ocimum sanctum L.*), Aloe vera (*Aloe barbadensis Miller*), and Neem (*Azadirachtaindica A. Juss.*) are widely recognized for their antimicrobial potential.

Tulsi (*Ocimumtenuiflorum*) belongs to the family Lamiaceae and contains phytochemicals such as eugenol, ursolic acid, rosmarinic acid, and flavonoids, which contribute to its antimicrobial and antioxidant activities. Neem (*Azadirachtaindica*), a member of the family Meliaceae, is rich in azadirachtin, nimbin, nimbidin, and gedunin, compounds known for their antibacterial, antifungal,

antiviral, and anti-inflammatory properties. Aloe vera (*Aloe barbadensis*), belonging to the family Asphodelaceae, contains anthraquinones, aloin, aloe-emodin, saponins, and salicylic acid that contribute to its wound-healing and antimicrobial effects.

Previous studies have reported the antimicrobial potential of these medicinal plants. Agarwal, Nagesh and (Murlikrishnan 2010) demonstrated significant antibacterial activity of Tulsi (Ocimum sanctum) against Streptococcus mutans. (Geeta et al. 2001) also reported the effectiveness of Ocimum sanctum against various enteric pathogens. The antibacterial efficacy of Neem has been documented by (Ghonmode et al. 2013), who observed inhibitory activity against Enterococcus faecalis. Similarly, (Bazvand et al. 2014) and Bhardwaj, Ballal and (Velmurugan 2012) reported the antimicrobial properties of Aloe vera against pathogenic microorganisms. (Subbiya et al. 2013) and (Vinothkumar et al. 2013) further confirmed the antimicrobial effectiveness of herbal extracts containing Ocimum sanctum.
The increasing prevalence of drug-resistant microorganisms has intensified the search for alternative antimicrobial agents derived from natural sources. In the present study, the antibacterial activity of these medicinal plant extracts was evaluated against two bacterial pathogens: *Escherichia coli*, a Gram-negative bacterium commonly associated with gastrointestinal and urinary tract infections, and *Bacillus subtilis*, a Gram-positive bacterium frequently used as a model organism in microbiological studies.
The agar well diffusion method is a widely accepted laboratory technique for assessing the in vitro antimicrobial efficacy of plant extracts. Therefore, this study aimed to investigate and compare the antimicrobial activity of Tulsi (*Ocimumtenuiflorum*), Neem (*Azadirachtaindica*), and Aloe vera (*Aloe barbadensis)* extracts against *Escherichia coli* and *Bacillus subtilis* using the agar well diffusion assay.

MATERIAL AND METHODS

Collection of plant Materials

Fresh and healthy leaves of Tulsi, Neem, and fleshy leaves of Aloe vera were collected from local gardens. The samples were thoroughly washed under running tap water, rinsed with sterile distilled water, and air-dried under aseptic conditions.

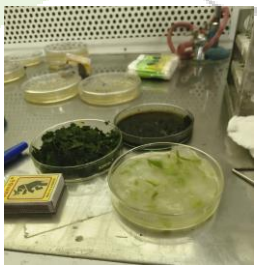

Figure 1: Collection of plant samples used for antimicrobial extract preparation

Extraction

Tulsi and Neem: Cleaned leaves were crushed using a sterile mortar and pestle with a minimal volume of sterile distilled water (or ethanol) to prepare a concentrated crude extract. The homogenate was filtered through sterile Whatman No. 1 filter paper, and the filtrate was collected.

Aloe vera: The thick outer epidermis of the leaf was carefully peeled off using a sterile scalpel. The inner clear parenchymatous gel was scraped out, homogenized aseptically, and used directly for the assay.
Preparation of Bacterial Inoculum

Pure cultures of Escherichia coli and Bacillus subtilis were maintained in Nutrient Broth incubated at 37°C to achieve optimal turbidity matching the standard standard optical density.

Preparation of culture

Preparation of Culture Media

Nutrient Agar (NA) was prepared according to standard microbiological procedures. The medium was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile Petri plates under a laminar airflow hood to solidify.

Agar Well Diffusion Assay

A sterile cotton swab was dipped into the bacterial suspension and evenly spread (lawn culture) across the surface of the solidified nutrient agar pates

Using a sterile cork borer (typically 6–8 mm in diameter), equidistant wells were punched into the agar plates.

Fixed volumes of the crude extracts (Tulsi, Aloe vera, and Neem) were introduced into the designated wells using a micropipette.

Sterile distilled water (or solvent) was added to a central well to serve as a negative control, while a standard antibiotic disk or solution was used as a positive control.

Incubation and Measurement

The plates were kept at room temperature for 1 hour to allow proper diffusion of the plant extracts into the agar matrix. Subsequently, the plates were incubated inverted at 37°C for 24 hours. After the incubation period, the plates were examined for the presence of a clear zone of inhibition around each well, and the diameters were measured in millimeter.

RESULTS

Figure 2: Natural petri plate results showing zones of inhibition of Neem, Tulsi and Aloe vera extracts against *Bacillus subtilis* and *Escherichia coli.*

Table1: Antibacterial Action of Different Plant Extracts

Plant Extract	Zone of Inhibition against E. *coli* (mm)	Zone of Inhibition against B. *subtilis* (mm)
Tulsi Extract	14 mm	17 mm
Aloe vera Gel	10 mm	12 mm
Neem Extract	16 mm	19 mm
Negative Control (Water)	0 mm	0 mm

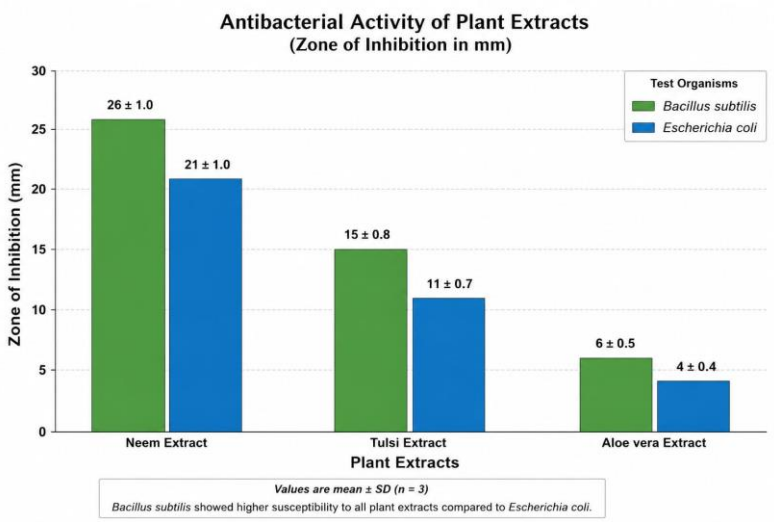

Figure 3. Antibacterial Activity of Different Plant Extract

DISCUSSION

The plant extracts exhibited clear antibacterial activity against the test organisms, as evidenced by visible zones of inhibition around the wells in the agar plates. Neem extract showed the strongest inhibitory effect against both Bacillus subtilis and Escherichia coli, producing comparatively larger zones of inhibition. Tulsi extract also demonstrated appreciable antimicrobial activity with moderate inhibition against both organisms. Aloe vera showed only slight inhibition, with smaller
zones of inhibition compared to Neem and Tulsi. Overall, Bacillus subtilis appeared more susceptible than Escherichia coli to the tested plant extracts.

The results obtained in the present investigation are consistent with earlier reports. (Agarwal, Nagesh and Murlikrishnan 2010) observed significant antibacterial activity of Tulsi extract, while (Geeta et al. 2001) reported its effectiveness against enteric bacterial pathogens. The strong inhibitory effect of Neem observed in the present study agrees with the findings of (Ghonmode et al. 2013), who demonstrated the antibacterial efficacy of neem leaf extract against Enterococcus faecalis.

The moderate antimicrobial activity exhibited by Aloe vera in the present study is in accordance with the observations of (Bazvand et al. 2014) and Bhardwaj, Ballal and (Velmurugan 2012), who reported inhibitory effects of Aloe vera against pathogenic microorganisms. Similar findings were also reported by (Lakshmi 2013), (Rani et al. 2015), and (Vinothkumar et al. 2013), highlighting the antimicrobial potential of herbal extracts against resistant microbial strains. Furthermore, (Subbiya et al. 2013) demonstrated the antibacterial efficacy of Ocimum sanctum leaves, supporting the results observed in the current investigation.

• Neem (*Azadirachtaindica)*: Showed the maximum zone of inhibition against both Bacillus subtilis and Escherichia coli. The zone was significantly wider against the Gram-positive Bacillus subtilis, indicating higher susceptibility of its cell wall structure to neem phytochemicals.
• Tulsi (Ocimum sanctum): Exhibited strong antibacterial activity, forming clear, well-defined zones of inhibition. It effectively limited
• Aloe vera (Aloe barbadensis): Demonstrated moderate inhibitory activity. While a zone of inhibition was visible, its diameter was smaller compared to Neem and Tulsi, suggesting a milder or more localized antiseptic effect under crude conditions.

The control well containing sterile distilled water showed absolutely no zone of inhibition, confirming that the inhibitory actions observed were entirely due to the bioactive constituents extracted from the medicinal plants.

The findings of this study validate that the chosen medicinal plants possess significant secondary metabolites capable of arresting bacterial proliferation. The active compounds diffuse through the porous agar matrix, establishing a concentration gradient that inhibits the lawn growth of the seeded bacteria.

The variations in the zone of inhibition diameters between Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli) bacteria can be attributed to the fundamental differences in their cell wall architecture. Gram-negative bacteria possess an outer lipopolysaccharide membrane that acts as an additional structural barrier, often rendering them slightly more resistant to plant extracts than Gram-positive bacteria.

The high potency of Neem is linked to its diverse array of triterpenoids, which disrupt bacterial cell membrane integrity. The efficacy of Tulsi is predominantly driven by phenolic compounds like eugenol, which denature microbial enzymes and proteins. Aloe vera's moderate action is likely due to the highly viscous nature of its crude gel, which can restrict rapid diffusion through the agar matrix compared to liquid extracts. Environmental parameters, plant maturity, and extraction solvents play vital roles in determining the final concentration of these bioactive principles.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the faculty members and laboratory staff of the Department of Microbiology for their valuable support, guidance, and cooperation throughout this work. We are especially thankful to Prof. G. Sunitha, Principal. For providing laboratory facilities, institutional support, and constant encouragement for the successful completion of this study.

CONFLICTS OF INTREST

The author declares no conflict of interest

CONCLUSION

The investigation successfully demonstrated the antimicrobial properties of Tulsi, Aloe vera, and Neem extracts using the agar well diffusion assay. Both Neem and Tulsi emerged as highly effective natural agents against the tested bacterial strains, while Aloe vera offered moderate protection. These results reinforce the scientific basis for utilizing traditional plant extracts as safe, accessible, and ecofriendly alternatives or supplements to conventional antibiotics. Further purification of these crude extracts could lead to the isolation of specific bioactive molecules for drug formulation

REFERENCES

1. Agarwal, P., Nagesh, L., &Murlikrishnan. (2010). Evaluation of the antimicrobial activity of various concentrations of Tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: An in vitro study. *Indian Journal of Dental Research, 21*(3), 357–359. https://doi.org/10.4103/0970-9290.70800
2. Arora, T., Kang, R. S., Mann, J. S., Khurana, N. S., Aggarwal, R., &Walia, G. (2015). Antimicrobial activity of herbal extracts against recalcitrant endodontic pathogens: An original in vitro study. *Saint International Dental Journal, 1*, 28–32.
3. Agarwal, P., Nagesh, L., &Murlikrishnan. (2010). Evaluation of the antimicrobial activity of various concentrations of Tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: An in vitro study. *Indian Journal of Dental Research, 21*(3), 357–359. https://doi.org/10.4103/0970-9290.70800
4. Bazvand, L., Aminozarbian, M. G., Farhad, A., Noormohammadi, H., Hasheminia, S. M., &Mobasherizadeh, S. (2014). Antibacterial effect of triantibiotic mixture, chlorhexidine gel, and two natural materials, propolis and aloe vera, against *Enterococcus faecalis*: An ex vivo study. *Dental Research Journal, 11*(4), 469–474.
5. Bhargava, K. Y., Aggarwal, S., Kumar, T., & Bhargava, S. (2015). Comparative evaluation of the efficacy of three antioxidants versus NaOCl and EDTA used for root canal irrigation in smear layer removal: SEM study. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*, 366–371.
6. Bhardwaj, A., Ballal, S., & Velmurugan, N. (2012). Comparative evaluation of the antimicrobial activity of natural extracts of *Morindacitrifolia*, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*: An in vitro study. *Journal of Conservative Dentistry, 15*(3), 293–297. https://doi.org/10.4103/0972-0707.97964
7. Geeta, Vasudevan, D. M., Kedlaya, R., Deepa, S., &Ballal, M. (2001). Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against enteric pathogens. *Indian Journal of Medical Sciences, 55*(8), 434–438.
8. Ghonmode, W. N., Balsaraf, O. D., Tambe, V. H., Saujanya, K. P., Patil, A. K., &Kakde, D. D. (2013). Comparison of the antibacterial efficacy of neem leaf extract, grape seed extract and 3% sodium hypochlorite against *Enterococcus faecalis*: An in vitro study. *Journal of International Oral Health, 5*(6), 61–66.
9. Hugar, S. M., Mistry, L. N., Hogade, S., &Badkar, C. M. (2015). An in vitro comparative evaluation of efficacy of disinfective ability of garlic oil (Lasuna), clove leaf oil (Lavang) and autoclaving on endodontic K-files tested against *Enterococcus faecalis*. *Indian Academy of Medical Journal, 3*, 2277–2284.
10. Lakshmi, T. (2013). Antibacterial activity of two herbal extracts and 2% sodium hypochlorite against *Enterococcus faecalis*: An in vitro comparative study. *Journal of Chemical and Pharmaceutical Research, 5*, 782–786.
11. Prabhakar, A. R., Basavraj, P., &Basappa, N. (2013). Comparative evaluation of *Morindacitrifolia* with chlorhexidine as antimicrobial endodontic irrigants and their effect on micro-hardness of root canal dentin: An in vitro study. *International Journal of Oral Health Sciences, 3*, 5–9.
12. Rani, A., Thakhu, S., Gupta, S., Gauniyal, P., Bhandari, M., & Gupta, H. (2015). Comparative evaluation of antimicrobial activity of different herbal extracts and 2% chlorhexidine gluconate against *Enterococcus faecalis* and *Candida albicans*: An in vitro study. *Indian Journal of Dental Sciences, 1*, 20–23.
13. Subbiya, A., Mahalakshmi, K., Pushpangadan, S., Padmavathy, K., Vivekanandan, P., &Sukumaran, V. G. (2013). Antibacterial efficacy of *Mangiferaindica* L. kernel and *Ocimum sanctum* L.

leaves against *Enterococcus faecalis* dentinal biofilm. *Journal of Conservative Dentistry, 16*(5), 454–457. https://doi.org/10.4103/0972-0707.117507

14. Vinothkumar, T. S., Rubin, M. I., Balaji, L., &Kandaswamy, D. (2013). In vitro evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real-time quantitative polymerase chain reaction. *Journal of Conservative Dentistry, 16*(2), 167–170. https://doi.org/10.4103/0972-0707.108208

15. Zakarea, N. A., Mohamad, T., &Taqa, A. A. (2014). Evaluation of antibacterial efficacy of newly prepared endodontic irrigant solution against *Enterococcus faecalis* (An in vitro study). *Al-Rafidain Dental Journal, 14*, 153–160.