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Original Research Article

Green synthesis of silver nitrate (AgNO_3) mediated herbal antimicrobial aqueous extracts of *Ocimum basilicum* (Basil Leaves)Kirti Rani^{1*}, Deepanshi¹¹Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

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ABSTRACT

Green antimicrobial herbal preparations have emerged as cost effective, non-toxic and sustainable approach that involves the use of eco-friendly and non-toxic plant herbs and spices extracts. Green synthesis of silver-treated herbal extracts of *Ocimum basilicum* (basil leaves extracts) is well known for its safe and cost-effective approach due to having antimicrobial activity to treat various influenza like symptoms, antistress and antidepressant properties. Herbal plant leaf extracts have also been reported to contain an active reducing agent that can reduce metal ions to synthesize biogenic metal nanoparticles. Silver itself, is well reported for their bactericidal properties and also enhance antimicrobial properties when used to prepare biogenic silver nanoparticles from herbal and other biological sources. Hence, we prepared silver nitrate (AgNO_3) mediated herbal formulations of (basil extracts) that showed significant antibacterial activity against gram-negative bacteria, *Escherichia coli* BL21 strain. Moreover, this green approach can be more promising to prepare antimicrobial of silver-treated formulations using *Ocimum basilicum* extracts. It might be safe and cost effective herbal based preparations including nanosuspensions that can further proposed for clinical practices to treat various microbial pathogenesis.

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1. Introduction

For the last many decades, natural plant sources have been exploited for the preparation of therapeutic herbal medicine due to having their excellent antimicrobial and antioxidant properties. However, plants are vulnerable to damage and depletion, so various researchers have more focus to find out green alternative to prepare nano/micro/submicron materials using safe and eco-friendly method over conventional.^{1,2} The green synthesis of herbal preparations is environmentally safe and economical which involves the use of mild reaction conditions and non-toxic precursors to carry out sustainable biochemical practices to synthesize nanoparticles.^{3–5} In addition to their antimicrobial properties, silver based various formulations

including nanoparticles have also been investigated for their potential use in cancer therapy inducing cell death via various biological pathways, such as apoptosis, necrosis, and autophagy.⁶ The use of jungli tulsi (*Ocimum canum* Sims.), *Ocimum canum* Sims. (hoary basil) Bapchi, Naked bapchi and Ban tulsi have been proposed in nanoparticles preparations in the countries like India, China, Indonesia, Malaysia, Myanmar, Philippines, Srilanka, Africa and south west Asia. Aromatic smell of basil plants and its leaves makes it conspicuous and medicinally potent to treat headaches, cough, cold, fever, sexual debility and urinary tract infections. Extracted essential oil from the leaves of basil leaves has found to contain 1,8-Cineole and eugenol that have insecticidal and antiplasmodial properties which has been reported effective to treat Malaria.^{7,8} Hence, we proposed green synthesis of silver nitrate mediated prepared various aqueous extracts of *Ocimum basilicum*

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(basil plant leaves) by the used of inherent antimicrobial activities of silver metal and basil plant leaves to enhance antimicrobial properties of prepared aqueous extracts. And silver nitrate mediated herbal formulations also studied for their significant antimicrobial activities against gram-negative bacteria, *Escherichia coli* BL21 strain that could further be proposed for safe clinical practices to treat various microbial pathogenesis.

2. Materials and Methods

2.1. Preparation of antimicrobial aqueous extracts of silver nitrated treated *Ocimum basilicum* (Basil leaves)

Fresh well handpicked leaves of *Ocimum basilicum* (Indian Holy or great basil) were washed thoroughly with double distilled water. Took 30 to 50 grams of *Ocimum basilicum* (basil leaves) and divided into three groups by weighing 10g to 15g of leaves for each three groups. For group A (Fresh leaves), B (overnight soaked leaves) and C (Boiled leaves) were crushed by using a mortar and pestle with 40ml of double distilled water and centrifuged at 5000rpm for 15 to 20 minutes. The supernatants were collected and stored at 4°C until further use.⁹ Silver nitrate (AgNO_3) solution of two different concentrations (0.40mg/ml and 0.50mg/ml) were prepared. Each 15ml of prepared all *Ocimum basilicum* (basil leaves) extracts were treated with 60 ml of prepared different concentration of silver nitrate by boiling at 80°C for 20 minutes.¹⁰

2.2. Antimicrobial activity assay

Antimicrobial assay was done by using BL21 strain of *Escherichia coli* that grown on LB agar plates by spread plating method (Figure 1). Microbial plates were incubated at 37°C for 24 hours and colonies were observed in prepared cultured plates. 30 μl of prepared concentration of silver nitrate (AgNO_3) treated herbal aqueous extracts of *Ocimum basilicum* (basil leaves) were testing for antimicrobial sensitivity on cultured plates. Cultured test Plates were incubated again for 24 hours at 37°C to observe zone of inhibition on cultured plates.^{10,11}

3. Results & Discussion

Zone of inhibition in controls and all tested cultured plates were observed and compared. Effectiveness against microbial growth was directly proportional to both prepared concentration (0.40mg/ml and 0.50mg/ml) of the silver nitrate treated (*Ocimum basilicum*) basil leaves aqueous extracts (Figures 3 and 4). It was observed that effectiveness of silver nitrate treated basil leaves aqueous extracts (sample A: fresh basil leaves extracts; sample B: basil overnight soaked leaves; sample C: basil boiled leaves extracts) were found excellent against microbial growth on test plates (Figures 3 and 4) as compared to controls (untreated basil



Figure 1: Pure colony of *Escherichia coli* BL21 strain on cultured plate

leaves aqueous extracts) (Figure 2).

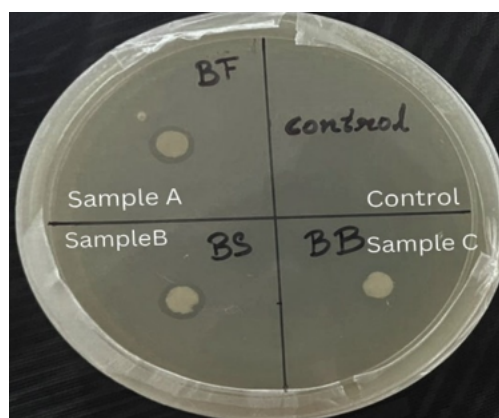


Figure 2: Control plates to observe Antimicrobial susceptibility results of untreated *Ocimum basilicum* (basil leaves extracts) by using *Escherichia coli* BL21 strain. Sample A: Fresh Leaves, Sample B: Overnight soaked leaves, Sample C: Boiled leaves.

The noted observations were studied to interpret effectiveness of antimicrobial properties of prepared silver nitrate treated various *Ocimum basilicum* (basil leaves extracts) against pathogenic microorganisms whose results were found comparable to previous findings.¹² (Table 1) Silver nitrate (AgNO_3) 0.50 mg/ml concentration treated Sample B (overnight soaked basil leaves extracts) were found be more effective to inhibit microbial growth as compared to respective 0.40mg/ml AgNO_3 treated sample

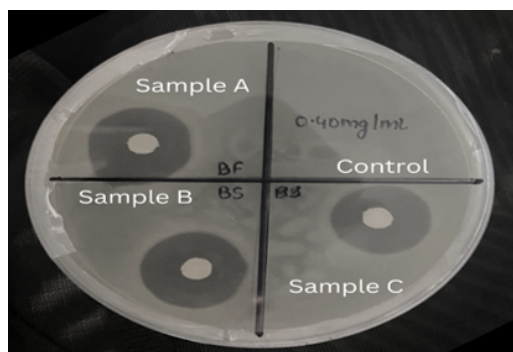


Figure 3: Antimicrobialsusceptibility results of 0.40 mg/ml concentration of silver nitrate treated Ocimum basilicum (basil leaves) extracts by using *Escherichia coli* BL21 strain; Sample A: Fresh Leaves, Sample B: Overnight soaked leaves, Sample C: Boiled leaves.

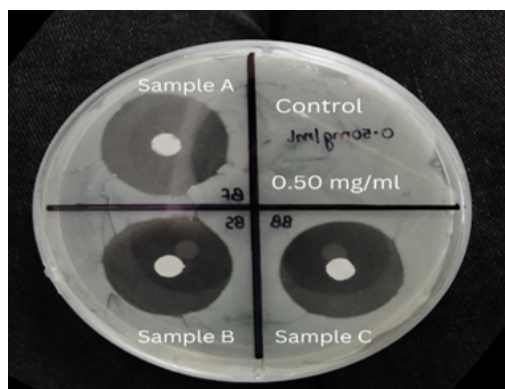


Figure 4: Antimicrobialsusceptibility results of 0.50 mg/ml concentration of silver nitrate treated Ocimum basilicum (basil leaves) extracts by using *Escherichia coli* BL21 strain, Sample A: Fresh Leaves, Sample B: Overnight soaked leaves, Sample C: Boiled leaves

Table 1: Observed effectiveness of antimicrobial activities of prepared concentrations of silver nitrate (AgNO₃) mediated Ocimum basilicum (basil leaves) herbal aqueous extracts by measuring the diameter of the zone of inhibition ring by using *Escherichia coli* BL21 strain.

Samples Ocimum basilicum (basil) leaves aqueous extracts	Silver nitrate conc. of 0.40mg/ml	Silver nitrate conc. of 0.50mg/ml
Sample A (basil fresh leaves)	2	2.6
Sample B (basil overnight soaked leaves)	2.1	2.9
Sample C (basil boiled leaves)	1.9	2.5
Effectiveness	Effective	<<Effective

A (fresh basil leaves extracts), sample B (basil overnight soaked leaves), sample C (basil boiled leaves extracts). And it was also found more effective 0.50mg/ml AgNO₃ treated sample A (fresh basil leaves extracts) & sample C (basil boiled leaves extracts) both extracts.¹³ Observed antimicrobial effectiveness of safe and cost-effective herbal silver nitrate mediated preparations of *Ocimum basilicum* (basil leaves extracts) were found to be much good against *Escherichia coli* BL21 strain which were very much comparable with previous reported data.¹⁴(Figure 5).

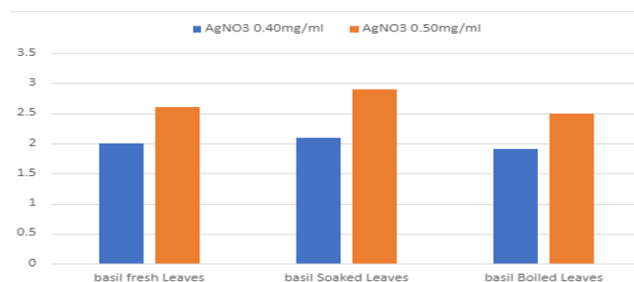


Figure 5: Comparative Effectiveness of observed antimicrobial activities of prepared concentrations (0.40mg/ml and 0.50mg/ml) of silver nitrate mediated Ocimum basilicum (basil leaves) herbal aqueous extracts

4. Conclusion

Silver nitrate concentrations of 0.50 mg/ml AgNO₃ treated fresh, overnight soaked and boiled preparation of *Ocimum basilicum* (basil leaves) extracts were found to have good effective antimicrobial susceptibility when observed zone of inhibitions compared with 0.40 mg/ml silver nitrate treated basil leaves aqueous extracts. 0.50 mg/ml concentration of silver nitrate treated Sample B (overnight soaked basil leaves) were found be more effective to inhibit microbial growth as compared to respective fresh and boiled leaves. Hence, green preparations of 0.50 mg/ml silver nitrate treated herbal aqueous extracts can be proposed for more potent herbal and cost-effective alternative against *E. Coli*. (gram-negative bacteria). These safe and nontoxic silver nitrate mediated herbal basil leaves aqueous extracts can be proposed for treatment of various pathogenesis caused by *E.Coli* or gram negative bacteria and may helpful to combat global health havoc caused by antibiotic or drug resistant microbes. More potent antimicrobial activities of herbal preparation can be attributed by treating with silver nitrate solutions and could be further proposed as a cost effective and safe alternative to conventional antibiotics treatment of various pathogenesis like pneumonia, peritonitis, UTIs, and bloodstream infections.

5. Source of Funding

None.

6. Conflict of Interest

None.

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