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RESEARCH ARTICLE

Green Synthesis of Zinc Oxide Nanoparticles in the Fruit Extract of *Hylocereus undatus* Linn. and its Antiurolithiatic Activity Against Struvite Kidney Stones

Vanathi Sundarapandiyan[1], I. Helen Diana[1*] and K. Manjula[2]

[1]Department of Botany, Holy Cross College, Tiruchirappalli – 620002, Tamil Nadu, India; helendiana1981@gmail.com

[2]Biotechno Solutions, Ponmalaipatti, Tiruchirappalli – 620004, Tamil Nadu, India

Abstract

Aim: The objective of the study is to synthesize and characterize the properties of zinc oxide nanoparticles from natural sources. The aqueous extract of *Hylocereus undatus* Linn. fruit was used to synthesize Zinc oxide nanoparticles that were taken for the investigation of antiurolithiatic activity against the struvite stones. **Methods:** The Preliminary analysis of phytochemical screenings was carried out to study the secondary metabolites of the fruit. The magnetic stirrer method was used to synthesize the Zinc oxide Nanoparticles then their characterizations were determined by Ultra Violet (UV) spectral analysis, Fourier Transform Infra-Red (FTIR) analysis, Scanning Electron Microscope (SEM), Energy-Dispersive X-ray Analysis (EDAX) and X-ray Diffraction (XRD) Analysis. Struvite kidney stones were synthesized by a single diffusion method to carry out the antiurolithiatic activity. **Results:** The results proved the availability of various secondary metabolites and their concentrations. The prepared Zinc oxide Nanoparticles were evaluated by the ultraviolet spectral analysis and the specific range by 274.90nm, organic compounds under the FTIR analysis gave the expected outcome. The external structures by SEM show the body-centred cubic structure. The clear formation of ZnO NPs was revealed by the EDAX analysis. The nature of the crystal by XRD revealed the satisfied outcomes. The struvite kidney crystals were developed and after the crystallization they underwent scaling and morphological observation, and then the rate of inhibition was recorded. **Conclusion:** It was concluded that this novel work shows extraordinary outcomes. The sample of *H. undatus* fruit extract has a potent inhibitor of struvite kidney stones. Another focus of the study is on reducing the toxic side effects of nanoparticles through the fabrication of ZnO NP by using fruit extract also successfully exhibited.

Keywords: Biofabrication, Magnetic Stirrer Method, Secondary Metabolites, Single Diffusion Method, Struvite Stones, Zinc Oxide Nanoparticles

1. Introduction

Nanoparticles are nano-sized objects like atoms, molecules, and supermolecules[1,2]. Their size distinguished them from fine and ultrafine particles. Ultrafine particles have a size of 1-100 nm and fine particles have 100-2500 nm[2]. A nanometer is a 'Billionth of a meter' or 109[3-5]. Zinc oxide Nanoparticles (ZnO NPs) are metal-oxide nanoparticles that have distinctive properties with enormous applications. Researchers widely use *Azadirachta indica*, *Aloe barbadensis*, *Cochlospermum religiosum*, *Zingiber officinale*, *Trifolium pratense*, and *Jacaranda mimosifolia* to synthesize ZnO NPs. They reduce inflammation, corrosion, and treat diabetes[1], and cancer[5]. This is because of their biodegradable and biocompatible ability[5]. They also have bio-imaging characteristics[5]. ZnO NPs fight against *Staphylococcus aureus* and *Escherichia coli*. This is because of their antimicrobial properties[1]. It plays an essential role in insulin secretion, synthesis, storage, and keeping the insulin structure[6,7]. Oral administration of ZnO NPs shows promising

*Author for correspondence

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anti-diabetic effects. Glucose tolerance enclosures demonstrated this[6-10]. ZnO NPs can be utilized as nano fertilizers. They promote plant growth, seedling vigour, germination, and increasing stem and root generation in peanuts[1]. Experiments have been conducted to study the applications of ZnO NPs obtained from plants and fruit extracts. They act as natural alternatives to treat urolithiasis[11].

Urolithiasis or Nephrolithiasis are formations of stones in the urinary tract and other areas, like bladders or the kidney itself[12]. Elderly people are more affected than young children. The mortality rate is 10-15 % and whites are more susceptible than Asians and blacks[13]. Calcium stones are prevalent. Calcium oxalate, calcium phosphates, and calcium urate are the causes[13,14]. Uric acid stones are smooth, pellucid, and yellowish-orange in colour caused by low intake of fluids and excessive fluid loss from the body[15]. Cystine stones are rare stones caused by the excretion of specific amino acids. This is a hereditary disease called cystinuria[16]. Protease stones are new and usually occur in 4-12 % of HIV patients. It is due to protease inhibitors such as indinavir sulfates[11]. Some gram-negative bacteria like pseudomonas and proteus species present in the urinary tract fabricate struvite. This is a hard mineral[12]. It is 10-15 % and is more common in women than men. The struvite stones block the ureter, and the bladder causing fatal damage to the kidney[14]. Individuals with metabolic disorders like hyperparathyroidism and idiopathic hypercalciuria have this condition. The crystals are large in size, crooked, and laminated[12,13]. Hematuria, unbearable pain, fever frequent urination, and pain while urinating are some symptoms. Nonsteroidal anti-inflammatories with opioids act as analgesics[13]. Various plants have antiurolithiatic activities. Some are, *Ricinus communis, Ocimum tenuiflorum, Tridax procumbens, Pedalium murex,* etc[12,13].

Strawberry pear is called a Dragon fruit which is *H. undatus.* It belongs to the family Cactaceae also known as the vine cacti family. Originating in the United States. Gujarat is the largest producing state in India[16,17]. It is an ornamental and epiphytic with a succulent greenish stem, and its roots are arial[18,19], long and showy flowers[20]. Fruit is edible, and pulps are bright colours and minute black seeds[21]. The fruits are effective in constipation, regulating cholesterol levels, uplifting the immune system, hair growth and skin glow, lifting haemoglobin, brain activity, and giving clear vision[12]. It prevents colon cancer and has chemoprotectants[17] to raise bone function. It reinforces kidney functions and keeps the body hydrated[18,19]. Improves the growth of healthy gut bacteria and lowers coronary artery disease[16]. Can immediately heal wounds and bruises, and promote teeth and gum activities[19]. The seeds contain 50% of essential oils which are very useful to biological functions[17]. They also have Vitamin C, Vitamin B2, Vitamin B3, B1, and loads of active compounds[20]. It shows the presence of distinct antioxidant capabilities, Chloroform test shows effective antimicrobial activities[19]. The leaves have effectual capillary resistance and can able to resist permeability[20,21]. The study focuses on the biological synthesis of zinc nanoparticles using the *H. undatus* fruit extract to bring out the natural alternative source to treat struvite kidney stones.

2. Materials and Methods

2.1 Plant Collection and Extraction

Hylocereus undatus Linn.[22,23] fruit was purchased from the local market of Tiruchirappalli. It was identified by widely using keys and plant descriptions from several articles and monographs[20,22]. The purchased fruit was washed and cut into pieces. Then it is weighed 15 g in two beakers separately. 100 ml of the aqueous solution was added to those beakers and kept in the water bath for 2-3 days. The extraction was filtered using filter paper and kept in a beaker[24].

2.2 Phytochemical Analysis

2.2.1 Qualitative Screening

To discover the presence of chemical compounds like terpenoids, flavonoids, saponins, alkaloids, tannin, steroids, glycosides, phlobatannins, proteins, coumarin, anthocyanin, anthraquinones, emodin, carbohydrates, leuco-anthocyanin, xanthoprotein, cardiac glycosides and phenols in the fruit extracts, this qualitative screenings[25,26] were carried out with specific tests for each chemical compound using specific solvents[27,28].

2.2.2 Quantitative Screening

To calculate the concentration or the number of phytonutrients present in the extract. This quantification exposed the availability of flavonoids, tannins[23], saponins, alkaloids, phenol, and phenols in the aqueous fruit extract this screening was carried out with specific tests of each compound[5,15,27,28].

2.3 Synthesis of Zinc Nanoparticles

The ZnO nanomaterial was synthesized by a magnetic stirrer method. About 50 ml of zinc acetate $(CH_3COO)_3$ $Zn.H_2O$ was taken in a conical flask and mixed with 15 ml of aqueous *H. undatus* fruit extract with continuous and constant stirring. It is stored in a tight container and placed in a magnetic stirrer for 45 minutes. This leads to the formation of yellowish colour precipitation. The formation of zinc oxide nanoparticles was corroborated by Ultra Violet spectral analysis[29-36].

2.4 Characterization Techniques

To observe the functional groups of zinc oxide nanoparticles and specify the wavelength, characterization techniques are executed. Ultra Violet (UV)[31,36,37] spectra and Fourier Transmitting Infrared analysis (FTIR)[38,39] were established to analyze the wavelength and functional group of the ZnO NPs. Its nature of crystalline, elemental constituents and size were determined by Scanning Electron Microscope (SEM)[40-42], Energy-Dispersive X-ray Analysis (EDAX)[43,44], and X-ray Diffraction (XRD)[36,45,46].

2.5 Struvite Crystal Growth

The occupied technique for crystal growth is a single diffusion reaction. The reactant used in the sample was 0.02194 mg of ammonium sulfate mixed with 100 ml of distilled water with continuous and constant stirring. The 15 ml of prepared ammonium sulfate solution was poured slowly into seven separate test tubes with an equal proportion of distilled water under the ratio of 15:15. The gel employed the PH of 7 at the beginning and furtherly maintained within the range of 6. The test tubes were air-tightened by using the cotton lids and left undisturbed for 5-6 days. A supernatant solution was prepared which means the various concentrated synthesized ZnO NPs with extract a control. After the gel transition process, 40 ml of 1M Magnesium acetate was prepared then added 10 ml in the control and 5 ml in each test tube along with the supernatant solution. It was kept at room temperature (37°C) and executed the complete experiment[47,48].

To investigate the Struvite crystal growth using aqueous fruit extract of *H. undatus* was employed by the sol-gel method. The preparation of various concentrations of Zn NPs with the aqueous extract in five test tubes was carried out depending upon the average growth of weight of the crystals, Table 1 shows the various concentrated test tubes were added furtherly unvarying amount of the supernatant solution and control[47-50].

% of inhibition was calculated by a formula

1%= [(TSI-TAI)] / TSA x 10

TSI → Number of crystals without inhibitors

TAI → Number of crystals with inhibitor

Table 1. Preparation of gel medium to grow struvite crystals

Crystal	Class	Analysis
Struvite	A	Control
	B	Control + Distilled Water
	C	Control + 1% Synthesized ZnO NPs
	D	Control + 2% Synthesized ZnO NPs
	E	Control + 3% Synthesized ZnO NPs
	F	Control + 4% Synthesized ZnO NPs
	G	Control + 5% Synthesized ZnO NPs

3. Results

3.1 Phytochemical Analysis of *H. undatus* Fruit Extract

3.1.1 Qualitative Analysis

The analysis expresses the active biomolecules present in the aqueous fruit extract. Based on this outcome the fruit of *H. undatus* was considered a hub for synthesizing bioactive compounds. The observed compounds are terpenoids, flavonoids, steroids, alkaloids, saponins, tannins, cardiac glucosides, phlorotannins, proteins, coumarin, emodin, anthraquinone, anthocyanin, carbohydrates, leucoanthocyanin, phenols, alkaloids, and xanthoproteins. Among these, except phlobatannins remaining constituents show the expected outcome. Similarly, Research conducted on fruit from the

Cactaceae family, *Opuntia ficus–indica* has effective phenolic constituents from the flavonoids group, tannins, phenolic acids, and organic acids[51]. Another study states that *Opuntia dillenii* is rich in phytonutrients like phenols, flavonoids, betanin, and ascorbic acids[52]. An investigation of the synthesis of biomolecules in 28 different plants from Africa shows efficacy in neuroprotection. Among these, the fruit extract of *Argemone mexicana* contains tannins, alkaloids, flavonoids, cardiac glucosides, terpenoids, and steroids. The *Xylopia aethiopica* fruit extracts show tannins, steroids, and leucoanthocyanin[53]. These investigations provide the required data to state that the aqueous extract of *H. undatus* shows prominent results.

3.1.2 Quantitative Analysis

The quantitative screening tests in Flavonoids, tannins, saponins, phenols, alkaloids, and terpenoids express the possible results. Among these, except flavonoids, the remaining phytonutrients showed greater results. Alkaloids found a higher amount of 0.147 mg/g. The empty and yield values were recorded (Table 2). Likewise, A study carried out quantitative tests in the aqueous fruit extract of *Citrus paradisi* and brought out estimated results in total flavonoids, total phenols, alkaloids, tannins, and saponins. Alkaloids were higher than the other bioactive compounds[54]. One more investigation carried out the quantitative screenings in an aqueous extract of *Mutingia calabura* and appraised the results in carbohydrates and protein[55]. The quantitative analysis on *Citrullus lanatus* shows total alkaloids and total phenols showing the expected results among the eleven compounds[56]. The above studies show that *H. undatus* contains an effective concentration of bioactive properties to carry out any potential applications in the medical field.

3.2 Characterization of Synthesized Zinc Oxide Nanoparticles

3.2.1 Colour-changing Observation

The aqueous extract of *H. undatus* was added to the glass vessel containing the zinc acetate, and a color-changing process occurred. The colourless zinc solution turns into yellow precipitations. This process occurs due to the complexation of zinc ions with specific compounds found in the aqueous sample. This process is also accompanied by pH changes. An investigation was conducted on the synthesis of ZnO NPs from the Aqueous extract of *Punica granatum* where they observed the pale yellow color-changing visuals to assess the presence of ZnO NPs[57]. Similarly, The synthesized ZnO NPs from *Berberis tinctoria* fruit were characterized by color-changing observations from yellow to light brown[58]. Another pale-yellow colour-changing visual was observed in another study conducted by Manokari M and colleagues in 2019[34]. Likewise, *H. undatus* peel extract shows a white to slight yellow colour change. It occurs during the ZnO NP synthesis[59].

3.2.2 Ultra Violet (UV) Spectra

The presence of specialized metabolites in the fruit converts the zinc ions into zinc oxide. The fruit extracts work both as a reducing and stabilizing agent. The UV analysis expressed the presence of ZnO NPs and proved this by analyzing the peak absorption between

Table 2. Quantitative analysis of *H. undatus* fruit

S. No.	Phytochemicals	Empty value	Yield value	Final value	Result
1	Flavonoid	20.777	20.782	0.005 mg/g	++
2	Tannin	18.704	18.757	0.053 mg/g	+++
3	Saponin	18.794	18.885	0.091 mg/g	+++
4	Alkaloid	19.904	20.057	0.147 mg/g	+++
5	Phenol	17.917	17.932	0.015 mg/g	+++
6	Terpenoid	20.837	20.889	0.052 mg/g	+++

+++ - Strongly detected, ++ - slightly detected, + - Trace, – - absent

200nm-1100nm. The characteristic peak of the surface plasma resonance of zinc is 274.90nm (Figure 1). Another study shows 311 nm which is considered the other specific characteristic peak for ZnO NPs that are obtained from an Aqueous extract of strawberry waste[37]. One more work in ZnO NPs synthesized from water extract of *Ficus benghalensis* fruits and other parts, expressed a particular wavelength like 292nm range in fruit extract[60] and also in *Moringa pubescens* shows 300nm in immature and ripened fruit extract[61]. A researcher investigated the spectrum analysis in ZnO NPs which are obtained from the Mulberry fruit showing the specified range of 350nm[62] and this range is also found in *Averrhoa carambola* fruit extract[63]. Comparatively, The results show quite the possible resemblances.

3.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR analysis was carried out between the range of 4000-400cm^{-1} to investigate the organic compounds or specialized metabolites that act as reducing, stabilizing, and capping agents of ZnO NPs. The report of this study exposes the extraordinary performance of secondary metabolites as a capping agent of ZnO NPs. The broad range was observed by 3437.39 cm^{-1} expressing the vibration of N-H stretching. The second sharp band was found at 1631.85 cm^{-1} showing the C=C stretching. Other moderate bands are found between 2000–1100 cm^{-1} showing the presence of Alkane, aldehyde, aromatic amine, etc. The weaker bands are observed between 1000–660 cm^{-1} showing the presence of fluoro and halo compounds (Figure 2) Table 3. Likely,

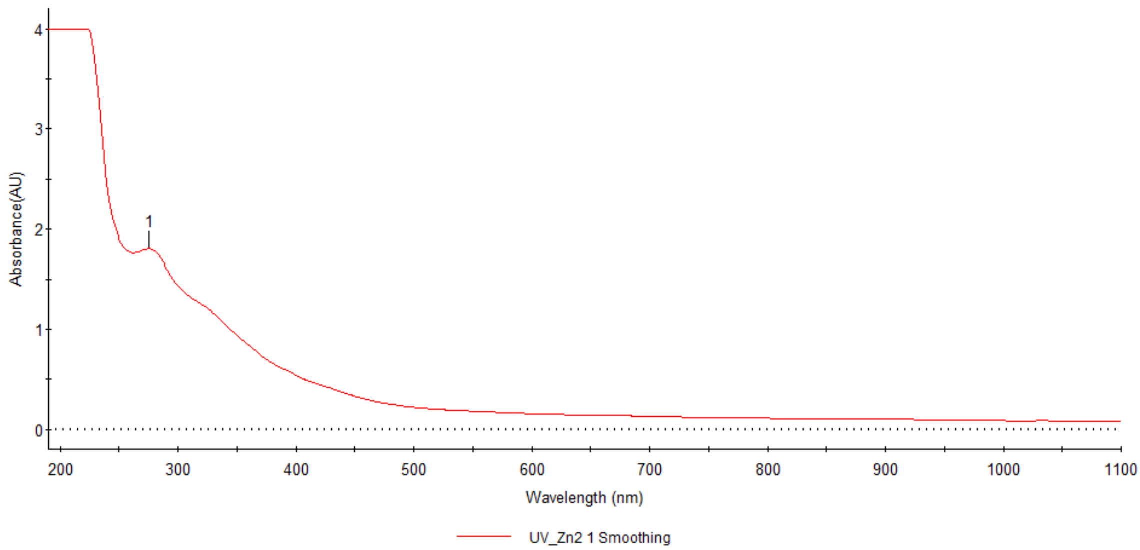


Figure 1. UV Analysis of synthesized ZnO NPs.

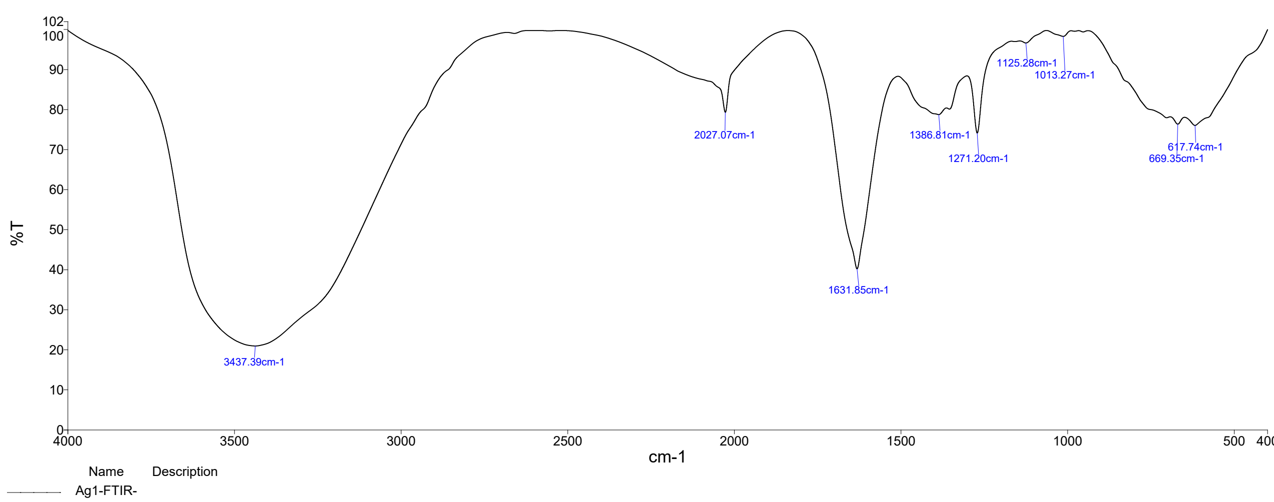


Figure 2. FTIR Spectroscopy on synthesized ZnO NPs.

Table 3. Available of Chemical compounds during FTIR analysis

S. No.	Absorption (cm-1)	Group	Compound Class
1	39 cm^{-1}	H Stretching	Aliphatic Primary Amine
2	2027.07 cm^{-1}	N=C=S Stretching	Isothiocyanate
3	1631.85 cm^{-1}	C=C Stretching	Alkane
4	1386.81 cm^{-1}	H Stretching	Aldehyde
5	1271. 20 cm^{-1}	C-N Stretching	Aromatic Amine
6	1125.28 cm^{-1}	C-O Stretching	Tertiary Alcohol
7	1013. 27 cm^{-1}	C-F Stretching	Fluro compound
8	669.35 cm^{-1}	C-Br Stretching	Halo compound
9	617.74 cm^{-1}	C--Br Stretching	Halo compound

The research focused on the liquid extract of *Garcinia mangostana* to synthesize the ZnO NPs representing the FTIR characterization showing almost similar outputs like alkane and aromatic compounds[35]. A study on ZnO NP fabrication using *Citrus sinensis* shows the C-H functional groups and aromatic rings of C–C stretching[64]. Relatively one more ZnO NP formation on *Allium cepa* peels described the broadband shows the Hydroxyl groups and a deep band shows the C-O Stretching[65]. The water extract of Gooseberry fruit shows the FT-IR analysis expressed the stabilizer and reducer agents of ZnO NPs stretches at C-Br, C=O, OH, and C-I bonds[66]. Overall, the FTIR characterization of zinc nanoparticles derived from *H. undatus* fruit extract provides valuable insights into their composition and potential applications.

3.2.4 X-Ray Diffraction (XRD)

The nature of the crystalline and shape of the ZnO NPs was examined by carrying out the XRD analysis. The XRD analysis of the Fruit extract is shown in Figure 3 and the resulting pattern was recorded in Table 4. The peaks were acquired at 32.35, 33.97, 36.85, 48.27, 56.32, 62.53, 67.52, and 68.76 in Table 3 and matched with the lattice plane values 100, 002,101,102,110,112,201 proved the structure of ZnO NPs were body centred crystals with cubic shape shows 43.75 nm that is calculated by Scherrer's equation. A study proposed the relative results of ZnO NPs which are obtained from cucumber fruit giving 20 nm and a chemical method showing 26nm[67]. A research work states that peculiar results of ZnO NPs that are obtained from cherry extract reported 20.18nm[68]. One of the relative investigations of ZnO NPs from Oak Fruit shows 34nm[69] and 24nm approximately in the Extract of Olive Fruit[29]. In the end, The *H. undatus* shows great results when compared to the above similar studies.

3.2.5 Scanning Electron Microscope (SEM)

SEM Analysis was carried out to expose the exterior morphology of the ZnO NPs It was exhibited by

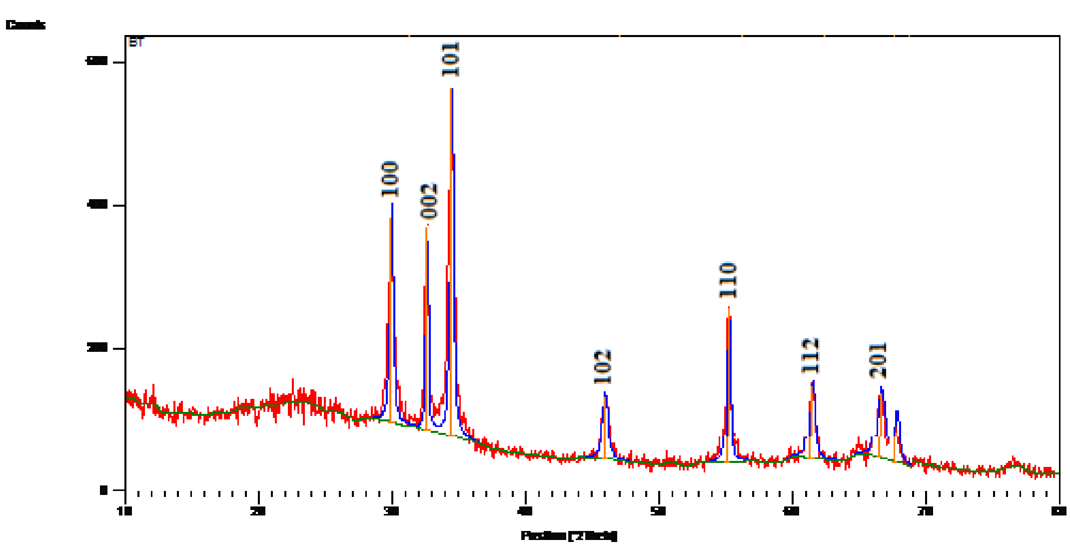


Figure 3. XRD Spectroscopy on synthesized ZnO NPs.

Table 4. Peaks recorded in XRD analysis

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
31.3562	352.32	0.2856	2.85412	67.46
33.9762	337.71	0.1945	2.63803	52.08
36.8544	542.86	0.2645	2.50541	100.00
48.2735	87.62	0.4820	1.93016	17.87
56.3247	262.69	0.2954	1.64576	53.65
62.5324	137.51	0.4281	1.48350	32.19
67.5249	128.36	0.5934	1.42674	18.17
68.7632	72.44	0.4900	1.38727	22.78

Pos. [°2Th.] - Position in degrees two theta, Height [cts] - height in counts, FWHM [°2Th.] - Full width at half medium in degrees two theta, d-spacing [Å] - Interplanar spacing in angstroms, Rel. Int. [%] - Relative intensity in percentage.

200nm. It exhibited a cubic shape under the 200 nm size (Figure 4). A study was carried out on the fabrication of ZnO NPs from the fruit extract of *Rosa canina* and carried out SEM analysis. It shows a spherical shape with 50nm[41] and in *Ananas comosus* fruit exhibited 100nm[38]. Similarly, one more study worked out the ZnO NP synthesis in *Myristica fragrans* fruit extract and underwent the SEM. Reported the semispherical shape in 43.3 nm- 83.1nm[70]. Comparatively, another study has conducted the ZnO NP synthesis using the *Ocimum tenuiflorum* leaves exposed to the hexagonal shapes of 11-25 nm[71]. Here, The different fruit extracts express various outer morphology of Zinc nanoparticles in the characterization.

3.2.6 Energy-Dispersive X-ray Analysis (EDAX)

The EDAX analysis helps to examine the formation of ZnO NPs clearly and reports the elemental numbers in the synthesized nanoparticles. In the graphical representation (Figure 5) the X axis shows the energy while the Y axis shows the counts of x-rays. The Atomic weight of O K is 54.39 while its weight was 24.52. Similarly, the atomic weight of Zn electrons which are found closest is 58.37 and the weight is 22.35. Then the atomic weight of Zn L is 17.11 and the weight is 23.26 (Table 5). The total values were recorded in Table 4. A study expressed the highly refined ZnO NPs through

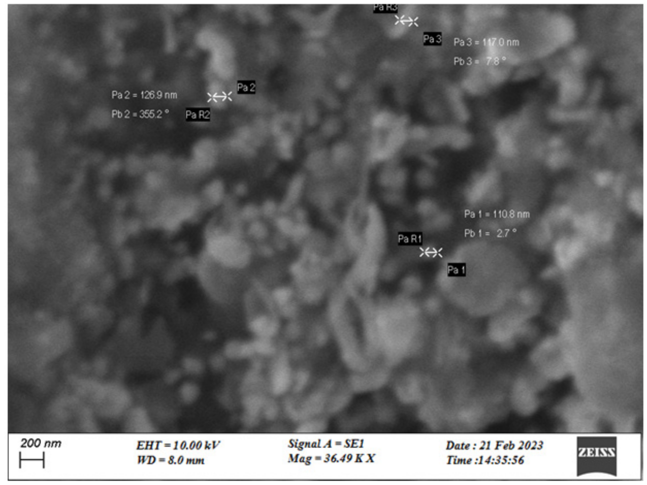


Figure 4. SEM Analysis on synthesized ZnO NPs.

EDAX which are obtained from the *Carica papaya* fruit extract. It shows approximately 49, 3, 47% and 45, 2, 52% in two samples ZnAc and Zncl[44]. Similarly, the purification of ZnCO obtained from Grapefruit shows 43% zinc, 30% carbon, and 26% oxygen[72]. One more study also states the clearance of ZnO NPs from *Puccinia granatum* juice is 35% zinc and 23% oxygen while 44% silicon[43] and in *Terminalia chebula* fruits show 80% zinc and 16% oxygen through EDX characterization[73]. The overall resemblances of this study's result are discussed in the above studies.

3.3 *In Vitro* Antiurolithiatic Activity of *H. undatus*

The formation of crystals and nucleation of struvite stones were described. It is carried out by examining the crystal's weight. The crystallization technique is carried out as a gel diffusion method. It contains the control with the 10 ml of magnesium acetate exhibits the high outcome in the growth of crystals. The formation of the crystals occurred within one day. Subsequently, the supernatant solution which is mixed with the fruit extract of *H. undatus* was added. Due to adding this synthesized ZnO NPs combined with extract, the process of nucleation holdup takes 4–5 days for the complete crystal growth. Their morphology and scaling of crystals were observed (Figure 6 a, b). The inhibition was gradually increased depending on the concentrations. Finally, the rate of inhibition which is recorded was 84.98% (Table 6). A Study reviewed that *Couroupita guianensis* shows effective inhibition against the struvite kidney crystals[74]. A

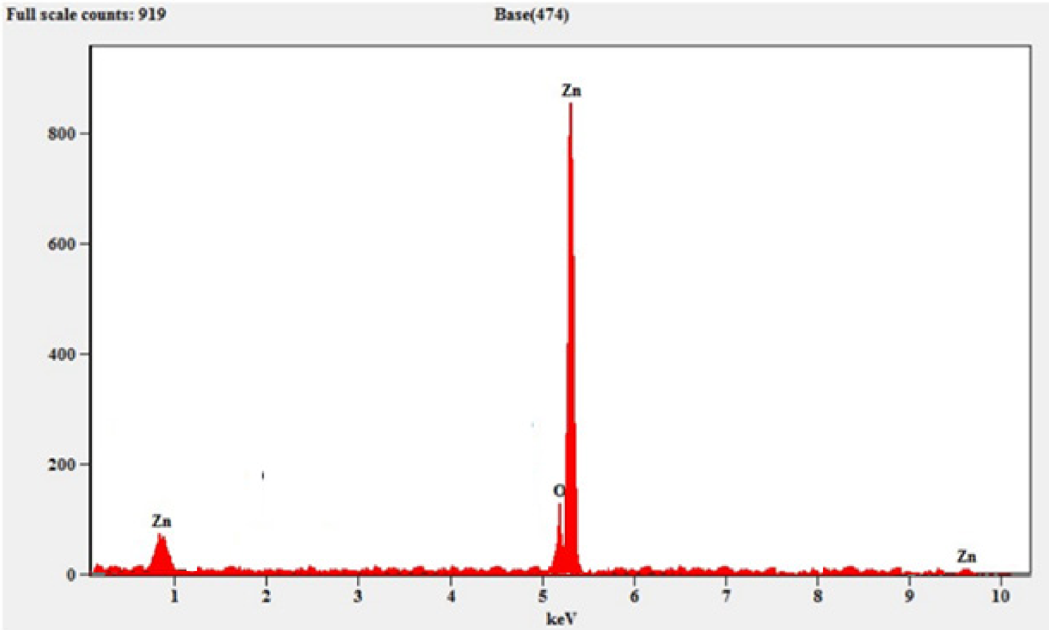


Figure 5. EDAX Spectrum on synthesized ZnO NPs.

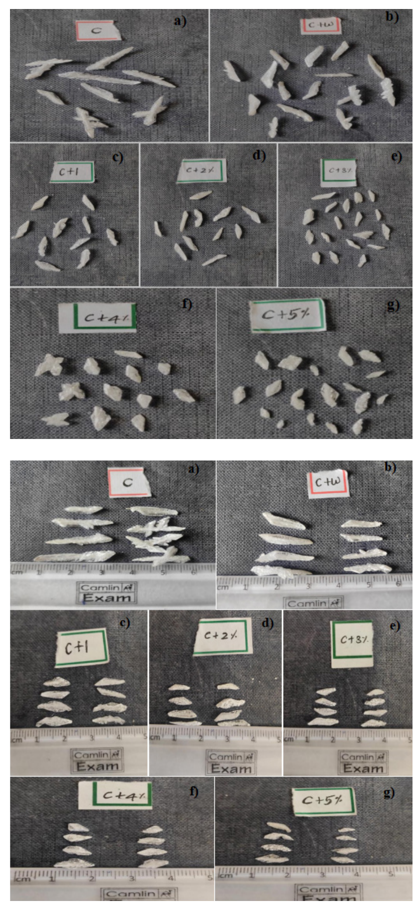
Table 5. Elemental composition of synthesized ZnO NPs

Element line	Weight %	Weight %	Atom %
O K	24.52	± 0.82	54.39
Zn K	58.37	± 1.54	22.35
Zn L	17.11	± 2.73	23.26
Total	100.00		100.00

Weight values uttered in ± mean(SEM)

similar *in vitro* antiurolithiatic study conducted with *Terminalia chebula* bark expressed the productive gradual inhibition of struvite stones at 87.9%[75]. Another *in vitro* antiurolithiatic evaluation was carried out in the *Pedalium murex* exposing the great size reduction rate of 0.17% of the struvite crystals[76]. The aqueous extract of *Linum usitatissimum* randomly shows 93% inhibition against struvite kidney stones[50]. The combined treatment of *H. undatus* extract and zinc nanoparticles solution demonstrated significant anti-urolithic activity. *In vitro* assays revealed marked inhibition of struvite crystal nucleation and growth, indicating the potential of the combined treatment to prevent stone formation. Phytochemical analysis identified several bioactive compounds in the *H. undatus* extract, including polyphenols and flavonoids,

(a)



(b)

Figure 6. **(a).** Morphology of struvite crystals; **(b).** Scaling of struvite crystals.

Table 6. Rate of inhibition of ZnO NPs against struvite stones

Crystal Name	Class	Analysis	Harvested crystals (Length)	Inhibition %
Struvite	A	Control	2.93	0 %
	B	Control + Distilled Water	2.76	5.80 %
	C	Control + 1% Synthesized ZnO NPs	0.88	69.96 %
	D	Control + 2% Synthesized ZnO NPs	0.74	74.74 %
	E	Control + 3% Synthesized ZnO NPs	0.66	77.47 %
	F	Control + 4% Synthesized ZnO NPs	0.52	82.25 %
	G	Control + 5% Synthesized ZnO NPs	0.44	84.98 %

which may synergistically enhance the anti-urolithic effects of zinc nanoparticles.

4. Conclusion

This novel study is focused on reducing the toxic side effects that are made by the physical and chemical methods of nanosynthesis. The basic intention of this study is the fabrication of ZnO NP by using the fruit extract of *H. undatus*. Then the synthesized ZnO NPs were used to investigate the Anti-urolithiatic activity against struvite kidney stones. The phytonutrients qualitative screenings proved the availability of various secondary metabolites and the quantitative screenings evaluated the weight of screened phytonutrients like alkaloids, saponins, flavonoids, phenols, terpenoids, and tannins. The prepared ZnO NPs were evaluated by the UV spectral analysis and the specific range was recorded. The combination of fruit extract and the ZnO NPs exposed the indication of organic groups under the evaluation of FTIR and provided the expected results. The external structures of ZnO NPs were examined by the SEM analysis. The clear formation of ZnO NPs was revealed by the EDAX analysis and brought out positive results. The nature of the crystallinity of synthesized ZnO NPs was determined by the XRD characterization and revealed the expected outcomes. The struvite kidney crystals were developed by single diffusion method and after the crystallization they underwent the scaling process and results were recorded. In the inference, the extraordinary outcomes exposed the extract of *H. undatus* fruit as a potent inhibitor of struvite kidney stones. Hence, The eco-friendly and toxin-less synthesis of nanoparticles has been applied to various pharmaceutical, drug discovery, and medical applications. This study is an example, that the future of health science and drug discovery is in the hands of natural compounds and eco-friendly fabrication of metal nanoparticles. It also provides safer and potentially more accessible options for patients with urolithiasis.

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