



## ANTIMICROBIAL STUDIES OF *CLITORIA TERNATEA* LEAVES WITH CdZnS NANOPARTICLES

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### ABSTRACT

*Clitoria ternatea* is a perennial herb that belongs to the family Fabaceae and Subfamily Papilionaceae and had been perceived to exhibit antibacterial and antifungal activities. The representation of synthesized CdZnS nanoparticles with *Clitoria ternatea* leaf extract were done by chemical precipitation method. The formation of CdZnS nanoparticles with leaf extract was confirmed by using XRD, EDAX. Antimicrobial studies were done and it is noted that the higher Zone of inhibition were observed against *Klebisella pneumonia* and *Candida tropicalis* which showed promising antibacterial and antifungal activity. The leaf extracts of *Clitoria ternatea* has many pharmaceutical applications for the control of deadly pathogens.

**Keywords:** *Clitoria ternatea*, *Candida tropicalis*, *Klebisella pneumonia*, *Salmonella typhi*.

### INTRODUCTION

Nanotechnology is one of the exciting fields with many applications in the modern technology (Xia et al. 2010). Nanoparticles are ranging in the size of 10-200 nm and are in the solid state either amorphous or crystalline in nature (Gardea-Torresdey et al. 2002,2003). Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles (Song and Kim, 2009).

Semiconductor nanoparticles (NPs) are made from a variety different compounds (Orhan et al. 2017). Based on the periodic table groups, they are referred to as II-IV, III-V or IV-VI semiconductor nanoparticles. Group 2-6

semiconductor such as ZnS, CdS has the properties in between those of insulators and conductors comprising elements of group II and VI of the periodic table. CdZnS is considered to be ternary alloyed semiconductor which has the properties in between CdS and ZnS.(Cinsy 2023).

Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary health care level (Malabadi et al. 2007). *Clitoria ternatea* commonly known as 'Butterfly pea' has been used in Ayurvedic medicine in which various parts of the plants are

used to treat health issues such as indigestion, constipation, arthritis, skin diseases, liver and the intestinal problems. *Clitoria ternatea* leaves possess anti-inflammatory, antipyretic and antimicrobial activities (Mukherjee et al. 2008). *Clitoria ternatea* leaves have high chlorophyll content. This study was carried out to investigate the antibacterial and antifungal potentials of leaf extracts of *Clitoria ternatea* with CdZnS nanoparticles. The antimicrobial activity was determined by the Kirby Bauer Method. The antibacterial and antifungal activities of *Clitoria ternatea* with CdZnS for 10 ml concentration was tested.

## MATERIAL AND METHODS

### Preparation of extract

The plant of *Clitoria ternatea* was collected and washed with water and again thoroughly washed with deionised water for several times and made to dry completely. Thoroughly dried, the whole plant was crushed using a mortar. The extract was collected by squeezing the crushed leaves.

CdZnS nanoparticles added with *Clitoria ternatea* were synthesized by the chemical precipitation method. The chemicals used for the synthesis are Cadmium acetate ( $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ ), Zinc acetate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ ), Thiourea ( $(\text{NH}_2)_2\text{CH}_2$ ), triethanolamine, TEA ( $\text{N}(\text{CH}_2\text{CH}_2\text{OCH}_3)_3$ ), and ammonia ( $\text{NH}_3\text{OH}$ ). Using a magnetic stirrer, a specific amount of Zinc acetate was added to water and mixed for 10 minutes. Triethanolamine was added to the solution after stirring and again stirred for 10 minutes. Likewise ammonia was added and stirred well for 20 minutes, then thiourea was added to the solution and it was colourless. Finally the desired amount of squeezed *Clitoria ternatea* extract was added to the solution and stirred well. In order to avoid any temperature gradient, the solution was placed in a constant temperature bath of  $80^\circ\text{C}$  for 1 hour. As a result, a greenish-yellow colored precipitated solution was

obtained. The obtained precipitate was then strained and washed with distilled water and dried in hot air at  $70^\circ\text{C}$  for 1.5 hours, a greenish yellow powder was obtained. This powder was finely powdered with the help of Agate mortar for  $\frac{1}{2}$  an hour.

## EXPERIMENTAL ANALYSIS

### XRD analysis

The structure and particle size was revealed by X-ray diffraction analysis. The figure 1(a) shows the X-ray analysis of CdZnS and CdZnS added with *Clitoria ternatea* extract of concentration of 10 ml. The diffraction peaks of the prepared nanoparticles are well indexed as the Zinc blende (cubic) structure. It is clearly seen that the three diffraction peaks of the lattice planes (111), (220) and (311), suggests the contents of *Clitoria ternatea* have been incorporated into CdZnS lattice. Using the Debye-Scherrer equation,  $\text{PS} = 0.9\lambda / (\beta \cos\theta)$ , the average particle sizes of pure CdZnS nanoparticles and *Clitoria ternatea* (10 ml) added CdZnS NPs, were calculated. Here  $\lambda = 0.15418 \text{ nm}$ , is the x-ray wavelength provided from a Cu ( $\alpha$ ) radiation,  $\beta$  is the FWHM in radians and  $\theta$  is the Bragg's angle.

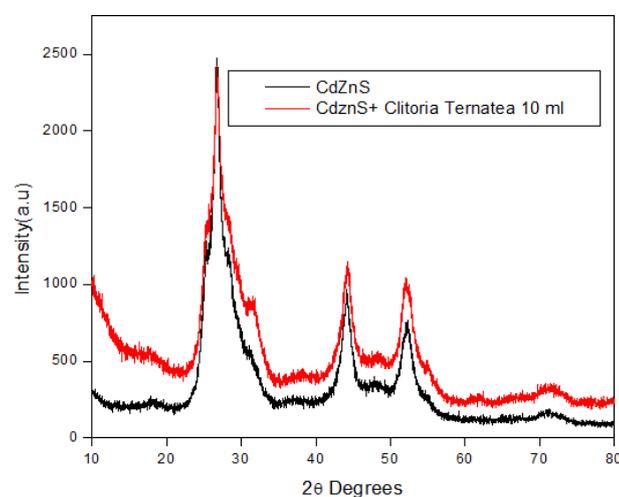


Figure 1(a) X-ray diffraction pattern of CdZnS and *Clitoria ternatea* (10ml) added CdZnS nanoparticles.

**Elemental studies**

The elemental composition of CdZnS and *Clitoria ternatea* added CdZnS nanoparticles were confirmed by EDAX spectrum. The peaks obtained from the spectrums for CdZnS and *Clitoria ternatea* (10 ml,) added CdZnS nanoparticles are shown in figure 2(a), (b) respectively. The peaks from the spectrum shows the presence of major elements Cadmium, Zinc and Sulphur and the presence of carbon, nitrogen and oxygen confirms the presence of *Clitoria ternatea* with CdZnS nanoparticles. The analysis of elemental composition of the sample is presented in table 1(a), (b).

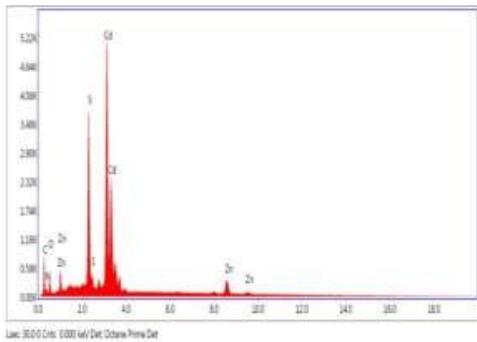
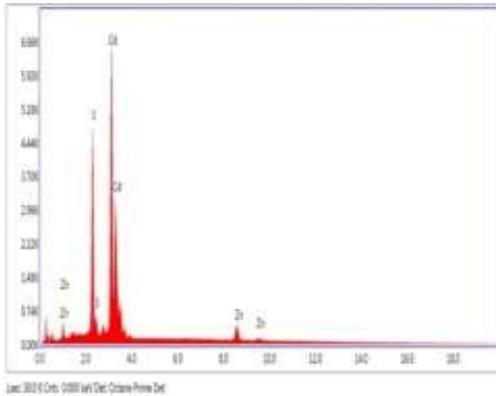


Figure 2 EDAX spectrum of (a) CdZnS nanoparticles (b) *Clitoria ternatea* (10 ml) added CdZnS nanoparticles.

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

Table 1 (b) EDAX analysis for CdZnS : *Clitoria ternatea* 10 ml

Element s	Weight % of CdZnS: <i>Clitoria ternatea</i> 10 ml	Atomic %
Cd	51.62	16.01
Zn	11.30	6.03
S	13.10	14.24
C	15.83	45.96
O	8.13	17.72
N	0.02	0.04

From table 1 the elemental analysis was confirmed by the presence of the elements Cadmium, Zinc, Sulphur, Carbon, Oxygen, Nitrogen.

**ANTIMICROBIAL ACTIVITY**

**Zone of inhibition test**

The emergence of antibiotic resistance microbes limits the effectiveness of current drugs significantly causing treatment failure of infections (Scheffler et al. 2013). In regard to this challenge there is a need to develop alternative approaches in addition to searching for new antimicrobial compounds. The antimicrobial test was performed by Kirby Bauer Method. The test organisms used for the investigations of antimicrobial activities are *Escherichia coli*, *Pseudomonas areginosa*, *Klebisella pneumonia*, *Salmonella typhi*, *Shigella sonnei*, *Bacillus substilis*, *Staphylococcus albus*, *Staphylococcus aures* and fungi, *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Aspergillus flaves*. For the fastidious organisms, defibrinated blood may be

necessary. The medium was allowed to cool to 50°C before 7% of blood is added. This medium was then poured into petri dishes. Poured plates are stored +40°C and used within one week of preparation.

In order to avoid the droplets of moisture, the plates should be dried with lidajar before inoculation. The pH of the medium should be 7.2 to 7.4. The growth is transferred to a test tube containing 1.5ml of sterile suitable broth. The tubes are incubated for 2 hours to produce a bacterial suspension of moderate turbidity. The plates must be inoculated within 15 minutes of preparation of suspension. After the inoculum has dried it was checked to ensure even contact with the medium. At 350 to 370°C, the plates are incubated for 16 to 18 hours aerobically for fastidious organisms. The diameter of zones are measured to the nearest millimeter with vernier callipers.

## RESULTS AND DISCUSSION

### Antibacterial activity

The antibacterial activity of CdZnS nanoparticles added with whole plant extract of *Clitoria ternatea* (10 ml) showed positive results and it is presented in the Table 2. The *Clitoria ternatea* extract was tested against 8 bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi*, *Shigella sonnei*, *Bacillus subtilis*, *Staphylococcus albus*, *Staphylococcus aureus*) and was found to have the most potent activity against *Klebsiella pneumoniae* with an maximum inhibition zone of 26mm. This shows that the extract was found to have good antibacterial activity against *K.pneumonia*.

Table 2. Zone of inhibition for bacterial species

Microorganisms	Zone of inhibition
<i>Escherichia coli</i>	23mm
<i>Pseudomonas areginosa</i>	19mm
<i>Klebsiella pneumonia</i>	26mm
<i>Salmonella typhi</i>	24mm
<i>Shigella sonnei</i>	21mm
<i>Bacillus subtilis</i>	24mm
<i>Staphylococcus albus</i>	19mm
<i>Staphylococcus aureus</i>	24mm

Among the microbes tested *Pseudomonas aeruginosa* and *Staphylococcus albus* are found to be moderately active with minimum zone of inhibition (19 mm). Thus the result showed that the extracts of *Clitoria ternatea* were found to be more effective against all the microbes tested.

### Antifungal activity

The rise in resistance towards most antifungal agents in diverse pathogens which calls for the need to identify new therapeutic agents (Perfect 2016). The *Clitoria ternatea* leaves added with CdZnS extract were tested against *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Aspergillus flavus* and is given in Table 3. Kirby Bauer method was used to determine the zone of inhibition for these fungal strains.

Table 3, Zone of inhibition for fungi species.

Microorganisms	Zone of inhibition (mm)
<i>Candida albicans</i>	1 8
<i>Candida tropicalis</i>	2 2
<i>Aspergillus niger</i>	2 0
<i>Aspergillus flavus</i>	1 7

The higher inhibition zone was recorded against *Candida tropicalis* and are found to

be highly active against *Clitoria ternatea* extract. The zone of inhibition of *Candida tropicalis* was 22mm which is higher than the standard used. The fungus that are moderately active are *Candida albicans* and *Aspergillus flavus*. The result of the present study conclude that the germination of the fungal strains are inhibited.

Medicinal plants are used by large proportion of Indian population. The reasons for this include true improvement, absence of harmful side effects, and high cost of other forms of treatment. Medicinal plants exhibit antimicrobial activity against by different mechanisms. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The present study shows that the *Clitoria ternatea* leaves acts as a antimicrobial agent to inhibit the microbial growth. The antimicrobial activity of *C.ternatea* with CdZnS nanoparticles is was shown in Figure 3. (Uma et al. 2009) investigated that the *Clitoria ternatea* flower extract is highly effective against *Klebsiella pneumoniae* with zone of inhibition, 26 mm. Our study was in concordance with the study conducted by (Uma et al. 2009). By disk diffusion method (Malabadi et al 2005) investigated antibacterial activity of *Bacillus subtilis*, *Staphylococcus aureus*, *E.coli* and *K.pneumoniae*. *K.pneumoniae* was recorded to have more potent for both Silver nanoparticles and Silver nitrate extract.

(Ethel et al. 2021) determined antifungal activity of methanol extract of *C.ternatea* flower(100 mg/ml) tested against *Candida albicans*, *Rhizopus* and *Penicillium*. Among them, *Candida albicans* had the

highest activity with an inhibition zone of 19 mm in agar diffusion method. Thus *Clitoria ternatea* extract was found to have many beneficial effects in various other studies such as anti-aging (Zakaria et al. 2018), hepatoprotective (Nithianantham et al. 2013), testicular damage protection (Iamsaard et al. 2014) and starch digestion (Chusak et al. 2018) activity. Thus different studies were taken into account to study the effectiveness of *Clitoria ternatea*.

## CONCLUSION

. Present study suggests that *Clitoria ternatea* was effective against the multidrug resistant microorganisms. Higher inhibition zones were observed against the bacterium *Klebsiella pneumoniae* and fungus *Candida tropicalis*. The current study can pave better ways to control pneumonia, blood stream infections, meningitis and other invasive infections caused by *K.pneumoniae* and *C.tropicalis*.



Figure 3(a) Antibacterial activity of *C.ternatea* on *E.coli*



Figure 3(b) Antibacterial activity of *Clitoria ternatea* on *P.aereginosa*



Figure 3(c) Antibacterial activity of *C.ternatea* on *K.pneumoniae*



Figure 3(d) Antibacterial activity of *C.ternatea* on



Figure 3(e) Antibacterial activity of *C.ternatea* on *Shigella sonnei*



Figure 3(f) Antibacterial activity of *C.ternatea* on *Bacillus subtilis*.



Figure 3(g) Antibacterial activity of *C.ternatea* on *Staphylococcus albus*



Figure 3(h) Antibacterial activity of *C.ternatea* on *Staphylococcus aureus*.



Figure 3(i) Antifungal activity of *C.ternatea* on *C.albicans*



Figure 3(j) Antifungal activity of *C.ternatea* on *C.tropicalis*.



Figure 3(k) Antifungal activity of *C.ternatea* on *Aspergillus niger*.



Figure 3(l) Antifungal activity of *C.ternatea* on *Aspergillus flavus*.

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