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Antimicrobial Activity of Strobilanthes in Urinary Tract Infection (UTI)

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Abstract: ***In both community and hospital settings, urinary tract infections (UTIs) are among the most prevalent bacterial illnesses globally. Even though there is a wide spectrum of clinical symptoms associated with UTIs, from simple (uUTIs) to complex (cUTIs), the majority of UTIs are often treated empirically. The primary cause of these infections is bacteria, while on rarer occasions, other microorganisms including fungus and some viruses have also been linked to UTIs. The most frequent cause of both uUTIs and cUTIs is Uropathogenic Escherichia coli (UPEC), which is followed by other pathogenic microbes as Proteus mirabilis, Enterococcus faecalis, Klebsiella pneumoniae, and Staphylococcus species. Furthermore, the frequency of multidrug - resistant UTIs (MDR) is rising, which has a substantial impact on the economic burden of these infections as well as the propagation of antibiotic resistance. Physicians have traditionally faced difficulties in diagnosing and treating upper and lower urinary tract infections (UTTIs) due to the high frequency of these infections, the potential for recurrence and inappropriate treatment, and the global rise in antibiotic resistance that calls for the adoption of appropriate antibiotic stewardship practices. Females are twice as likely as males to get urinary tract infections, and the frequency rises with age. Many Indian plants are used in traditional medicine to treat urinary tract infections (UTIs), but little is known about their therapeutic potential against the bacteria that cause UTIs. In order to identify the plant species that are utilized in traditional Indian medicine to treat urinary tract infections (UTIs), a thorough assessment of the literature was conducted. This research lists the effectiveness of Strobilanthes kunthiana to treat urinary tract infections.***

Keywords: Urinary Tract Infection, Pathogenic microbes, Uropathogenic, Traditional medicine, Strobilanthes kunthiana

1. Introduction

Among the most prevalent illnesses in humans worldwide are urinary tract infections (UTIs). In fact, estimates place the number of people who have at least one UTI annually at close to 800 million, or over 11% of the world's population [1, 2]. Women are predicted to have a substantially higher prevalence of such conditions than men, making them far more common in women than in men [3]. Urine was once thought to be a sterile liquid, and numerous studies were conducted by scientists to both predict and confirm urinary tract infections (UTIs). Urine frequently contains urinary bacteria; it is not sterile [4]. Roughly 40 and 50 percent of women acquire a UTI at least once in their lifetime [5]. In few continents, like Africa and Asia, recorded higher incidence of UTI mainly in pregnant women [6]. The occurrence of UTIs is largely influenced by environmental and lifestyle factors. Due to their numerous illnesses, older persons may be more susceptible to UTIs from treatment and administration programs. Specifically, the use of catheters raises the risk of urinary tract infections, especially when gram - negative bacteria are the causal agent. Additionally, using antibiotics for extended periods of time to treat other illnesses impairs immunity, which makes people more vulnerable to UTIs. Higher sexual activity in younger women between the ages of 18 and 39 raises the frequency of recurrence as well as the incidence of UTIs [7]. Any region of the urinary tract, including the kidneys, bladder, urethra, and ureter, can get infected. In extreme cases, the infection spreads to the kidneys and circulation, causing uremia and bacteremia [8]. Cystitis, or bladder infection, is the term for urinary tract infections that affect the lower urinary tract. Kidney infections are frequently used to describe infections of the upper urinary tract (pyelonephritis) [9]. A urinary tract infection (UTI) is a condition characterized by inflammation and development of germs within the urinary tract. This is brought on by germs that go from the gastrointestinal tract to the urethra, where they grow and spread illness. UTIs are caused by bacterial pathogen invasion of the epithelium lining the urinary tract, which extends from the small calyx to the prostatic urethra [10].

Gram - negative bacteria, the most common cause of urinary tract infections (UTTIs), are mainly responsible for the spread of infections, especially Escherichia coli [11]. Numerous factors, including, genetic inheritance, intestinal population, vaginal biological processes, behavioural factors, uropathogenic virulence traits, and host - barrier factors, influence the complex causative agents of urinary tract infections (UTTIs) [12]. Enterococcus spp., K. pneumoniae, Staphylococcus aureus, Proteus spp., Pseudomonas aeruginosa, and Enterobacter spp. are among the additional bacterial species that can be identified from UTIs and are very harmful [13]. Due to the emergence of a resistant microbe from the improper use of antibacterial medicines, UTIs are frequently not treated with broad - spectrum antibiotics [14]. To treat UTIs, many regimens have been employed. As of right now, trimethoprim and nitrofurantoin are the first - line medications. Prostate patients are advised to use second - line antibiotics, such as quinolones [15]. Herbal remedies have been shown to be quite effective in treating urinary tract infections. Different functional groups may be found in the

structures of many plant compounds, and these compounds' antibacterial properties are explained by a variety of processes [16]. Plants have drawn interest from all across the world for millennia as remedies for a variety of illnesses [17]. Furthermore, a number of studies have demonstrated the effectiveness of medicinal plants in treating and preventing a wide range of illnesses [18 - 20]. The aim of this study is to characterize and to target the causative agents using the medicinal plant Strobilanthes kunthiana, which can be used as alternative medicine for their prevention and treatment.

2. Literature Survey

Every year, millions of individuals worldwide suffer from urinary tract infections (UTIs), which are among the most prevalent bacterial illnesses. Uropathogenic Escherichia coli (UPEC) is the main cause, with Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis, and Staphylococcus species following in order of occurrence. Alternative therapeutics must be investigated since the growing number of multidrug - resistant (MDR) bacteria has reduced the effectiveness of traditional antibiotic treatments.

The antibacterial qualities of medicinal plants have been extensively researched. One promising substitute for treating MDR infections is the application of plant - based extracts and nanoparticles made from therapeutic plants. Because it's economical and environmentally benign, green synthesis of nanoparticles utilizing plant extracts has become more and more popular. The antibacterial, antioxidant, and anti - inflammatory qualities of plant - derived nanoparticles have been documented in a number of studies.

A plant that has been traditionally utilized in Indian medicine, is Strobilanthes kunthiana. The plant has demonstrated possible antibacterial properties. Its efficacy against germs that cause UTIs hasn't been well investigated, though. Zinc oxide (ZnO) nanoparticles made from plant extracts have been shown to have strong antibacterial qualities in earlier studies. The current work assesses the effectiveness of ZnO nanoparticles against UTI pathogens and focuses on their green manufacture utilizing Strobilanthes kunthiana.

3. Materials and Methods

Collection of plant
Strobilanthes kunthiana leaves were collected from the Munnar town located in the Idukki district of the Southwestern Indian state of Kerala. The plant was identified and authenticated by Anna Arch Siddha Hospital, Chennai, Tamil Nadu (Authentication number: 715.05012401)

Figure 2.1: Leaves of Strobilanthes kunthiana plant

Preparation of plant extract:
The sample leaves obtained was washed and allowed to shade dry for 3 - 5 days.300 grams of dried leaves were grounded using mechanical blender and fine powder is obtained for further studies.

Figure 2.2: Dried leaves and powdered leaves

Green synthesis of zinc nanoparticles:
Modified procedures of previous work on green synthesis to produce ZnO nanoparticles have been taken for study.10mM of precursor and 90ml of deionised water (solution A) is added to the conical flask. The solution B is prepared by mixing 0.5g of extract is weighed and mixed with 100 ml of deionised water (solution B). Further the solution B is heated for 3 mins in the microwave oven and cooled for further use.10 ml of the clear solution is taken from the solution B and added to the conical flask containing solution A. The conical flask is mixed thoroughly and kept in stirrer for 5hrs at 1000 rpm. The sample was analysed every 5hrs using UV visible Spectrometry to obtain the wavelength and the optical density of the nanoparticles. After the synthesis of NP's, the solution is transferred to the centrifuge tubes and centrifuged at 6000 rpm for 15 mins. To the pellet obtained, deionised water is added and the tubes were further centrifuged at 6000 rpm for 15 mins. For the third time of centrifugation process, ethanol is added to the obtained pellet and centrifuged at 6000 rpm at 15 mins. Finally, the obtained pellet is transferred to the petri plate and allowed to air dry for 2 days and the nanoparticles obtained is used for further studies.

Fourier Transformed Infra - Red Spectrophotometry:
The Zn nanoparticles were portrayed for the functional group analysis using FTIR spectrophotometer at room temperature by employing attenuated total reflection procedure with diamond crystals. For the analysis of spectral range of 4000 - 500 cm^{-1} was picked. The peaks established in the range of 500 to 700 cm^{-1} are the trademark of tetragonal zinc oxide vibrations.

Transmission Electron Microscopic Analysis:
The TEM study was carried out to understand the crystalline characteristics and size of the nanoparticles. Qualitative measurements of the synthesized nanoparticles, such as particle size, size distribution, and morphology, can be obtained using TEM (Pyrz & Buttrey, 2008). TEM conducts imaging by passing the particles between 50 - 200nm.

Scanning Electron Microscopic Analysis:
The SEM images of the ZnO nanoparticles were analysed at VIT, using JSM - 6360. All the samples were analysed and carried out to find the surface morphology of zinc oxide nanoparticles at different magnification levels.

Anti - bacterial activity:
The anti - bacterial activity of Strobilanthes kunthiana is assessed against pathogenic bacteria namely, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas species.10 µl of the fresh cultures were inoculated in sterile Hi - veg broth medium and incubated for 18 hours in an orbital shaker at 120 - 150 rpm. Muller Hinton agar was prepared and 5mm wells were made using sterile tip. Different concentrations of biosynthesised zinc nanoparticles such as 25µl, 50µl, 100µl were added along with the positive control. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured. The zone of inhibition and the activity of the sample is correlated with the standard antibiotic streptomycin. The width of the zone was deliberated and documented.

Time kill curve Assay:
The time kill curve assay can be used to assess an antimicrobial agent's bactericidal or bacteriostatic activity over time measuring how well it works against the particular strain of bacteria. Nutrient broth was prepared, sterilised and 6ml was added to three sets of tubes. Bacterial suspension was added to all three sets of tubes in the range of $5x10^5$CFU/ml. First and the second tube contains Acorus calamus extracts (Aqueous, Ethanolic, Methanolic) tested usually at a final concentration of 0.25 x MIC and 1 x MIC and the third tube is considered to be the growth control tube. The incubation is done under suitable conditions for varied time intervals (0, 4, 6, 8, 10, 12&24 hours). The percentage of dead cells is calculated at wavelength of 600nm at regular intervals.

Antioxidant Activity of Strobilanthes kunthiana synthesised ZnO nanoparticles – DPPH Assay:
1, 1 - diphenyl - 2 - picryl - hydrazil (DPPH) assay was mediated using Strobilanthes kunthiana Zn nanoparticles (Rajeshkumar, 2017). Different concentrations of Strobilanthes kunthiana intervened zinc nanoparticles was mixed with 1ml of 0.1 mM DPPH in methanol and 450µl of 50mM Tris HCl buffer of pH 7.4 and incubated for 30 mins. After incubation, the reduction in the amount of DPPH free radicals was evaluated based on the absorbance of 517nm. BHT was used as control. The percentage inhibition was calculated from the following equation given below.
% Inhibition = (Abs of control - Abs of test sample/Abs of control) x 100 - - - - - - - - - - (1)

Anti - inflammatory Activity of Strobilanthes kunthiana synthesized ZnO nanoparticles:
The anti - inflammatory activity of Neelakurinji mediated zinc particles were performed by the method reported in (Chithralekha et al., 2019).2ml of 1% bovine serum albumin was mixed with 400 µl of biosynthesised silver nanoparticles in different concentrations (500 - 100 µg/ml) such as 10µl, 20µl, 30µl, 40µl, 50µl. The pH was suggested to 6.8 using 1N HCl. The mixture was incubated at room temperature for 20 mins and then heated at 55°C for 10 mins in water bath. After cooling, the absorbance values are observed at 660nm. Dimethyl sulphoxide (DMSO) is used as control. Diclofenac sodium in various concentrations is used as standard. The percentage of inhibition was calculated using the formula:
% Inhibition = (Control OD – Sample OD/Control OD) x 100 - - - - - - - - - - - - - - - - - - - (2)

Lethality Assay using BRINE SHRIMP (Cytotoxic Effect):
The brine shrimp (Artemia saline) eggs were purchased from Aqua remedies, Chennai. To prepare artificial sea water, 15 gms of iodine free salt was dissolved in 1000ml of deionised water. The saline water is prepared using 1N NaoH and pH was adjusted to 8.5. The brine shrimp were added and kept under constant aeration for 24 hours. After 24 hours, the nauplii's hatched from the eggs were used to study the toxicity level of Strobilanthes kunthiana mediated zinc nanoparticles. The ELISA plates wells are filled with saline water (2gms in 200 ml of distilled water), 10 - 12ml in each well. The nauplii is added to each well using a capillary tube along with Zinc mediated Strobilanthes kunthiana nanoparticles (5µl, 10µl, 15µl, 20µl, 25µl). Further the ELISA plates were incubated at room temperature for 24 hours. The number of live nauplii's present is observed and calculated using the formula:

% Cytotoxicity = (Number of dead nauplii / (Number of dead nauplii + Number of live nauplii)) x 100 - - - - (3)

4. Results and Discussion

Green synthesis of zinc nanoparticles
Green synthesis is a method of creating nano - materials that is safe, economical, ecologically benign and clean. Microorganisms that serve as substrate for the environment, are known to be a friendly production of nanomaterials including bacteria, yeast, fungi, algal species, and certain plants. Furthermore, the process of green synthesis yields nanomaterials with natural reduction, stabilization and antibacterial characteristics [21].

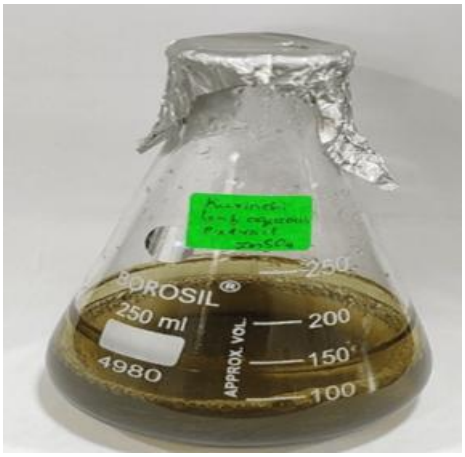

Figure 3.1: Zinc Nanoparticles synthesised extract

UV Visible Spectroscopy analysis:
Reflectance spectroscopy or absorption spectroscopy in the UV Vis spectral region is cited as UV - Vis spectroscopy. It aids to help the light in the visible and adjacent ranges. The green synthesis of Zinc oxide nanoparticles was examined from 250 to 750nm. The absorbance peaks at 440 nm, which marked to be the green synthesis of Zinc oxide nanoparticles using Strobilanthes kunthiana.

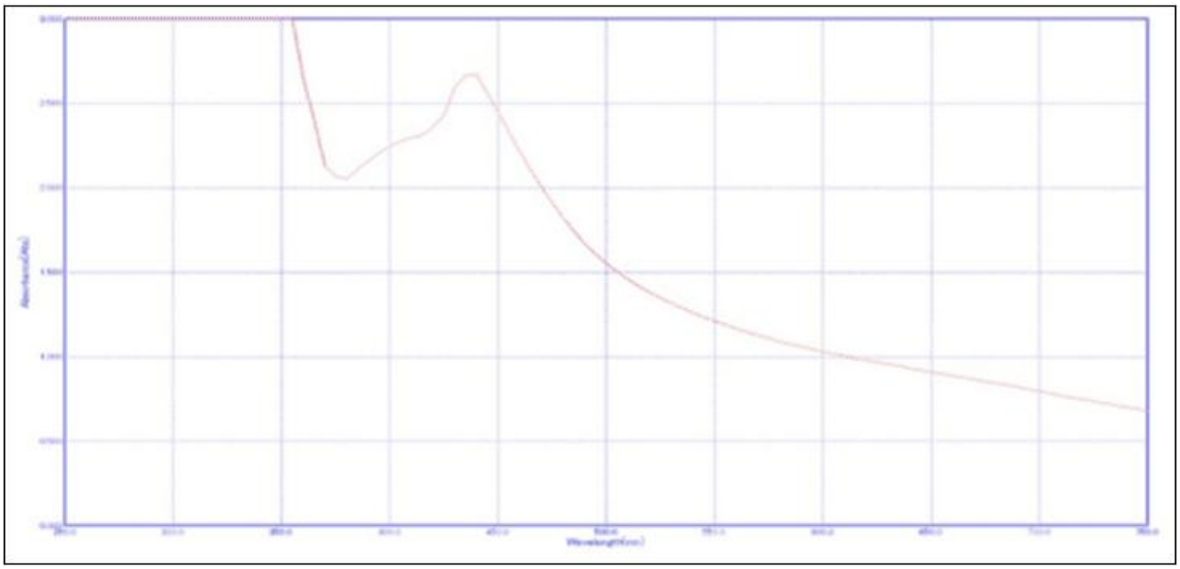

Figure 3.1.1: UV - Vis spectroscopy analysis

Fourier Transformed Infra - Red Spectrophotometry:
An Infrared absorption spectrum is produced using Fourier Transform Infrared Spectroscopy, which is used to determine the chemical bonds of a molecule. The profile of the sample, which is been created by the spectra, turns to be unique molecular fingerprint that may be used to screen and scan samples for a variety of constituents, for the purpose of identifying functional groups and characterising the covalent bonding formation [22 - 23]. The nanoparticles were portrayed for the functional group analysis using FTIR spectrophotometer at room temperature by employing the attenuated total reflection procedure with diamond crystal. For the analysis the spectral range of 4000 - 500 cm^{-1} was picked. The peaks were established in the range of 500 to 700 cm^{-1} are the trademark of tetragonal zinc oxide vibrations [24].

The peak 3430.78 corresponds to amine group (primary), 2922.67 corresponds to C - H group, 1564.17 corresponds to C - N amide 2 group, 1639.47 corresponds to beta sheet amide 1 group, 1384.31 corresponds to CH & CH_2 aliphatic bonding group, 1076.87 corresponds to PO_2 stretching which is symmetric mainly to phospholipids, 899.05 corresponds to C - O - C group, 1154.97 corresponds to alkyl amine group, 1409.68 corresponds to alkane group.

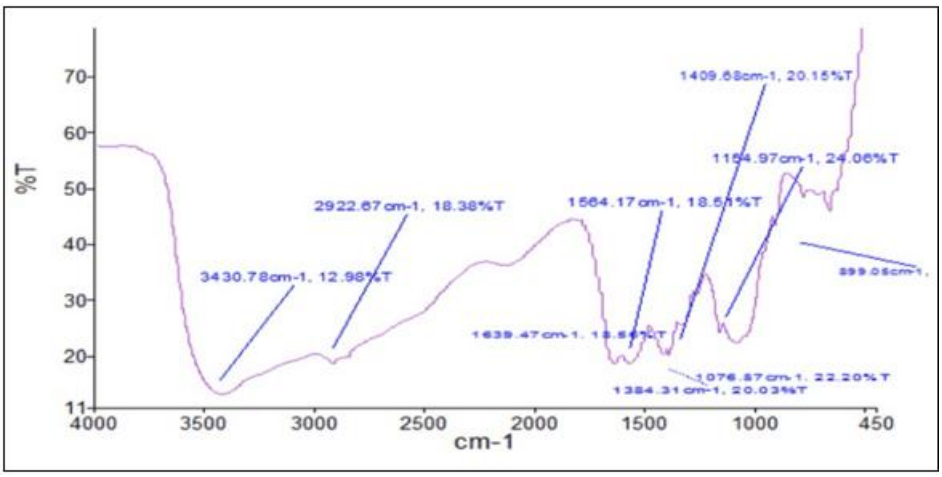

Figure 3.2: Fourier Transformed Infra - Red Spectroscopy

Transmission Electron Microscopic Analysis:
The TEM study was carried out to understand the crystalline characteristics and size of the nanoparticles. The TEM images of ZnO confirm that the particles are almost hexagonal with slight variation in thickness, which supports SEM results [25]. The average particle size by histogram was found to be 50 - 200nm.

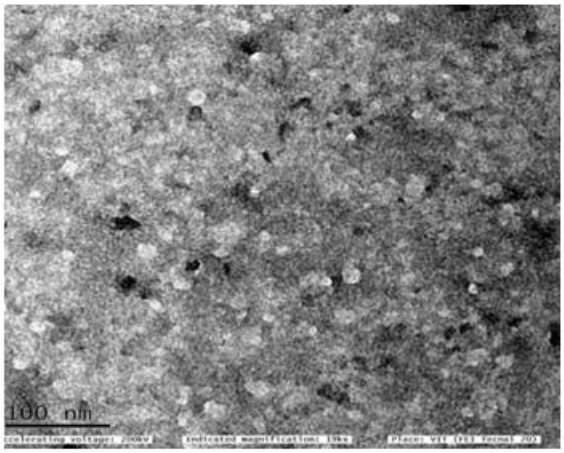

Figure 3.3: Transmission Electron Microscopic Analysis

Scanning Electron Microscopic Analysis:

SEM Analysis was carried out to find the surface morphology of zinc oxide using JSM - 6360 scanning electron microscope at different magnification levels and results were shown in the figure 3.4. The micrographs showed that the network formation occurred at the zinc oxide nanoparticles and clearly indicates that agglomeration has been taken place. It further confirms that the synthesised zinc oxide nanoparticles were in well arrangement and moreover the synthesised zinc oxide nanoparticles had a spherical shape.

Figure 3.4: Scanning Electron Microscopic Analysis

Anti - bacterial Activity:

The antibacterial activity of Strobilanthes kunthiana is assessed against the pathogenic bacteria and the zone of inhibition around the well - marked the absence of bacterial growth [26]. The activity of the sample is compared with the antibiotic streptomycin and the width of the zone was documented below. In the results E. Coli and Pseudomonas, 100µl concentration showed higher zone of inhibition when compared to other concentrations. Similarly, in E. faecalis 50µl concentration showed higher zone of inhibition when compared and in S. aureus, 50µl and 100µl showed higher zone of inhibition. The graph obtained represents the zone of inhibition formed using ZnO nanoparticles and antibiotic used against UTI. It further indicates that the activity of ZnO nanoparticles against microbes is increased with increase in volume of sample used.

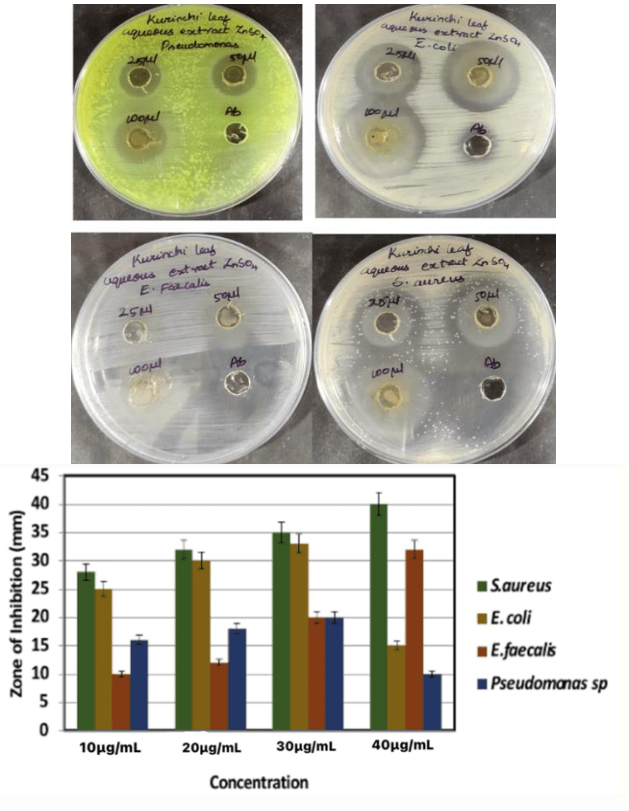

Figure 3.5: A Visual observation of antibacterial activity and 3.5 b Zone of inhibition of zinc oxide nanoparticles against UTI pathogens

Time kill curve Assay:

The time kill curve assay can be used to assess an antimicrobial agent activity or bacteriostatic activity over time by measuring how well it works against a particular strain of bacteria. The time kill assay can monitor the effect of various concentrations of an antimicrobial agent over time in relation to the stages of the growth of the bacteria in lag, exponential and stationary phase [27 - 29]. Time kill kinetics is used for agents like antiseptics as they require a shorter time kill kinetic study and further follows different morphology in contrast to the multiple time points. The Minimal Bactericidal Concentration (MBC) test is defined as a 99.9% or greater killing efficacy at a specified time. The graph represents the time kill curve assay, in which S. aureus gives better results when compared to all other species.

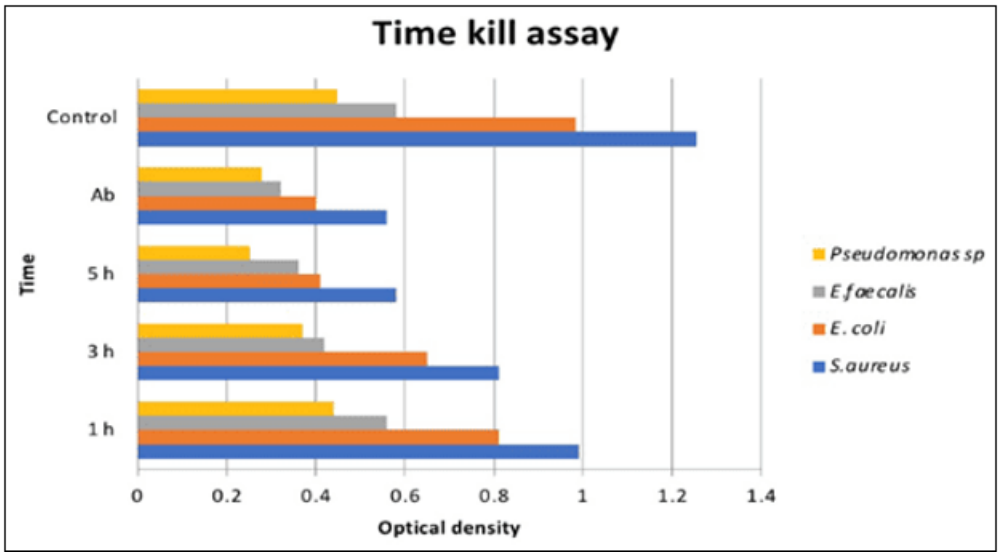

Figure 3.6: Time kill curve assay

Antioxidant Activity of Strobilanthes kunthiana synthesised ZnO nanoparticles – DPPH Assay:
The graph shows that the percentage of inhibition of ZnO nanoparticles constantly increases with the increase in the volume of sample used. This clearly shows that the nanoparticles synthesised from Strobilanthes kunthiana have the ability to act as an anti - oxidant agent.

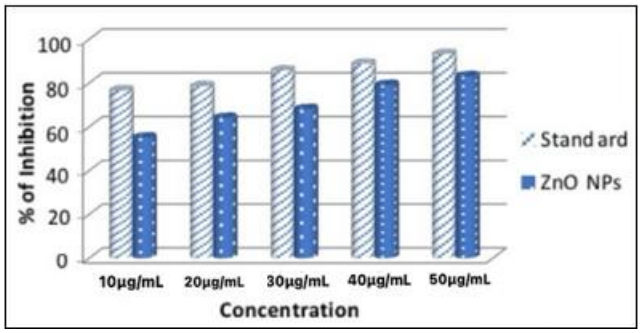

Figure 3.7: Anti - oxidant Activity of Strobilanthes kunthiana synthesized ZnO nanoparticles using DPPH Assay

Anti - inflammatory Activity of Strobilanthes kunthiana synthesised ZnO nanoparticles:
The graph represents the anti - inflammatory activity of ZnO nanoparticles mediated from Strobilanthes kunthiana with comparison to a standard solution. It clearly suggests that the percentage of inhibition increases with increase in the sample volume and it is considered to be the potential anti - inflammatory agent.

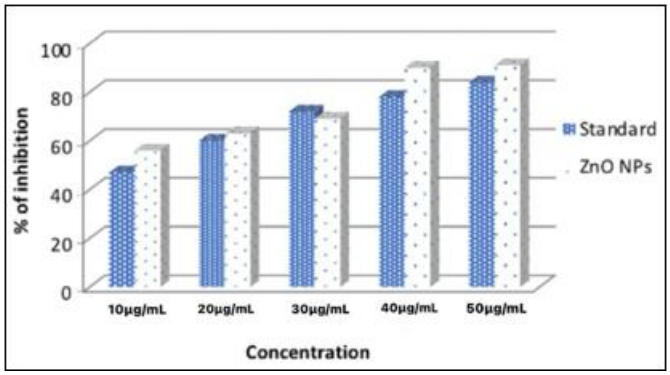

Figure 3.8: Anti - inflammatory Activity of Strobilanthes kunthiana synthesised ZnO nanoparticles

Lethality Assay using BRINE SHRIMP (Cytotoxic Effect):
A straightforward, high throughput cytotoxicity test for bioactive substances is the brine shrimp lethality bioassay. It is predicated on test substances capacity to kill brine shrimp, a basic zoological creature. Several organisations have further improved this assay after it was initially proposed by Michael et al [30]. The cytotoxicity assay for brine shrimp was thought to be a useful probe for a first assessment of toxicity. One significant physiological response to a wide range of harmful substances is inflammation. The graph represents the cytotoxic effect of day 1 and day 2 live nauplii. The graph indicates that day 1 (% of live nauplii) has high cytotoxicity activity in all range of concentrations and in the case of day 2 (% of live nauplii) has high cytotoxicity effect in 5µl and 10µl concentrations, based on the results obtained it clearly indicates that as the concentration increases its cytotoxicity gets decreased.

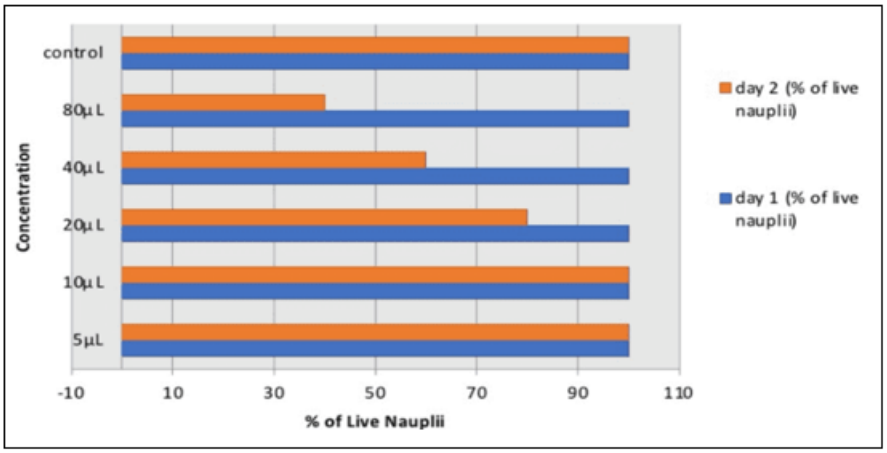

Figure 3.9: Cytotoxicity effect of zinc oxide nanoparticles using Brine Shrimp – Lethality Assay

5. Conclusion

Strobilanthes kunthiana is used for the green synthesis of ZnO nanoparticles. In the present study, it is concluded that the synthesis of zinc oxide nanoparticles from extract was confirmed by characterization studies such as UV visible spectroscopy, FTIR, SEM and TEM analysis. The synthesised zinc oxide shows close results with antibiotics which are treated with different types of bacteria which are used for antibacterial activity. They even have the ability to act as an anti - oxidant agent. Through the anti - inflammatory assay it is evident that the extract can be used as anti - inflammatory agent. The increased lethality of brine shrimp is due to the increase in cytotoxicity activity. The lethality of the bacteria in time kill assay indicates that different concentrations of the ZnO nanoparticles produced from the extract has the ability to kill the bacteria. Hence, the antimicrobial properties of the plant nanoparticles will help pharma industries develop modifiers or any kind of precursors for synthesizing new therapeutic alternatives to treat UTI.

6. Future Scope

1) **Further Characterization of Nanoparticles**
- Advanced techniques such as X - ray diffraction (XRD) and dynamic light scattering (DLS) can be used to further characterize the synthesized ZnO nanoparticles and their properties.

2) **Mechanistic Studies on Antibacterial Activity**
- Investigating the exact mechanism of action of ZnO nanoparticles on bacterial cells, including reactive oxygen species (ROS) generation, membrane disruption, and intracellular damage.

3) **Clinical Trials and In Vivo Studies**
- Conducting in vivo studies to assess the safety, efficacy, and bioavailability of ZnO nanoparticles synthesized using *Strobilanthes kunthiana*.

4) **Synergistic Studies with Antibiotics**
- Evaluating the potential of combining ZnO nanoparticles with conventional antibiotics to enhance their effectiveness and reduce resistance development.

5) **Application in Drug Formulations**
- Developing nanoparticle - based formulations such as ointments, oral drugs, or urinary catheter coatings for effective prevention and treatment of UTIs.

6) **Exploration of Other Medicinal Plants**
- Investigating the antimicrobial potential of other traditionally used medicinal plants for nanoparticle synthesis and their effectiveness against UTIs.

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Conflict of Interest
The authors declare that there is no conflict of interest.

Authors Contribution
All the authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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