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ANTIBACTERIAL STUDIES OF OCIMUM TENUIFLORUM LEAVES WITH CdZnS NANOPARTICLES

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ABSTRACT

Ocimum tenuiflorum, also known as *Ocimum sanctum* is a perennial fragrant plant in the Lamiaceae family. The "Queen of Herbs," tulsi, is referred to in ancient literature as a sacred and therapeutic herb. Many scientific investigations have revealed the anti-inflammatory, antibacterial, antifungal, antistress, and antioxidant effects of *Ocimum sanctum*. The characterizations of synthesized CdZnS nanoparticles with *Ocimum tenuiflorum* leaf extracts were done. The structure and particle size were revealed by XRD. The elemental compositions of CdZnS and *Ocimum sanctum* added CdZnS nanoparticles were confirmed using the EDAX spectrum. *Ocimum tenuiflorum* leaves possess antibacterial activity against bacterium *Salmonella Typhi* at the concentration of 20ml and bacterium *E.coli* at the concentration 10ml, which shows the maximum zone of inhibition. Tulsi was very useful to the wine industry for the production of herbal wine or medicinal wine which can also be taken as a healthy tonic. The Antibacterial activity of *Ocimum tenuiflorum* with CdZnS nanoparticles is used for the treatment of Typhoid fever also known as enteric fever, life threatening infection.

Keywords: *Ocimum tenuiflorum*, CdZnS nanoparticles, Chemical Precipitation Method, *Escherichia Coli, Salmonella Typhi.*

INTRODUCTION

In today's science and technology, nanotechnology is viewed as a distinct area of study with a wide range of applications. One of the most exciting areas of research right now is how nanotechnology can be used for medical applications. Many different substances can be used to create semiconductor nanoparticles (NPs). According to the periodic table groups these elements are formed into, they are known as II-VI, III-V, or IV-VI semiconductor nanoparticles. Band gaps in insulators are often more than 5 eV, whereas they are typically between 1 and 4 eV for semiconductors. [1] Semiconductor nanoparticles have applications in various fields like biological labeling and diagnostics (Cinsy et al. 2020). The direct band gaps of group 2-6 semiconductors CdS and ZnS, which have values of 2.4 eV and 3.7 eV, respectively. Cadmium Zinc Sulphide (CdZnS) is a ternary alloyed semiconductor that has fine and tunable absorption in the visible region of solar energy so that they are widely used as wide band gap materials. [2]

India has a rich heritage of developing both curative and preventive medications based on plant science. A country like India is very much suited for development of drugs from medicinal plants [3]. The Indian subcontinent is excellent for the growth of a vast number of medicinal and aromatic plants that may be utilised as raw materials for the food, flavour, fragrance, cosmetics, and agrochemical sectors due to its broad variances in soil and temperature. The use of plants as medicines could be traced as far back as the beginning of human civilization (Bharathi et al. 2014).

An aromatic plant called *Ocimum tenuiflorum,* commonly referred to as Holy basil, tulasi, or *Ocimum sanctum* is an aromatic perennial plant in the family Lamiaceae [4]. It is an upright, heavily branched subshrub that is 30 to 60 cm tall. It has hairy stems and simple, fragrant green or purple leaves. It is cultivated extremely in tropical and semitropical region of India and other Asian countries. It is believed to be air purifier. Tulsi is referred to as "The Incomparable One," "The Mother Medicine of Nature," “The Queen of Herbs” and “Elixir of life” because it promotes longevity and is used in many formulations for the prevention and cure of various ailments. [5]

Ocimum sanctum have varied pharmacological properties that include antimicrobial, (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial) antioxidant, antibiotic, anti-diabetic, anti-inflammatory, analgesic, chemopreventive and antipyretic properties. [6]

Plants contain several secondary metabolites that have been shown to have antimicrobial effects in vitro, including tannins, terpenoids, alkaloids, flavonoids, glycosides etc. [7] (Nayan R Bhalodia et al 2023). The purpose of this study was to carried out with the antibacterial potentials of leaves of *Ocimum tenuiflorum*. The aim of the study is to evaluate the antibacterial activity and identify the zone of inhibition of extracts on various bacterial strains. In the present study, the antibacterial activity of *Ocimum tenuiflorum* (an ethno medicinal plant) leaves with CdZnS nanoparticles was evaluated for potential antibacterial activity against medically important bacterial strains. The antibacterial activity was determined by employing the agar disc diffusion method in the extracts. A disc diffusion test was conducted using standard procedure by Kirby-Bauer method. The antibacterial activities of different concentration (10ml, 20ml) of *Ocimum tenuiflorum*

were tested against bacterium such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Salmonella typhi, Shigella sonnei, Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus*. Zone of inhibition for the above bacterium among the different concentration were compared for antibacterial activity. The results demonstrated that the examined object caused a considerable suppression of bacterial growth organisms.

MATERIALS AND METHODS:

Preparation of Extract:

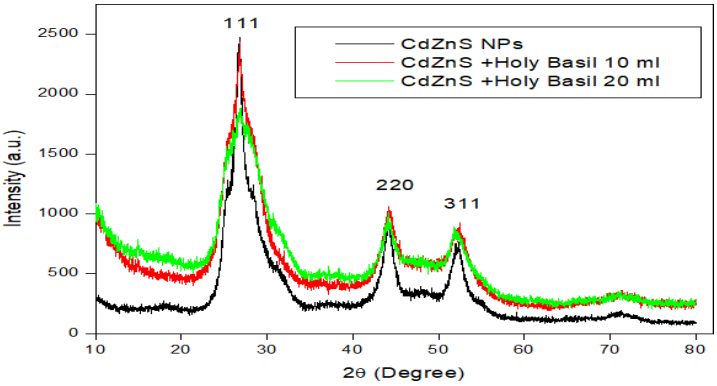
The Holy basil extract was prepared as follows, holy basil leaves were collected, washed thoroughly with water and then with deionized water. Then the water content was removed and then crushed using a mortar. The extract was then extracted from the crushed leaves.

Synthesis of Nanoparticles:

CdZnS nanoparticles added with *Ocimum tenuiflorum* (holy basil) were synthesized by the chemical precipitation method. The following substances were employed in the synthesis: Thiourea (NH2)2.CN, ammonia (NH3OH), cadmium acetate (Cd(CH3COO)2.H2O), zinc acetate (Zn(CH3COO)2.H2O), and triethanolamine. A certain quantity of Cadmium acetate and Zinc acetate were added with water and mixed for 10 minutes using a magnetic stirrer. After stirring desired amount of triethanolamine was added to the solution and stirred for 10 minutes. Then we have to add ammonia to the solution and stirred well for 20 minutes, then thiourea was added to the solution and it was colourless. The desired amount of holy basil extract was added and stirred well. Then, in order to prevent any temperature gradients and produce a precipitated solution with a greenish-yellow colour, the solution was immersed in a constant temperature bath of 80°C for 1 hour. The precipitate obtained is CdZnS nanoparticles mixed with *Ocimum tenuiflorum* (holy basil) extract. The resultant precipitated solution was centrifuged and then rinsed with deionized water. A greenish-yellow powder was produced after being dried in hot air at 70^0C for 1.5 hours. This powder was finely powdered with the help of Agate mortar for ½ an hour.

ANTIBACTERIAL ACTIVITY:

Determination of zone of inhibition:



In vitro antibacterial activities were examined for *Ocimum tenuiflorum* added CdZnS nanoparticles extracts. Antibacterial activities of extracts against eight pathogenic bacteria were investigated by the Kirby-Bauer method. In order to tests on fastidious organisms, defibrinated blood must be necessary for the process. Human blood is not recommended as it contain many antimicrobial substances. The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4. The plates are incubated for 16 to18 hours at 35 to 37^0C aerobically or in CO_2 atmosphere. All the extracts were screened for their antibacterial activities among different concentration against the *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Salmonella typhi, Shigella sonnei, Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus.* The diameters of zones are measured to the nearest millimeter with vernier calipers or a thin millimeter

scale. The zone of inhibitions is measured and tabulated.

EXPERIMENTAL

XRD analysis

The XRD patterns of pure CdZnS nanoparticles and CdZnS with Ocimum tenuiflorum extract are indicated in Figure 1. All the peaks in the X-Ray diffraction patterns of nanoparticles shows a cubic-zinc blende phase structure of CdS. The three diffraction peaks were found to be associated with the lattice planes of (111), (220), and (311), respectively, indicating that the Ocimum tenuiflorum's components had been integrated into the CdZnS lattice. The XRD patterns show that the diffraction's FWHM (Full Width at Half Height), peaks increases with the concentration of extract added which results in the degradation and change in the crystalline size. Based on the FWHM, the average particle sizes of pure CdZnS nanoparticles and Ocimum tenuiflorum added CdZnS nanoparticles, were calculated using the Debye-Scherrer equation, $D=0.9\lambda/(\beta\cos\theta)$; where $\lambda=0.15418$ nm, λ is the x-ray wavelength produced by a from a Cu (α) radiation, β is the FWHM in radians and θ is the Bragg's angle. The particle size obtained was in the nanometer scale.

Figure 1 X-Ray diffraction patterns of CdZnS nanoparticles and Ocimum tenuiflorum (10 ml, 20 ml) added CdZnS nanoparticles

Elemental analysis

The elemental compositions of CdZnS and Ocimum tenuiflorum added CdZnS nanoparticles were confirmed using the EDAX spectra. The peaks from the spectrums for CdZnS nanoparticles and Ocimum tenuiflorum (10 ml,20 ml) added CdZnS nanoparticles are shown in Figure 2 a, b, c respectively, shows the presence of major elements Cadmium, Zinc and Sulphur, and the presence of carbon, nitrogen and oxygen and it confirms the presence of Ocimum tenuiflorum with CdZnS nanoparticles. The elemental composition analysis for CdZnS and Ocimum tenuiflorum 10ml, 20ml added to CdZnS nanoparticles are shown table 1 (a), (b), (c) respectively.

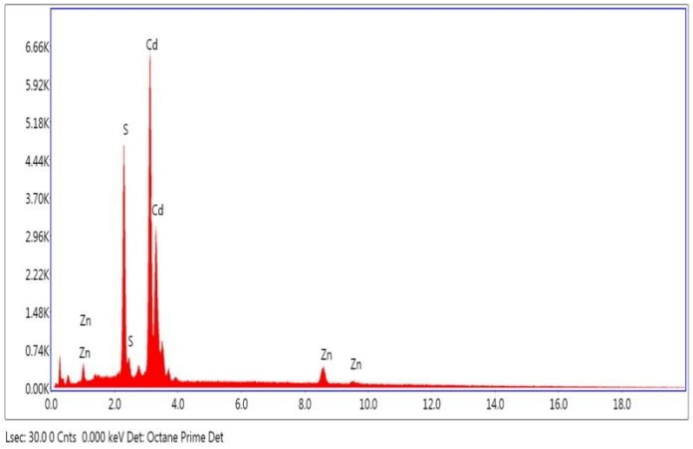


Figure 2 (a) EDAX spectrum of CdZnS nanoparticles

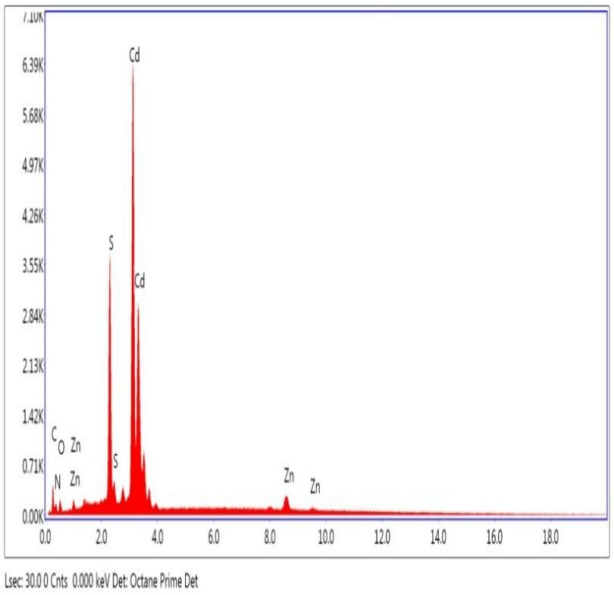


Figure 2 (b) EDAX spectrum Ocimum tenuiflorum of (10 ml) added CdZnS nanoparticles

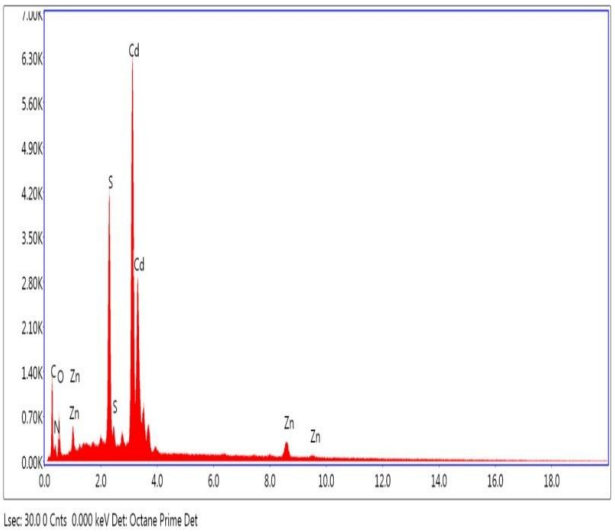


Figure 2 (c) EDAX spectrum Ocimum tenuiflorum of (20 ml) added CdZnS nanoparticles

Table 1 (a) EDAX elemental analysis of CdZnS nanoparticles

Element	Weight %	Atomic %
Cd	70.60	46.12
Zn	11.53	12.95
S	17.88	40.94

Table 1 (b) EDAX elemental analysis Ocimum tenuiflorum(10 ml) added CdZnS nanoparticles

Elements	Weight% of CdZnS:Ocimum tenuiflorum 10 ml	Atomic %
Cd	67.63	30.15
Zn	7.75	5.95
S	13.65	21.34
C	7.32	30.56
O	2.31	7.23
N	1.33	4.77

Table 1 (c) EDAX elemental analysis Ocimum tenuiflorum(20 ml) added CdZnS nanoparticles

Elements	Weight% of CdZnS:Ocimum tenuiflorum 20 ml	Atomic %
Cd	51.92	14.42
Zn	5.98	2.85
S	11.99	11.68
C	18.50	48.07
O	10.47	20.43
N	1.15	2.55

RESULTS AND DISCUSSION

Evaluation of Antibacterial activity

The antibacterial activity of *Ocimum tenuiflorum* added CdZnS nanoparticles were studied in different concentrations (10 ml, 20 ml) against eight pathogenic bacterial strains, such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Salmonella typhi, Shigella sonnei, Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus.* These strains have been chosen in order to be used in this application and for future formulation research. The antibacterial potential of extracts was evaluated in terms of the bacterial growth inhibition zone. The antibacterial activity' zone of inhibition is shown in Table 2. The diameters of the inhibition zone in the Agar plates are given in mm. Escherichia coli and Bacillus subtilis were more sensitive as compared with other bacterium for 10 ml concentration, and *Klebsiella, Salmonella typhi* and *Shigella sonnei* were more sensitive as compared with other bacterium for 20 ml concentration. The zone of inhibition is 25 mm for more sensitive bacteria for both 10 ml and 20 ml concentrations. The findings demonstrate that the extracts of *Ocimum tenuiflorum*

added CdZnS nanoparticles, which were found to be more effective against all the bacterium tested.

Table 2: Zone of inhibition for different bacterial strains

Name of the Bacteria	**Tulsi 10 ml**	**Tulsi 20 ml**
Escherchia coli	25 mm	22 mm
Pseudomonas aeruginosa	18 mm	16 mm
Klebsiella	23 mm	25 mm
Salmonella typhi	24 mm	25 mm
Shigella sonnei	23 mm	25 mm
Bacillus subtilis	25 mm	22 mm
Staph albus	21 mm	21 mm
Staph aureus	24 mm	23 mm

Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as source of medicine (Bharathi et al. 2014) [3]. In the present study, the extract obtained from *Ocimum tenuiflorum* added CdZnS nanoparticles shows strong activity against most of the tested bacterial strains.

The antibacterial activity of CdZnS nanoparticles added to *Ocimum tenuiflorum* was tested, and the findings are shown in Table 2. Among the two concentrations (10 ml, 20 ml) used, maximum zone of inhibition observed was 25 mm for both the concentration for different bacterium. The zone of inhibition obtained for different bacterium are *Escherichia coli* (25 mm, 22mm), *Pseudomonas aeruginosa* (18 mm, 16 mm), *Klebsiella* (23 mm, 25 mm), *Salmonella* typhi (24 mm, 25 mm), *Shigella sonnei* (23 mm, 25 mm), *Bacillus subtilis* (25 mm, 22 mm), *S. albus* (21 mm, 21 mm), *S. aureus* (24 mm, 23 mm). For 10 ml concentration, maximum antibacterial activity was observed in the bacterium *Escherichia coli* and *Bacillus subtilis*. Similar results were observed by (V Jalander et al. 2014) in case of ethanolic extract of *Ocimum sanctum* [9]. The bacterium *S. aureus* and *Salmonella typhi* have similar zone of inhibition as 24 mm, followed by bacterium Klebsiella and *Shigella sonnei* have similar zone of inhibition as 23 mm for 10 ml concentration. (Bishnu joshi et al. 2009) [8].

The maximum zone of inhibition at 20 ml concentration for bacterium *Klebsiella, Salmonella* typhi, *Shigella sonnei* are 25 mm, followed by bacteria *S. aureus* shows zone of inhibition as 23 mm, followed by bacterium *Bacillus subtilis* and *S. albus* shows zone of inhibition as 22 mm and 21 mm. Similar outcomes were reported by Bharathi et al. 2014 among the different concentration (100, 200, 300 mg/ml) used, maximum zone of inhibition observed at 200 and 300 mg/ml concentration for *Bacillus subtilis* but for 100 ml the second maximum zone of inhibition as compared to other bacterium.[3]

The bacteria *Pseudmonas aeruginosa* shows least zone of inhibition as 18 mm and 16 mm for 10 ml and 20 ml concentration. Antibacterial activities of *Ocimum tenuiflorum* (Tulsi) leaves with CdZnS nanoparticles on certain bacterium *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Salmonella typhi, Shigella sonnei, Bacillus subtilis, Staphylococcus aureus,* and *Staphylococcus albus.*

The chart for Antibacterial activities of *Ocimum tenuiflorum* leaves with CdZnS nanoparticles on microorganisms and its zone of inhibition was shown in Figure 3 and it is noted that among the different concentration (10 ml, 20 ml) , Escherchia coli and *Bacillus subtilis* shows maximum zone of inhibition for 10 ml concentration and *Klebsiella, Salmonella Typhi, Shigella sonnei* shows maximum zone of inhibition for 20 ml concentration.

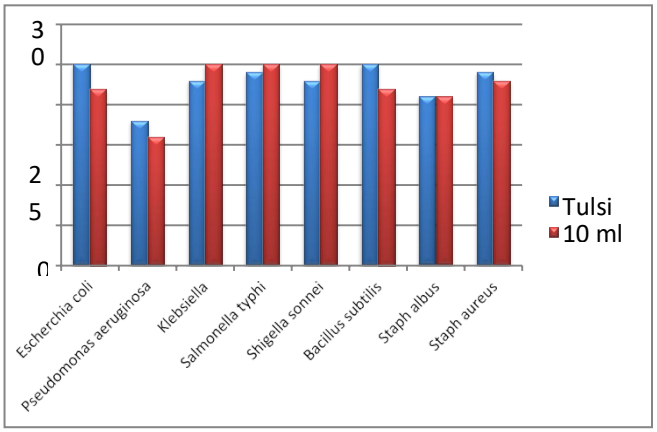


Figure 3 Antibacterial activities of *Ocimum tenuiflorum* leaves with CdZnS nanoparticles on microorganisms and its zone of inhibition.

CONCLUSION

The study of Antibacterial activity of herbal plant extract of *Ocimum tenuiflorum* with CdZnS nanoparticles for different concentration (10 ml, 20 ml) showed the promising antibacterial activity against bacterial human pathogens. The zone of inhibition which is greater than 20mm shows highly active against bacterium such as *Escherichia coli, Klebsiella, Salmonella typhi, Shigella sonnei, Bacillus subtilis, Staph albus, Staph aureus* and between the 16-20mm shows moderately active against *Pseudomonas aeruginosa*. Among the different concentration of plant extract, maximum inhibition activity was exhibited by the bacteria *Escherichia coli* for 10 ml concentration and *Salmonella typhi* 20 ml concentration. The results indicated that Antibacterial activity of *Ocimum tenuiflorum* with CdZnS nanoparticles is used for the treatment of Typhoid fever also known as enteric fever, life threatening infection. Also, as a result of Antibacterial activity of *Ocimum tenuiflorum* with CdZnS nanoparticles are highly active against bacterium *Escherchia coli, Klebsiella, Shigella sonnei, Bacillus subtilis, S. albus, S. aureus* is used for the treatment of UTI, *Pneumonia, Endocarditis*, skin infections.

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