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ANTIMICROBIAL, ANTIOXIDANT AND ANTIUROLITHIATIC ACTIVITY OF SYNTHESIZED ZINC OXIDE NANOPARTICLES USING PETALS OF *ROSA CENTIFOLIA* ON URINARY TRACT INFECTION CAUSING STRUVITE CRYSTALS

VARSHINI R[1], SNEHAPRIYA S[1], MOHAMED JUBEER P[1], MANJULA K[2*]

1: Department of biotechnology, Bharathiar University, maruthamalai road, Coimbatore-641046

2: Bio Techno Solutions Training and Research Institute, 4/271, Thalamboo Street, Thirunagar West, Ponmalaipatti, Trichy-620004

***Corresponding Author: Dr. P. Mohamed Jubeer: E Mail:** mohamedjubeer2001@gmail.com

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ABSTRACT

Struvite stone, which is often associated with urinary tract infection, is one of the types of urinary calculi that affects approximately 8-10% of the global population. The recurrence rate is significantly higher with the treatments that are currently available. So, this study focused on the analysis of the inhibitory potential of synthesized zinc oxide nanoparticle using the petals extract of *Rosa centifolia* on the gel grown struvite crystals as well as their antimicrobial activity against the urinary tract infection causing pathogens. Additionally, their antioxidant activity was also explored. The synthesized zinc oxide nanoparticle was characterized by UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray diffraction (XRD), scanning electron microscopy (SEM) and Energy dispersive X ray (EDX). The growth of gel grown struvite crystal was analysed by subjecting them with different concentration of synthesized zinc oxide nanoparticle. This revealed that the growth of the crystal was gradually decreased from 1.45cm to 0.45cm on increasing the concentration from 1% to 5% respectively. Then the antimicrobial activity of synthesized zinc oxide nanoparticle against major urinary tract infection causing pathogens such as *Escherichia coli(3cm), Klebsiella pneumonia(4cm),*

Enterococcus faecalis(3cm), Staphylococcus aureus(4cm), Candida albicans(4cm) and Candida vulgaris(3cm) was examined by measuring zone of inhibition in cm using disc diffusion method. The antioxidant activity also recorded in terms of IC50 which is 21.7% using DPPH assay. As a result, this study confirms that synthesized zinc oxide nanoparticle using the petals extract of *Rosa centifolia* has antiruolithiatic, antimicrobial and antioxidant activity on gel grown struvite crystals.

Keywords: Medicinal plants, *Rosa centifolia*, Green synthesis, Nanotechnology, Zinc oxide nanoparticle, Urinary tract infection, Urinary stone, Struvite crystals, Urolithiasis, antimicrobial activity, antioxidant activity

INTRODUCTION:

Nanotechnology is a multidisciplinary field that deals with nanoparticles whose size ranges from 1-100nm **[1] [2]**. Currently, nanoparticles are being applied in various fields such as medical imaging, drug delivery, cancer therapy, environment, electronics, food, agriculture, cosmetics, etc. **[3]**. It is because of its specialized properties such as larger surface area to volume ratio and increased reactivity **[4]**. Physical, chemical and biological methods can be used for the synthesis of nanoparticles **[5]**. In biological method (green synthesis) the nanoparticles are synthesized from bacteria, fungi, plants which is an eco-friendly, non-toxic, cheap and higher efficiency in nature **[6]** and it is preferred over physical and chemical methods which are often costly and toxic **[4]**. Top-down and bottom-up are the two approaches being carried out for the synthesis of nanoparticles. Majorly nanoparticles can be classified into three types (1) organic which includes dentrimers, micelles, liposomes and ferritin etc. (2) inorganic which includes metal (Zn, Al, Ag, Cu, Co, Fe, etc.) and metal oxides (ZnO, Al_2O_3, etc) (3) carbon-based nanoparticles include fullerene, carbon nanotubes, graphene, carbon nanofibre and carbon black, etc. **[7]**. Generally metal oxides are more reactive and has increased efficiency **[8]** in which zinc oxides, also known as zincite **[9]** have unique physiochemical properties **[10]** that has been used as folk medicine and in different industries **[11]**. Nowadays these synthesized zinc oxide nanoparticles from the plant extract have been used for vast applications such as antimicrobial, antioxidant, anticancer, antidiabetic activities, in drug delivery, as ROS scavenging, in gene delivery, in biosensor, in crop improvement and in plant protection **[3]**.

Urolithiasis has been one of the most challenging diseases for the past two decades **[12]**. Approximately 12 % of the population has been affected by urinary stone **[13]**. Men are more likely to be

affected by kidney stones for about 2 -4 times higher than women **[14]**. But in the case of struvite stones, women are mostly affected as they are more susceptible to urinary tract infection **[15]**. Urolithiasis occurs due to the supersaturation of urine with salts or insoluble metabolites or due to the lack of stone forming inhibitors **[16]**. It occurs in five stages such as urinary supersaturation, crystal nucleation, crystal growth, crystal aggregation and crystal cell interaction **[17] [18] [19]**. The major types of urinary stones are calcium oxalate, calcium phosphate, uric acid, struvite, cystine and medication induced stone **[20]**. Struvite stone which is also known as triple phosphate stone or infection stone is composed of magnesium ammonium phosphate. It is formed due to infection causing urea splitting bacteria such as proteus, klebsiella and pseudomonas which split urea into ammonia and carbon dioxide that makes the urine more alkaline which increases the rate of infection **[21]**. In adults it causes loin pain, gross haematuria, vomiting, sometimes fever and also be asymptomatic. In children it is often unclear **[22]**. Nowadays the most commonly available treatments are percutaneous nephrolithotomy, ureteroscopy and shockwave lithotripsy **[23]** which are costly, painful, with recurrence rate of 50-80% and have many side effects etc. **[24]**. As an alternative, herbal plants can be used as diuretic agents which are safe, affordable, readily available and don't have any complications **[20]**. As pharma industries are still depend on plants for their 60% drug production **[25]**

Rosa centifolia which is commonly known as cabbage rose belongs to the family rosaceae **[25]**. It can be found in temperate and cold regions and grows upto 1.5-2 meter in height **[26]**. The most important chemical constituents present in this species are phenyl ethanol, geranyl acetate, geraniol, Linalool, benzyl alcohol, benzaldehyde, nerol, citronellyl acetate **[25]**. It has been used as ornamentals, laxative, diuretics, cosmetics and in treating intestinal, ear, nose, throat problems, haemorrhages, cough, fever, insomnia, asthma, hypertension, urinary tract infection, kidney stones and also have anti-microbial, anesthetic, anti-septic, anti-inflammatory, anti-cancer, anti-pasmodic, anti-obesity, anti-oxidant, anti-rheumatism, antigout, nephroprotective, cardioprotective, neuroprotective and hepatoprotective properties **[25] [27] [28] [29]**.

MATERIALS AND METHODS

The chemicals such as ethanol, chloroform, sulphuric acid, hydrochloric acid, ferric chloride, glacial acetic acid, ammonia solution, sodium hydroxide, benzene, ethanolic alpha napthol, isoamyl alcohol, methanol, potassium ferro cyanide, diethyl ether, n-butanol, acetic acid, petroleum ether

were used for qualitative and quantitative analysis of phytochemicals and sodium meta silicate, ammonium dihydrogen ortho phosphate, anhydrous magnesium acetate, ascorbic acid, DPPH, potato dextrose agar, muller-hinton agar, which were used for gel formation. Antioxidant and antimicrobial studies were purchased from sigma-aldrich, New Delhi, India. The glass wares such as test tubes, boiling tubes, petri dishes, beakers, measuring cylinder and funnel were mainly used.

Collection of plant material:

The petals of *Rosa centifolia* were collected from the local market in Tiruchirappalli district **[30]**.

Preparation of ethanolic extract of *Rosa centifolia*:

The petals of *Rosa centifolia* were collected and dried at room temperature then the dried petals were made into powder. Hot percolation method was carried out to prepare the extract where, 15g of plant sample should be added with 100ml of ethanol followed by 24 hours incubation at 65˚C **[31]**.

Phytochemical screening of *Rosa centifolia*:

Based on precipitation and colouration method major phytochemicals of *Rosa centifolia* was analysed **[32]**.

1.Test for terpenoids:

To 2ml of extract few drops of chloroform followed by concentrated sulphuric acid should be added. If terpenoids is present, reddish-brown colour can be observed.

2.Test for flavonoids:

On the addition of few drops of concentrated sulphuric acid to 2ml of extract turns yellow shows the presence of flavonoids.

3.Test for saponins:

The appearance of froth formation when few drops of distilled water added to 2ml of extract shows the presence of saponins.

4.Test for tannins:

If the addition of few drops of distilled water followed by few drops of Ferric chloride to 2ml of extract forms green colour or green precipitation, it shows the presence of tannins.

5.Test for alkaloids:

To 2ml of extract few drops of glacial acetic acid followed by ammonia solution should be added. If the solution turns yellow it confirms the presence of alkaloids.

6.Test for steroids:

The addition of few drops of chloroform followed by concentrated sulphuric acid to 2ml of extract forms reddish-brown ring confirms the presence of steroids.

7.Test for glycosides:

When few drops of chloroform followed by few drops of glacial acetic acid added to 2ml of extract shows violet or blue or green colouration, confirms the presence of steroids.

8.Test for phlobatannins:

The Appearance of red colour precipitate on the addition of few drops of Hydrochloric acid to 2ml of extract confirms the presence of phlobatannins.

9.Test for protein:

To 2 ml of extract few drops of concentrated sulphuric acid should be added. The appearance of white precipitate shows the presence of protein.

10. Test for Coumarins

Few drops of sodium hydroxide added to 2 ml of extract turns the solution in to yellow shows the presence of Coumarins.

11.Test for emodin:

The appearance of red colour on the addition of few drops of ammonia solution to 2ml of extract shows the presence of emodin.

12.Test for antraquinone:

The appearance of pink or violet or red colour on the addition of few drops of benzene followed by few drops of ammonia solution confirms the presence of antraquinone.

13.Test for anthocyanin:

To 2ml of extract few drops of hydrochloric acid followed by few drops of ammonia solution should be added. The appearance of pinkish-red to bluish-violet shows the presence of anthocyanins.

14.Test for carbohydrates:

On the addition of few drops of distilled water followed by pinch of ethanolic alpha napthol and few drops of concentrated sulphuric acid forms reddish-violet ring confirms the presence of carbohydrates.

15.Test for leucocyanins:

When few drops of isoamyl alcohol added to 2ml of extract leads to the formation of red colour in the organic layer shows the presence of leucocyanins.

16.Test for cardiac glycoides:

To 2ml of extract few drops of glacial acetic acid followed by ferric chloride and concentrated sulphuric acid should be added. The formation of violet brown ring shows the presence of cardiacglycosides.

17.Test for xanthoproteins:

The appearance of blue or black coloration on the addition of ferric chloride to 2ml of extract shows the presence of xanthoproteins.

18.Test for phenolics:

If the addition of ammonia solution to 2ml of extract forms reddish-orange precipitate shows the presence of phenolics.

Quantitative analysis of phytochemicals in *Rosa centifolia*:

The phytochemicals of *Rosa centifolia* can be quantified in mg/g by standard protocol, in which the filtered phytochemical weighed in watch glass using electronic balance. Before weighing the filtered phytochemicals, empty watch glass should be weighed. Then to find the weight of phytochemical in mg/g the weight of the empty watch glass should be subtracted

from the weight of the watch glass with yield **[32]**.

1.Flavanoids:

To 0.5g of extract, 3ml of methanol should be added. After filtration, the filtrate should be poured in watch glass and left at room temperature.

2.Tannins:

To 0.5g of extract, 3ml of distilled water should be added. After filtration, the filtrate should be added with one drop of ferric chloride followed by 0.008M potassium ferrocyanate. Then it should be dried in watch glass at room temperature.

3.Saponins:

To 0.5g of extract, 3ml of ethanol should be added. After filtration, the filtrate should be added with 1ml of diethyl-ether and shaken well for the formation of double layer. Then the upper layer should be discarded and to the layer at bottom one drop of n- butanol should be added. At last, it should be dried in watch glass at room temperature.

4.Alkaloids:

1ml of acetic acid and 2ml of ethanol should be mixed well. This mixture should be added to 0.5g of extract. After filtration, one or two drops of ammonia solution should be added to the filtrate and dried in watch glass at room temperature.

5.Phenol:

To 0.5g of plant sample, 3ml of distilled water should be added. After filtration, the filtrate should be added with one or two drops of ammonia solution followed by one or two drops of isoamyl alcohol. Then it should be dried in watch glass at room temperature.

6.Terepenoids:

To 0.5g of extract, 3ml of ethanol should be added and filtered. Then the filtrate should be added with one or two drops of petroleum ether which should be dried in watch glass at room temperature.

Synthesis of zinc oxide nanoparticles:

1mM zinc acetate was prepared for 50ml then it was added with 10 ml of the extract of *Rosa centifolia*. Then the mixture was subjected to continuous stirring for about 2 hours. The appearance of pale-precipitate shows the formation of zinc oxide nanoparticles **[32]**.

Characterization of synthesized Zinc oxide nanoparticles:

The synthesized zinc oxide nanoparticles can be characterized based on the determination of its wavelength using UV-VIS Spectra, its functional groups using FTIR, its size using SEM, its crystalline nature and elemental composition using XRD and EDX respectively **[33]**.

UV spectroscopy:

The unique optical property of synthesized ZnO nanoparticle can be used for its characterization. If the absorbance range is recorded between 350-500nm using UV spectrophotometer, it confirms the presence of ZnO nanoparticles.

FTIR:

Fourier Transform Infrared Spectroscopy (FTIR) gives the information of multiple functional groups in synthesized ZnO nanoparticles for the characterization by scanning the particles using infrared light. The absorbance range of FTIR ranges between 400 and 4000cm-1. The specific ranges of absorbance represent the specific functional group reveals the nature of particle.

XRD:

X-ray Diffraction (XRD) is an analytical study of nanoparticles based on its crystallinity. By analysing the intensities and angles of scattered x-ray from the particles reveal the nature of that particle.

SEM:

By the determination of surface morphology of nanoparticles, the nature of the particle can be characterized using scanned electron microscope (SEM).

EDX:

Energy dispersive X-ray can be used for the characterization of nanoparticles as it determines the elemental composition of that nanoparticle.

Formation of struvite crystals:

The struvite crystals were formed by the addition of 15 ml of 0.5M ammonium dihydrogen ortho phosphate slowly along the side of the test tube to 15 ml of sodium meta silicate (30g in 30ml) (1:1 ratio) then it should be incubated for 4-5 days for gelation. 1M of magnesium acetate was prepared for the formation of struvite crystals **[34]**.

Analysis of growth of struvite crystals:

The analysis of the growth struvite crystals was carried out in different concentration (1%-5%) along with control represented in **Table 1** given below **[34]**.

Table 1: The compostion of samples with which the struvite crystals was grown

S. No.	Group	Composition
1	Control	10ml of magnesium acetate
2	Control + water	5ml of magnesium acetate + 5ml of water
3	Control + ethanol	5ml of magnesium acetate + 5ml of ethanol
4	1% of synthesized ZnO nanoparticle	1ml of synthesized ZnO nanoparticle+ 4ml of ethanol
5	2% of synthesized ZnO nanoparticle	2ml of synthesized ZnO nanoparticle + 3ml of ethanol
6	3% of synthesized ZnO nanoparticle	3ml of synthesized ZnO nanoparticle+ 2ml of ethanol
7	4% of synthesized ZnO nanoparticle	4ml of synthesized ZnO nanoparticle+ 1ml of ethanol
8	5% of synthesized ZnO nanoparticle	5ml of synthesized ZnO nanoparticle

Antioxidant activity by DPPH assay:

DPPH (2,2-diphenylpicrylhydrazyl) is a potential free radical that can be used for the determination of Antioxidant activity of synthesized ZnO nanoparticles from petal of *Rosa centifolia* where, the different concentrations of ascorbic acid (20-100µg/mL) and the ZnO nanoparticles (20-100µg/mL) were made upto 1ml with ethanol in the test tubes followed by 0.5ml

of DPPH solution was added to all the test tubes, then,1.5 ml of DPPH solution was used as control. The change in colour of the solution from purple to yellow represents the antioxidant activity which can be recorded at the absorbance of 540nm using calorimeter and the percentage of inhibition was calculated in each sample by the following formula.

Percentage of inhibition of DPPH activity = (A1-A2/A1) × 100

Where, A1 was OD value of the control and A2 was OD value of the sample. A curve of concentration of samples corresponded to the inhibition of DPPH were plotted and the IC50 value was calculated **[35]**.

Antimicrobial activity of synthesized zinc oxide nanoparticle from the petals of *Rosa centifolia*:

Collection of test pathogens:

The bacterial species *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus* and the fungal species *Candida albicans, Candida vulgaris* were taken as samples which were purchased from Microbial Type Culture and Collection (MTCC), Chandigarh, India and National Chemical Laboratory (NCL), Pune, Maharashtra, India respectively.

Antibacterial activity by disc diffusion method:

3.8g of Mueller-Hilton agar was dissolved in 100 ml of distilled water was prepared as media to grow the bacterial species. After media preparation, the culture should be added to the plate by swab method. Then at the top of the plate the sterile paper discs with 6mm diameter which have soaked in different concentration (60,80 and 100 µl) of ZnO nanoparticles along with positive (amoxicillin) and negative (ethanol) controls was placed. After 24 hours inhibition the zone of inhibition was recorded in millimetre. Here, the diameter of zone of inhibition is directionally proportional to the antibacterial activity **[36]**

Antifungal activity by disc diffusion method:

Potato dextrose agar was prepared at the concentration of 2.9g in 100 ml distilled water to grow fungal species. After the culture added in a plate by swab method, the sterile paper discs with 6mm diameter which have soaked in different concentration (60,80 and 100 µl) of ZnO nanoparticles along with positive (fluconazole) and negative (ethanol) controls was placed at the top of the plate. Then the plates were incubated for 24hrs for the formation of zone of inhibition whose diameter was recorded in millimetre because the diameter of zone of inhibition is directionally proportional to the antibacterial activity **[37]**.

RESULTS AND DISCUSSIONS:

Qualitative analysis:

Phytoconstituents present in the extract of *Rosa centifolia* were analysed using different tests shown in **Table 2 and Figure 1**.The obtained result was highly supported by the previous literature of Simatupang *et al*., 2021 who recorded the presence of major phytochemicals such as cardioglycosides, alkaloids, flavonoids, anthocyanins, betacyanins, coumarins, glycosides, phenolics, quinones, tannins and terpenoids from the methanolic extract of *Rosa chinensis* **[38]** and the work of singh *et al*., 2021 also recorded the presence of alkaloids, glycosides, flavonoids, steroids, cardiac glycosides from the ethanolic extract of *rosa centifolia* **[39]**.

Table 2: Phytochemical analysis of petals of *Rosa centifolia*

Test no	Phytochemicals	Observation	Result
1.	Terpenoid	Reddish brown colour	+++
2.	Flavanoid	Yellow colour	+++
3.	Saponin	Froth formation	+++
4.	Tannin	Green precipitate	+++
5.	Alkaloid	Yellow colour	+++
6.	Steroids	Reddish brown ring	+++
7.	Glycosides	Violet blue colour	+++
8.	Phlobatannins	Red colour precipitate	+++
9.	Protein	White precipitate	+++
10.	Coumarin	Yellow colour	+++
11.	Emodin	Red colour	+++
12.	Anthroquinone	Pink violet colour	+++
13.	Anthocyanin	Pinkish red colour	+++
14.	Carbohydrate	Reddish violet colour	+++
15.	Leucoanthocyanin	Organic layer into red	+++
16.	Cardiacglycosides	Brownish violet ring	+++
17.	Xanthoprotein	Bluish black colour	+++
18.	Phenol	Reddish orange precipitate	

+++ - Strongly present ++ - moderately presen + - slightly present A- Absent

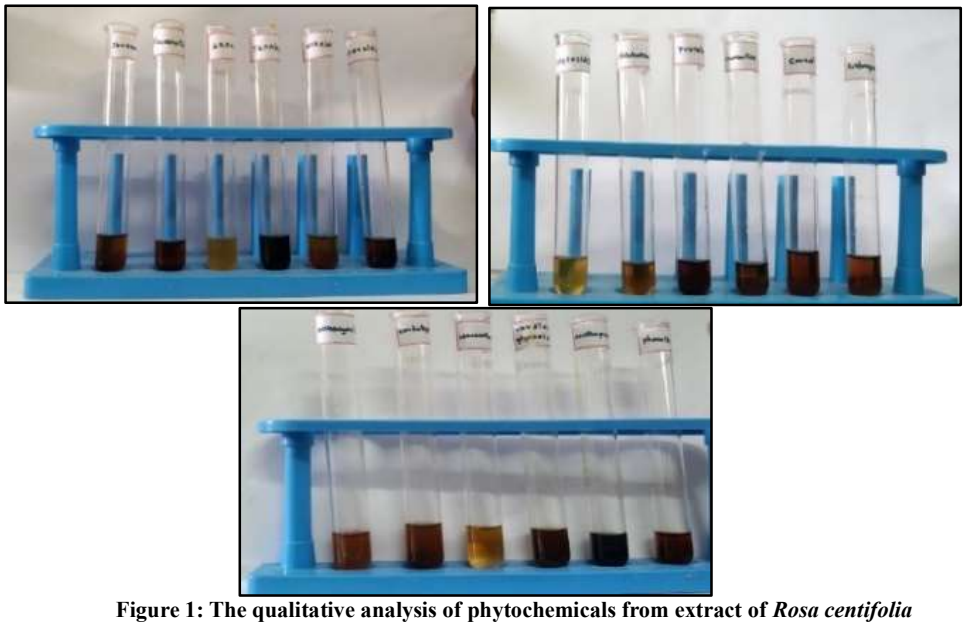


Figure 1: The qualitative analysis of phytochemicals from extract of *Rosa centifolia*

Quantitative analysis:

Quantification of phytoconstituents from the extract of *Rosa centifolia* was carried out and its concentration was determined for tannin(0.027mg/g), alkaloids(0.026mg/g), phenol (0.018mg/g), terepenoids (0.014mg/g) and flavonoids (0.010mg/g) which was showed in **Figure 2**. This same protocol was used for the quatification of phytochemicals in the previous study carried out by Vidhya *et al*., 2021 who determined the total amount of phytochemicals such as phenol (0.096 mg/g), flavonoids (0.063mg/g), saponins (0.048mg/g), terepenoids (0.042 mg/g), tannins (0.027mg/g) and alkaloids (0.003 mg/g) in *Terminalia chebula* **[34]** and in another study alsothe total amount of phytochemicals such as alkaloids (0.93mg/g), saponins (0.089 mg/g), phenols (0.011mg/g), tannins (0.008mg/g), flavonoids (0.006 mg/g) and terepenoids (0.003 mg/g) of *Linum usitatissimum* was recorded by Bykanmani *et al*., 2021 **[41]**.

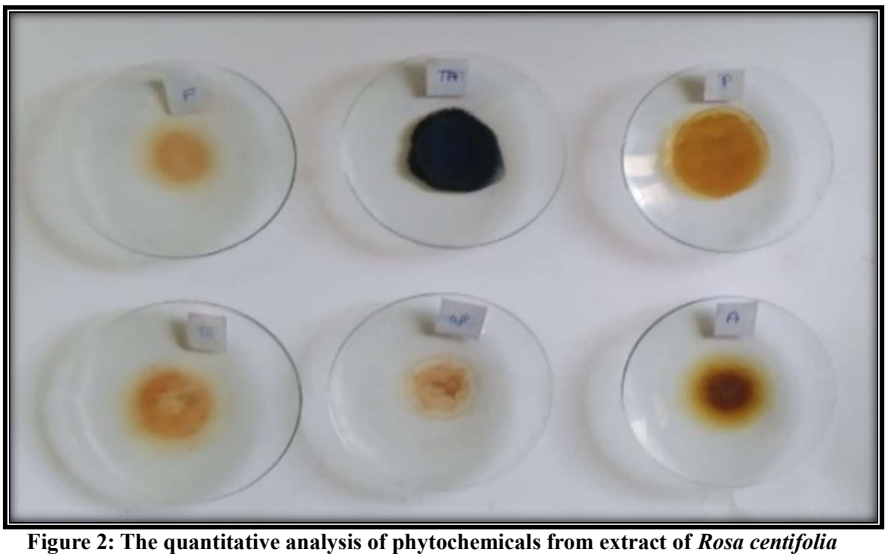


Figure 2: The quantitative analysis of phytochemicals from extract of *Rosa centifolia*

ZnO nanoparticle synthesis:

Green synthesis method is used for the synthesis of Zno nanoparticle. when zinc acetate solution was added with ethanolic extract of Rosa centifolia and subjected to magnetic stirrer, the zinc acetate was reduced to zinc oxide. This reduction was observed as a pale yellow colour precipitate which indicates the presence of Zno nanoparticle that was showed in **Figure 3**. In the previous literature of Tiwari *et al*., 2017, synthesized Zn nanoparticles from the petals of *Rosa indica* was observed as creamish-white precipitate by the addition of 10Mm of zinc nitrate with 20g of plant extract **[32]**. In another study by Raj *et al*., 2018, synthesized Zn nanoparticles from the plant *Rosa indica* was observed as pale-yellow precipitate as 10 ml of extract was added with 2mM solution of zinc acetate solution **[9]**.

Figure 3: Green synthesis of zinc oxide nanoparticle from the petal extract of *Rosa centifolia*

Characterization of ZnO nanoparticle:

1.UV-Vis spectroscopy:

The synthesized ZnO nanoparticles from the extract of *Rosa centifolia* were analysed in the range of 200-1100nm through UV spectrometer and the peak obtained at 278.65nm which was showed in **Figure 4** confirms the presence of Zno nanoparticle. The previous study carried out by Aaisha *et al.,* 2018 has obtained sharp peak at 279nm shows the presence of synthesized Zno nanoparticle from the extract of *Luffa acutangala* **[42]**.

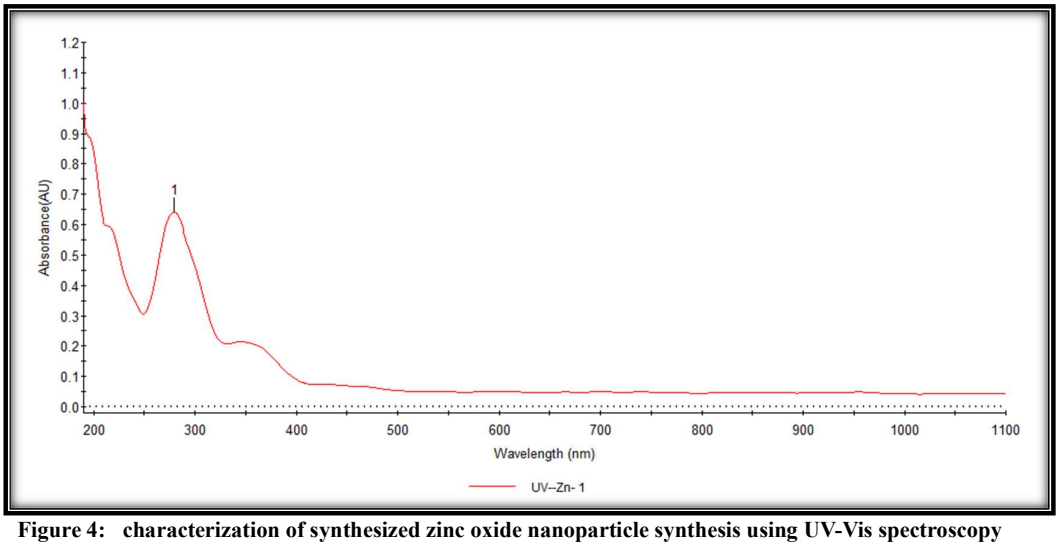


Figure 4: characterization of synthesized zinc oxide nanoparticle synthesis using UV-Vis spectroscopy

2.FTIR analysis:

The functional groups of synthesized Zno nanoparticles were analysed through FTIR and the peak obtained at 3459.82cm^{-1}, 2087.10cm^{-1}, 1757.92cm^{-1}, 1638.18cm^{-1}. 1384.21cm^{-1},1248cm^{-1}, 1051.58cm^{-1} and 639.74cm^{-1} which was showed in figure 5indicates OH stretching of amide group, H-O-H, C=O carboxylic acid and its derivatives, C=C stretching of alkane group, C-H bending of alkane group, O-C carboxylic acid and its derivatives, stretching of C-N, C- alkyl chloride respectively. Earlier study of analysis of synthesized zinc oxide nanoparticle using *Papaver somniferum* by Muhammad *et al.,* 2019 recorded the peaks at 3380cm-1, 2370cm-1, 1652cm-1, 1387cm-1, 1012cm-1,664cm-1 using FTIR which indicates OH of amide group, H-O-H, C=C stretching of alkane group, C-H bending of alkane group, stretching of C-N and C- alkyl chloride **[31]**. Another study carried out by Aaisha *et al.,* 2018 synthesized ZnO nanoparticles from the extract of *Luffa acutangala* has obtained peak at 1756.02cm-1, 1213.77cm-1 shows C=O carboxylic acid and its derivatives and O-C carboxylic acid and its derivatives **[42]**.

Table 3: FTIR analysis of synthesized zinc oxide nanoparticle

Important compound species of struvite crystal	IR frequency of control (cm^{-1})	IR frequency of synthesized ZnO naoparticle (cm^{-1})	Functional group
Absorption peak due to water of crystallization	3240.49	3247.45	H-O-H stretching vibration of water of crystallization
	2365.95, 2026.41	2365.95, 2026.77	H-O-H stretching of cluster of water
	1628.78	1630.74	H-O-H bending mode of vibration
Absorption peaks due to NH_4^+ units	2925.29	2924.93	*V1* symmetric stretching vibration of N-H in NH_4^+ units
	1437.50	1437.58	*V*4 asymmetric bending vibration of N-H in NH_4^+ units
Absorption peaks due to PO_4^{3-} units	1003.65	1004.08	*V*3 asymmetric stretching vibration of PO_4^{3-} units
	570.49	570.48	*V*4 asymmetric bending modes
	759.99	759.89	Liberation of water
Metal oxygen bonds	893.40	891.76	Deformation of OH linked to Mg^{2+}
	460.07	459.28	Metal oxygen bond

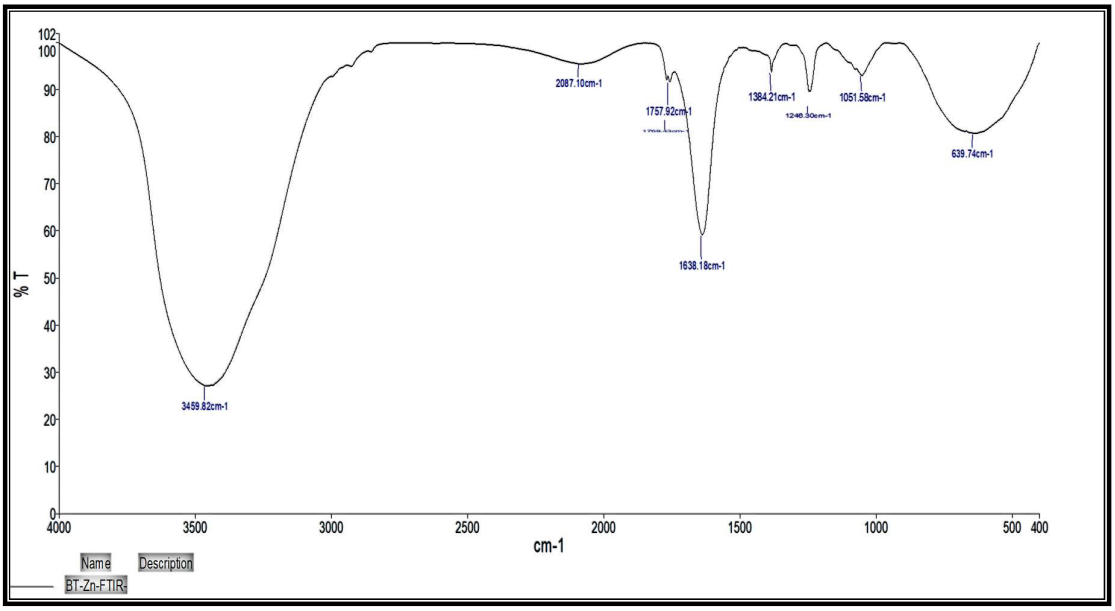


Figure 5: FTIR analysis synthesized zinc oxide nanoparticle

3.XRD Analysis:

The crystallographic structure of synthesized Zno nanoparticles was determined using XRD analysis. The diffraction pattern obtained at 2θ = 31.67° (100), 34.38° (002), 36.29° (101), 47.69° (102), 56.53° (110), 62.92° (103), and 68.78° (112) that was showed in **Figure 6** correspond to hexagonal wurtzite structure of the Zno nanoparticle. The peaks obtained shows good planar agreement with the JCPD files. In the previous study, the X ray diffraction pattern of synthesized ZnO nanoparticle from the leaf extract of *Ixora coccinea* was carried out by Yedurkar *et al*., 2016 in which the 2θ values were recorded at 31.84˚, 34.52˚, 36.38˚, 47.64˚, 56.7˚, 63.06˚ and 68.1˚which agreed with JCPDS Data Card No: 36-1451 **[43]** and in the another study carried out by chikanna *et al.,* 2018 revealed the 2θ values of synthesized ZnO nanoparticles from goat fecal matter which was recorded at 31.77˚, 34.44˚, 36.27˚, 47.62˚, 56.73˚, 62.96˚, 68.06˚ and from sheep fecal matter at 31.79˚, 34.47˚, 36.26˚, 47.67˚, 56.66˚, 62.94˚, 68.01˚ which were agreed with JCPDS :80-0075 card ICSD#:067849) **[44]**.

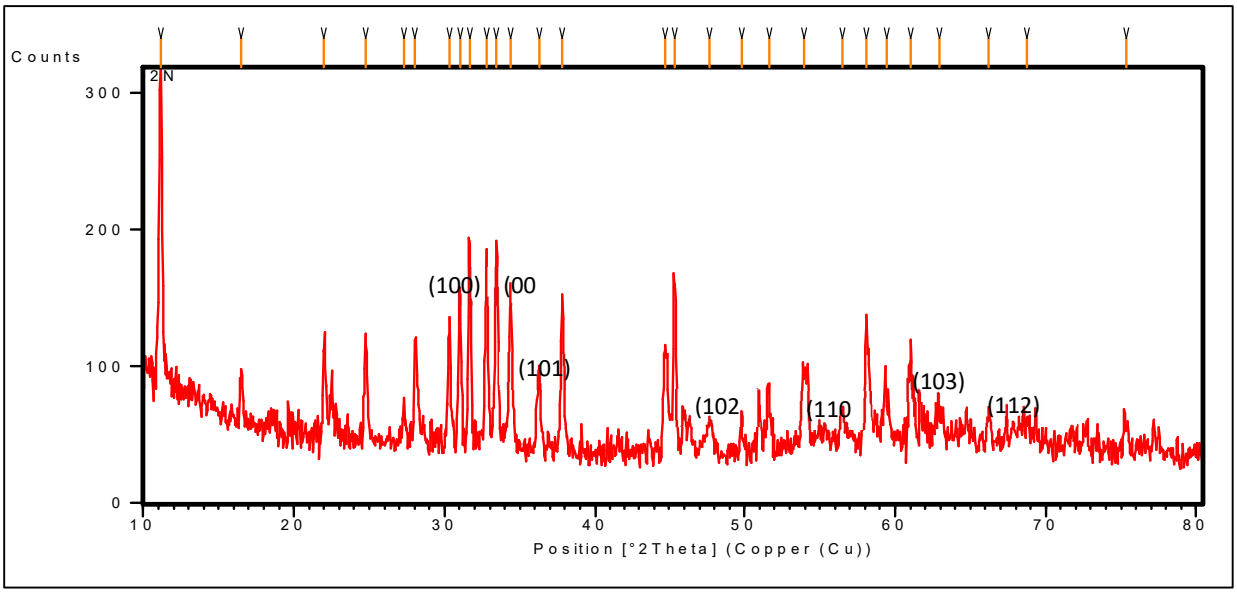
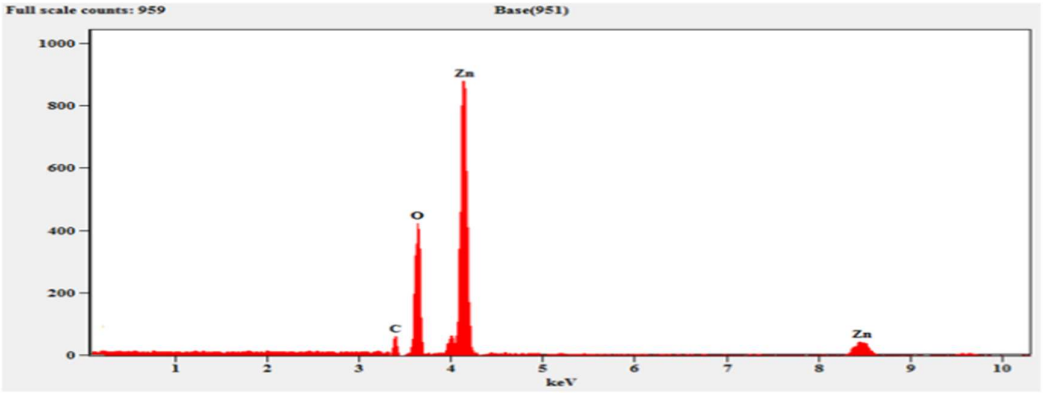


Figure 6: XRD analysis of synthesized zinc oxide nanoparticle

4. EDX Analysis:

In Energy dispersive X ray (EDX) analysis, the synthesized ZnO nanoparticles was characterized by its elemental composition. In the **Figure 7** the highest peak of zinc followed by oxygen was recorded which shows its purity and the small peak of carbon may due to the substrate carbon tape that was reported by the study of Islam *et al*., 2019 **[48]** or due to the interaction of phytochemicals during nanoparticle synthesis as per the previous study carried out by Santhoshkumar *et al*., 2017 where it showed the composition of 75.36% of zinc, 22.36% of oxygen and 2.29% of carbon in the synthesized ZnO nanoparticle from the leaf extract of *Passifloraceae caerulae* **[47]**.



	Weight %	Weight % Error	Atom %
C K	10.23	± 0.89	25.18
O K	26.48	± 0.79	44.26
Zn K	52.16	± 1.61	19.44
Zn L	11.13	± 1.81	11.12
Total	100.00		100.00

Figure 7: EDX analysis of synthesized zinc oxide nanoparticle

5. SEM Analysis:

Scanning electron microscopy revealed the surface morphology of synthesized nanoparticle. From the **Figure 8**, it was confirmed the synthesized ZnO nanoparticle has spherical shaped structure which was supported by the study of pillai *et al.,*2020 **[48]** who recorded that the synthesized nanoparticle from Beta vulgaris shows spherical shape.

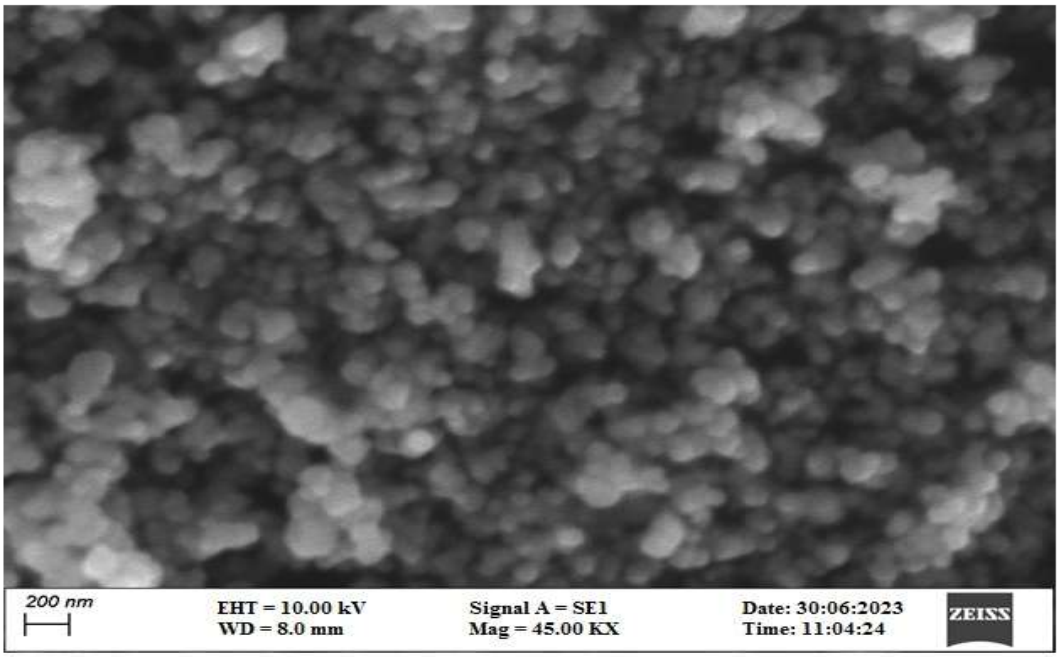


Figure 8: SEM analysis of synthesized zinc oxide nanoparticle

Characterization of struvite crystal:

FTIR analysis of struvite crystal was obtained with and without the addition of synthesized ZnO nanoparticles which was shown in the **Figure 9**. The frequency obtained and its functional group are listed below in the **Table 3**. The obtained result was highly supported by the study carried out by Das *et al*., 2016 who listed the already reported absorption spectrum of struvite crystal grown by gel method **[49]** and the statistical analysis also recorded using ANOVA which was represented in **Table 4**. The influence of herbal extract of *Orthosiphon aristatus* on struvite crystal was recorded by Muryanto *et al*., 2016 where the peak value of struvite crystal shifted from 2889.25 cm^{-1}, 1700 cm^{-1}, 1432.45 cm^{-1},1164.26 cm^{-1},991.98 cm^{-1},683.97 cm^{-1} to 2969.78 cm^{-1}, 1739.64 cm^{-1}, 1436.52 cm^{-1},1226.74 cm^{-1},1008.48 cm^{-1}, 685.17 cm^{-1} respectively **[50]**. Another study carried out by mammate *et al.,*2023 also recorded the peak shift when the struvite crystal was subjected to the different concentration of herbal extract of *Saussurea costus* **[51]**.

Table 4: statistical analysis of struvite crystals by ANOVA

	Groups	Treatments	Mean (gm) ± SD
Struvite crystals	A	Control (magnesium acetate)	2.86 ± 1.3736
	B	Control + water	2.73 ± 1.775
	C	Control + ethanol	2.81 ± 1.84
	D	Control + 1% of synthesized zinc oxide nanoparticle	0.94 ± 0.29a
	E	Control + 2% of synthesized zinc oxide nanoparticle	0.8 ± 0.58 ^{a,b,c}
	F	Control +3% of synthesized zinc oxide nanoparticle	0.68 ± 0.336 ^{a,b,c,d}
	G	Control +4% of synthesized zinc oxide nanoparticle	0.52 ± 0.35 ^{a,b,c,d,e}
	H	Control +5% of synthesized zinc oxide nanoparticle	0.39 ± 0.288 $^{a,b,c,d,e,f\text{-ns}}$

At 0.05 level, the population means are significantly different

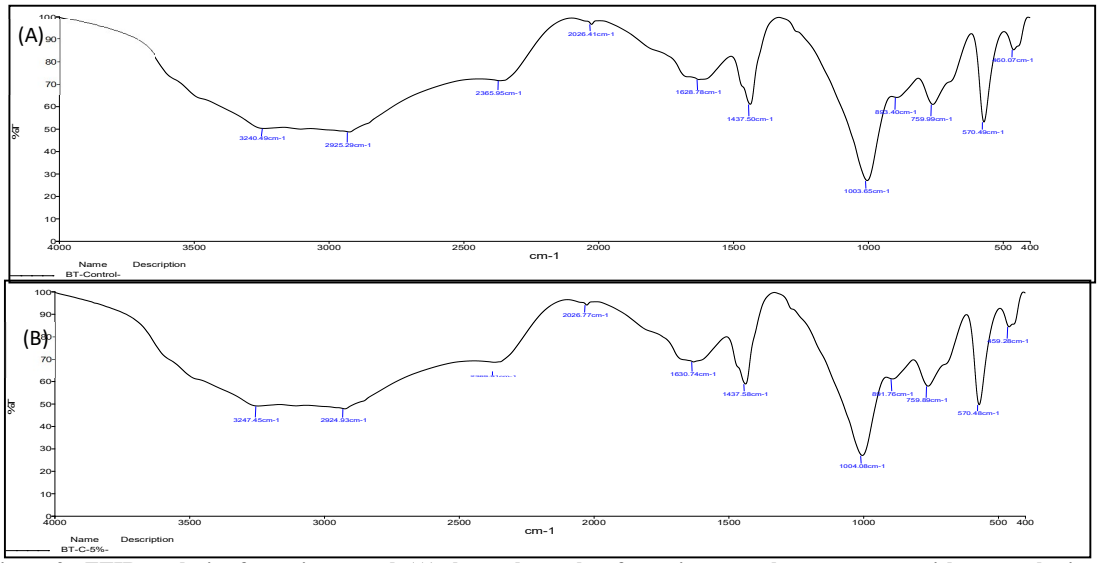


Figure 9: FTIR analysis of struvite crystal. (A) shows the peaks of struvite stone that was grown without synthesized zinc oxide nanoparticle (B) shows the peaks of struvite crystals that was grown with 5% of synthesized zinc oxide nanoparticle

Effect of synthesized zinc oxide nanoparticles on struvite crystal:

The growth analysis of gel grown crystals that were subjected to different concentrations of synthesized ZnO nanoparticle revealed their effect on struvite crystals. The maximum crystal growth was observed in control which was magnesium acetate and the gradual reduction of the sizes of the crystals were observed with increasing the concentration of synthesized ZnO nanopaticle that was showed in the **Figure 10** and the harvested struvite crystals were showed in **Figure 11**. The dimensions of crystals were recorded in cm that was showed in **Figure 12**, from which it was clearly understood that there was gradual reduction in the growth of crystals that were subjected to 1% - 5% of synthesized nanoparticles when compared to the growth of crystals in control, control + water and control + ethanol. In which the largest crystal (3cm) was recorded in control and the shortest crystal(1cm) was recorded in

5% concentration of synthesized zinc oxide nanoparticle. An ANOVA statistical analysis was performed to evaluate various parameters. The results shown in table 3indicated a significant correlation with a p-value of less than 0.05. The previous study recorded on antiurolithiatic activity of silver nanoparticle on *Terminalia chebula* bark by vidhya*et al*., 2021 determined that reduction of struvite stone growth by 87.9% using T.chebula mediated silver nanoparticle**[34]**.

Figure 10: The inhibition effect of different concnetration of struvite crystals. (a) control – magnesium acetate (b) control with water (c) control with ethanol (d) control with 1% (e) control with 2% (f) control with 3% (g) control with 4% (h) control with 5% of synthesized zinc oxide nanoparticle

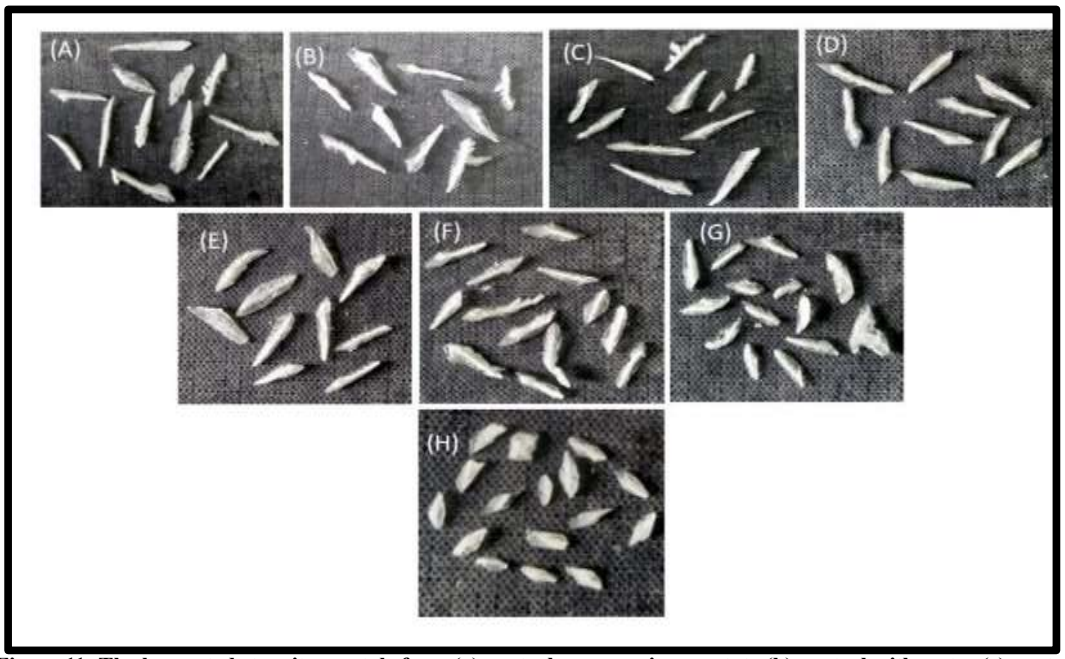


Figure 11: The harvested struvite crystals from (a) control – magnesium acetate (b) control with water (c) control with ethanol (d) control with 1% (e) control with 2% (f) control with 3% (g) control with 4% (h) control with 5% of synthesized zinc oxide nanoparticle

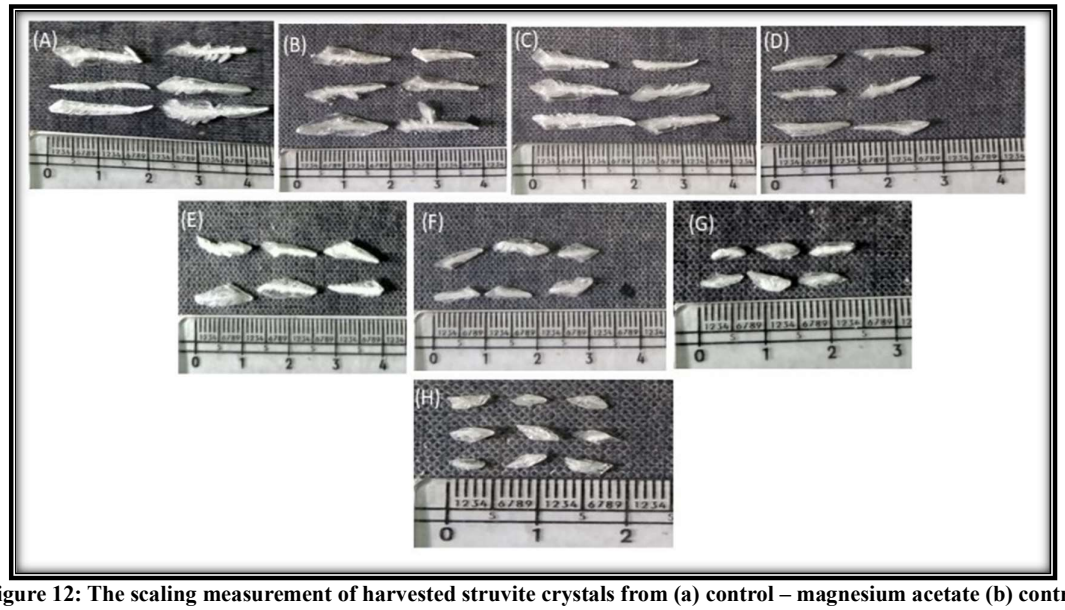


Figure 12: The scaling measurement of harvested struvite crystals from (a) control – magnesium acetate (b) control with water (c) control with ethanol (d) control with 1% (e) control with 2% (f) control with 3% (g) control with 4% (h) control with 5% of synthesized zinc oxide nanoparticle

Antioxidant activity of synthesized ZnO Nanoparticle from *Rosa centifolia* by DPPH assay method

The antioxidant activity with different concentration was represented in **Table 5**. The synthesized ZnO Nanoparticles from *Rosa centifolia* showed 83.49±3.6626% of inhibition at the concentration of 100µl/ml and the standard ascorbic acid showed 91.26±7.00482% of inhibition at the concentration of 100µl/ml. The previous study for antioxidant activity using DPPH assay was carried out by Sushma *et al*., 2015 reported that 300µg/ml synthesized ZnO nanoparticles from *ocimum tenuiflorum* have 65.23% of inhibition **[45]** and in another study of Rehana *et al*., 2017 the antioxidant activity of chemically synthesized ZnO nanoparticle and biologically synthesized ZnO nanopartcile from 5 different plant extract such as *Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii, Moringa oleifera*, and *Tamarindus indica with* IC_{50} *values of >100, 37.8* ± 0.200, 14.67± 0.244, 36.46 ± 0.115, 11.55 ± 0.100, 11.49 ± 0.221 respectively along with standards such as ascorbic acid (7.65 ± 0.300) and rutin (8.19 ± 0.195) were recorded using DPPH assay where they found that the ZnO nanoparticle from *Tamarindus indica* showed more antioxidant activity compared to others and all other biologically synthesized ZnO nanoparticles showed more activity than chemically synthesized one **[46]**.

Table 5: Antioxidant activity of synthesized ZnO Nanoparticle from *Rosa centifolia* by DPPH assay method

Concentration (µg/ml)	Ascorbic acid	ZnO Nanoparticle
20	53.39±5.50644	50.48±7.00482
40	65.04±6.61218a	58.25±5.25236[a]
60	76.69±6.40631ab	62.13±3.27424[a,b]
80	87.37±5.91728abc	73.78±6.56969[a,b,c]
100	91.26±7.00482abcd	83.49±3.6626[a,b,c,d]
IC50	9.55	21.7

At 0.05 level, the population means are significantly different

Antimicrobial Activity

The antimicrobial activity of synthesized zinc oxide nanoparticle against bacterial species such as *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus,* and fungal species such as *Candida albicans and Candida vulgaris* were recorded in **Table 6 and 7** respectively. From the **Table 6 and 7** given below, the inhibitory action of synthesized zinc oxide nanoparticle was recorded in terms of zone of inhibition that was measured in mm. it was observed that the zone of inhibition was increased with increase in concentration (60,80 and 100µg/ml) of synthesized zinc oxide nanoparticle which was showed in the **Figure 13 and 14**. In the previous literature of Murugan *et al.,* 2021, synthesized Zn nanoparticles from the leaf of *Limonia acidissima,* zone of inhibition of 19mm and 16mm against on *Klebsiella pneumonia and Staphylococcus aureus* were observed **[36]**. In another report, the synthesized Zno nanoparticle from fresh leaf extract of *Passiflfloracaerulea* bySanthoshkumar*et al.,*2017 wasrecorded with the zone of inhibition of 13mm and 9.33mm at 75ul against on *E.coli* and *Enterococcus sp***[47]**. In the previous study reported that Zno NP synthesized from *Cinnamomum tamala* and *Braassica olerocea* var. italica by pillai *et al.*, 2020 the zone of inhibition of 8mm against *Candida albicans* was observed **[37]**.

Table 6: Antibacterial activity of synthesized zinc oxide nanoparticle

Concentration	*Zone of inhibition in mm*			
Samples	*Escherichia coli*	*Klebsiella pneumonia*	*Enterococcus faecalis*	*Staphylococcus aureus*
Control (Ethanol)	0mm	0mm	0mm	0mm
Standard (Amoxicillin)	8mm	10mm	10mm	12mm
60µg/ml	3mm	5mm	3mm	4mm
80µg/ml	4mm	6mm	4mm	5mm
100µg/ml	5mm	8mm	5mm	6mm

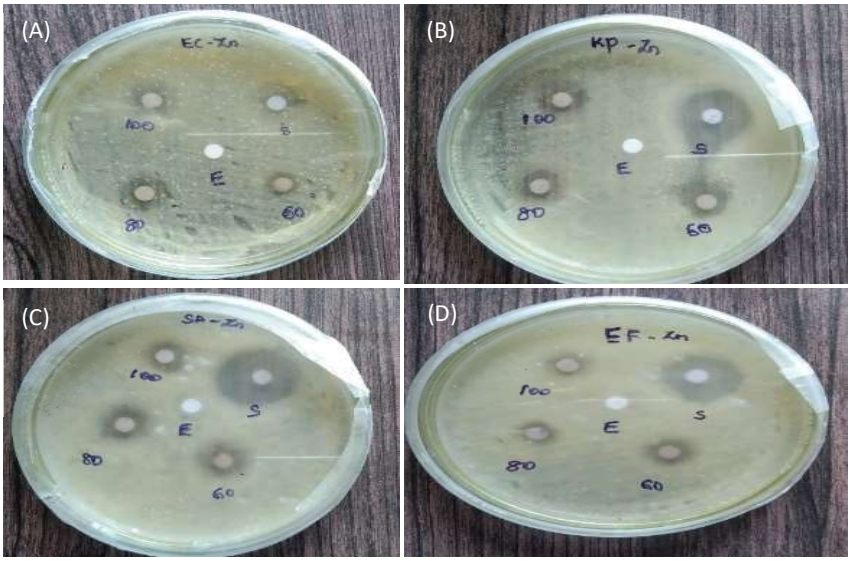


Figure 13: The antibacterial activity of synthesized zinc oxide nanoparticleon (a)*Escherichia coli* (b)*Klebsiella pneumonia* (c)*Enterococcus faecalis* (d)*Staphylococcus aureus*

Table 7: Antifungal activity of synthesized zinc oxide nanoparticle

Concentration	**Zone of inhibition in mm**	
Sample	***Candida albicans***	***Candida vulgaris***
Control (ethanol)	0mm	0mm
Standard(fluconazole)	12mm	12mm
60µg/ml	6mm	5mm
80µg/ml	7mm	7mm
100µg/ml	9mm	8mm

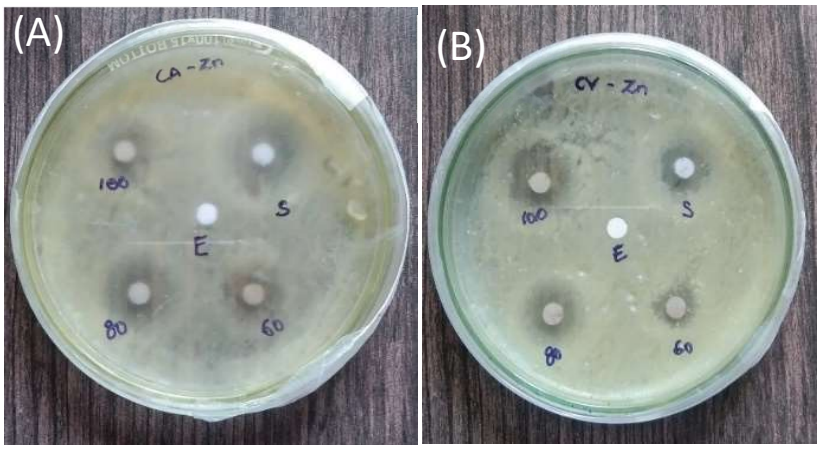


Figure 14: The antifungal activity of synthesized zinc oxide nanoparticle on (a) *Candida albicans (b) Candida vulgaris*

CONCLUSION:

The growth of gel grown struvite crystals was analysed using different concentration of biosynthesized zinc oxide nanoparticle which was characterized by UV-Vis spectroscopy, FTIR, XRD, SEM and EDX revealed that the increasing concentration of zinc oxide nanoparticle will gradually reduce the size of the struvite crystal. At the same time, it was found that they have

maximum inhibition effect on urinary tract infection causing microbes such as*Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus, Candida albicans and Candida vulgaris, Candida albicans, Candida vulgaris and Klebsiella pneumonia* as well as they have antioxidant activity with 21.7% of IC50. Thus, this was the first study that revealed the antiurolithiatic, antimicrobial and antioxidant activities of biosynthesized zinc oxide nanoparticle from the petal extract of *Rosa centifolia.*

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