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ANTIOXIDANT, ANTIUROLITHIATIC AND ANTIMICROBIAL ACTIVITY OF SYNTHESIZED ZINC NANOPARTICLES USING *MILLINGTONIA HORTENSIS* FLOWER ON URINARY TRACT INFECTION CAUSING STRUVITE CRYSTAL

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ABSTRACT

Urinary tract infections (UTIs) are a major public health concern throughout the world, and they are frequently associated with the occurrence of urinary stones, specifically struvite stones. The present study concentrated on an economical and environmentally friendly method for producing zinc nanoparticles utilizing *Mellingtonia hortensis* flower extract as a reducing and capping agent. We evaluated the potential of these nanoparticles as Antioxidant, Antiurolithiatic and Antimicrobial activity. These nanoparticles were characterized by UV-Visible spectroscopy and it shown the absorption band at 256nm. According to FT-IR spectroscopic investigations, phytoconstituents have a major role in the reduction and capping of zinc nanoparticles, *Mellingtonia hortensis* mediated zinc nanoparticles were crystallized and had a spherical shape, in XRD and SEM. The EDX analysis showed the elemental composition and the amount of zinc nanoparticles. The zinc nanoparticles mediated by *Millingtonia hortensis* were found to have an inhibitory efficiency of 88.3%. This results showing that they were an effective inhibitor for

destruvite crystals. The maximum zone of inhibition was observed against at a concentration 100µg/ml. In conclusion, the synthesized Zinc Nanoparticles using *Millingtonia hortensis* shown most promising Antioxidant, Antiurolithiatic and Antimicrobial activities against struvite urinary stones and UTI Infection

Keywords: *Millingtonia hortensis,* Zinc nanoparticles, Antiurolithiatic activity, FTIR, EDAX, Phytoconstituents

1. INTRODUCTION

Nanotechnology is the study of atomic and molecular scale and also used for medicine, electronic, energy production and many new materials. It is used for nanotechnology in various daily uses and industry uses of major application in research and development, renewable energy, environment, grapheme transistor, assented computing, CNT based Nanosensors **[1]**. The zinc nanoparticle is environmentally sustainable and economically viable. Zinc nanoparticles have range of antibacterial, anticancer, antioxidant, anti-inflammatory and wood healing property. The zinc nanoparticles composed in many methods chemical, physical and biological compounds. It is used to modern analytical tools such as X- ray differentiation, spectroscopy, and many electron microscopies **[2]**. Nanoparticles synthesis uses a toxic precursor for chemical methods. Energy-efficient an environment friendly that do not require the use of toxic and expensive chemical **[3]**.

Kidney stone is a worldwide healthcare problem that affect the many people **[4]**. An epidemiology of Kidney stone is common throughout the world around 12% significantly. In Europe, kidney stone increased in adults from 3.35 % in the year 2019-2023 **[5].** Kidney stone are containing of calcium oxalate, uric acid struvite and cysteine. There are many types of kidney stone such as Calcium stone, Uric acid stone, Struvite stone, Cysteine stone **[6].** Treatment of kidney stone depending to the cause, Gland surgery **[7].** There are many side effects such as bleeding, infection, severe pain, nausea, vomiting, fever, chills and blood in your urine **[8]** effects of medicine are medical expulsive therapy and dissolving medicine can cause dizziness or feeling light–headed, sinus congestion, or a runny nose. The herbal Plant was the primary role in the earth because nature was produce to medicine plant in the various diseases. The herbal treatment is the

very safe and most comfortable in many diseases **[9].**

Millingtonia hortensis are commonly known as cork tree and belong to the family *Bignoniaceae* and is widely distributed in South Asia, India, Thailand, and South China **[10]**. The medicinal uses of flower buds are used to treat Asthma, Sinusitis, Chologegue tonic and it used to tobacco for the treatment of smoking **[11]**. The flower also used in rituals and having Antimicrobial, Antimutagenic, Antipyretic, Antituberculer, Larvicidal, Anticancer and Antifungal properties **[12]**. The presence of chemical compounds like tannins, flavonoids, alkaloids, carbohydrates, glycosides, saponin, phenols, Terpenoids, and aminoacids **[13].** The current research seems to investigate the antiurolithiatic and antibacterial properties of zinc nanoparticles generated from *Millingtonia hortensis*plants against UTIs caused by struvite urinary stones.

2. MATERIALS AND METHODS

2.1 Sample Collection

The flower of *Millingtonia hortensis* were collected from the local market in Salem district and dried at room temperature and the dried petals were made into the powder. 20g of sample added with 100 ml of ethanol solution. Then it is extracted using hot percolation methods (Soxhlet) and the sample is condensed using rotary evaporation.

2.2 Phytochemical Screening

Several tests have been carried out using the standard methodology for ethanolic extracts from plant powdered samples to ascertain the qualitative phytochemical analysis for carbohydrates, steroids, saponins, glycosides, flavonoids, alkaloids, proteins, and resins **[14]**.

2.3 Synthesis of zinc oxide nanoparticles

ZnO nanoparticles were synthesized by the co-precipitated method with some modification 0.1mM zinc acetate was prepared for 50ml then it was added with 10ml of extract *Millingtonia hortensis* and subjected to continuous stirring for about 2hrs **[15]**.

2.4. Characterization of synthesized zinc oxide nanoparticles

The synthesized zinc oxide nanoparticles were characterized using UV-VIS, FTIR, SEM, XRD and EDX respectively **[16].**

2.5 Antioxidant Activity - DPPH Assay

The ZnO nanoparticles are encounter to DPPH assay to study for their antioxidant activity **[17].**

2.6 Antimicrobial Activity by Disc Diffusion Method

The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C. A MHA plate was cultured with standardized microbial culture broth. Each well was filled with varying concentrations from 100, 125, 150 µg/ml of the samples with positive control as streptomycin 25 mcg and negative/solvent control as DMSO, respectively. The plate was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested samples **[18].**

3. RESULT AND DISCUSSION

3.1 Qualitative and Quantitative Analysis of Phytocompounds

The qualitative analysis of ethanolic extract of *Millingtonia hortensis* flower was subjected to different qualitative test for the identification of phytoconstituents present **(Table 1)**.

The phytochemical composed of screening the quantitative analysis of flower extract *Millingtonia hortensis* was different amount have been reported. The higher quantity present in flower is phenol followed by Flavanoids (0.003), Tannins (0.007), Saponin (0.002), Alkaloids (0.004), Phenol (0.005), Terpenoids (0.006) **(Figure 1)**. Researchers reported that the quantitative analysis of phyto constituents with higher quantity present in phenols (0.096mg/g), flavonoids (0.063mg/g), saponins (0.048mg/g), terpenoids (0.042mg/g), tannins (0.027mg/g), and alkaloids (0.003mg/g) **[19]**. The methanol extract leaves of *Millingtonia hortensis* showed by the phenol content of leaves was 190.5±10.38 **[20]**.

Table 1: Phytochemical Screening of *Millingtonia hortensis*

S. No.	Phytochemical Constituents	Flower of *Millingtonia hortensis*
1.	Terpenoids	++
2.	Flavonoids	+++
3.	Saponins	+++
4.	Tannins	+++
5.	Alkaloids	+++
6.	Steroids	+++
7.	Glycosides	+++
8.	Phlobatannins	++
9.	Protein	+++
10.	Coumarins	+++
11.	Emodin	+++
12.	Antraquinone	+++
13.	Anthocyanin	++
14.	Carbohydrates	+++
15.	Leucocyanins	Absent
16.	Cardiacglycoides	+++
17.	Xanthoproteins	+++
18.	Phenolics	+++

+++: Strongly present+ +: Moderately present+: Slightly present

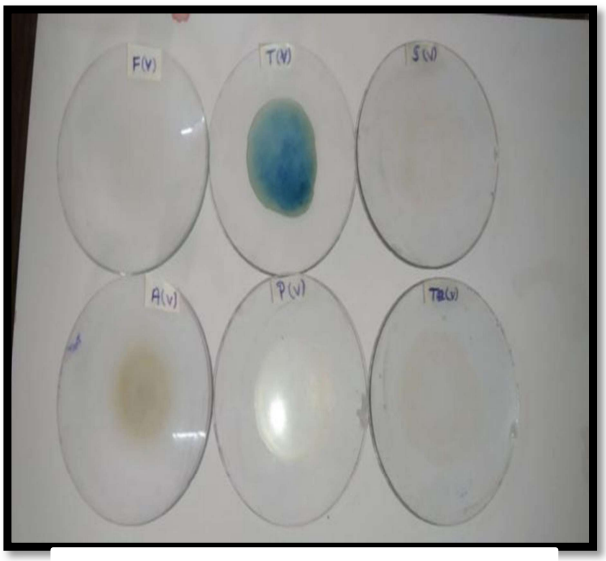

Figure 1: Quantitative Analysis

3.2UV-VIS Spectroscopy

The UV visible absorption spectrum was eminent at the range of 277.4nm clearly denotes the presence of ZnO nanoparticles in the reaction which the continuous synthesis of ZnO nanoparticles. The UV–VIS confirmed the formation of zinc nanoparticles by using the *Millingtonia hortensis* **[21].** It's showed the absorption band at 357nm to using a formation of zinc nanoparticles. *Ipomoea asarifolia* leaves extract was added to the Zinc sulphate solution **[22]**. **(Figure 2a)**.

3.3 Functional Group Determination Using FTIR Spectroscopy

The FT-IR spectrum of synthesized zinc oxide nanoparticles gives information above the functional group of involved in the zinc ions reduction FT-IR, range was established on $1cm^{-1}$ progress by Fourier transform infrared spectroscopy by applying the potassium bromide tablet method. The following peaks of obtained in the flower ZnNPs, the peak obtained $3448.46cm^{-1}$ corresponded to OH stretching vibration, peaking the range of $2083.10cm^{-1}$, $1637.74cm^{-1}$ corresponded to C=C stretch in aromatic ring C=O stretch in polyphenols and $1414.27cm^{-1}$ C-N stretch of amide-I in protein **(Figure 2b)**. It is analysis of these spectra strongly suggested presence of flavanoids and terpenoids, which was mainly responsible for the formation of zinc oxide nanoparticles by reducing zinc ions **(Figure 3a)**. Some researchers reported that by the synthesis of ZnNPs used in FT-IR spectrum of *Ipomoea asarifolia* mediated zinc nano particles is shown in absorption bands at 2853 cm^{-1} corresponds to C-H saturated strecting vibration, peak at$1609cm^{-1}$, $1500cm^{-1}$ and $1417cm^{-1}$ for aromatic stretching frequencies and peak at $3172cm^{-1}$ **[23]**. The bands are due to presence of polyphenols, proteins, tannins, and flavonaids in *Ipomoea asarifolia* **[24]**.

3.4 X-RAY DIFFRACTION (XRD)

XRD takes the crystalline nature of the ZnO NPs. The intensity of the different rays the function of the diffraction angles. The spectra gives the details of the crystals planes **(Figure 2c)**. The X-ray distraction peaks obtained at 31.6987, 34.7816, 37.7100, 48.9198, and 57.9632 is corresponded to lattice planes of (100), (002), (101), (102), (110), (112) and (201). The X-ray distraction peaks were obtained various angles. XRD gives the *Cayratia pedata* in crystalline nature of ZnO nanoparticles. The spectra showed the details of the crystal planes to the lattice planes (100), (002), (101), (110), (103), (112), and (201). The X- ray diffraction peaks were obtained at various angles as 31.57, 34.24, 36.07, 47.36, 56.42, 62.69, 67.77 and 68.91 **[25]**.

3.5 SEM Analysis

SEM analysis was performed by using ZnO and confirmed that the synthesized ZnO NPs using flower are almost common in size and shape. SEM image of the ZnO nanoparticles lie between 107.9-145.5nm region of the flower –ZnO nanoparticles, the average size of the nanoparticles is 124.1nm, were as the shapes were spherical shaped nanoparticles **(Figure 2d)**. The surface morphology and size of the ZnO nanoparticles, were confirmed and revealed that the Hexogonal particle backing. The SEM images at high magnification at 10µm showed the aggregates of nanocrystallizes **[26]**.

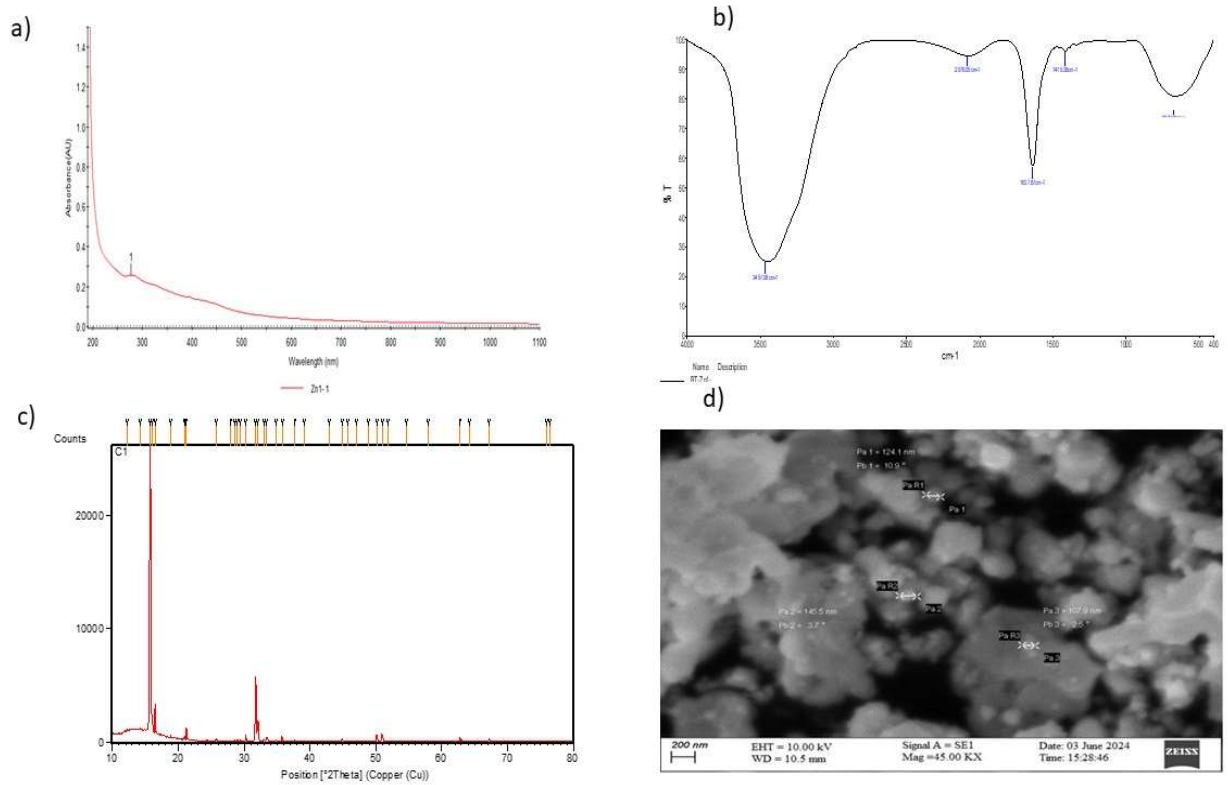

Figure 2: Characterization of Zinc Nanoparticles using *Millingtoniahortensis*
a) UV-VIS b) FTIR c) XRD and d) SEM

3.6 EDAX Analysis (Energy-dispersive X-ray Spectroscopy)

Diminutive stable nanoparticle reorganization was pointed on the portion of micro glass slip fond of to a pliable interlace enclosed by carbon layer, then allowable to dehydrate steadily at 37°C. This EDAX analysis was performed to confirm the elemental signal of zinc nanoparticles. The Y-Axis (vertical) represent the number of X-Ray counts while X-Axis (horizontal) shows the energy in VEDAX spectra synthesized ZnO from flower extract was shown in **(Figure 3)**, with supplementary peak of O as these

represent the biomolecule attached along with the ZnO nanoparticle surfaces the weight of the ZnO NPs reduced by the flower extract was found to be 100% which was summarize in the **(Table 2)** use in EDAX spectra. The purity of ZnO was determined via the EDAX analysis showed that the EDAX spectrum of ZnO NPS revealed that the EDAX data was composed of two elements which are Zn (76.3%) and O (23.7%) **[21]**.

Table 2: Elemental Composition of *Millingtonia hortensis* ZnO Nanoparticles

Element Line	Weight %	Weight % Error	Atom %
O K	37.65	± 0.82	42.62
Zn K	62.24	± 1.24	57.35
Zn L	---	---	---
Total	100.00		100.00

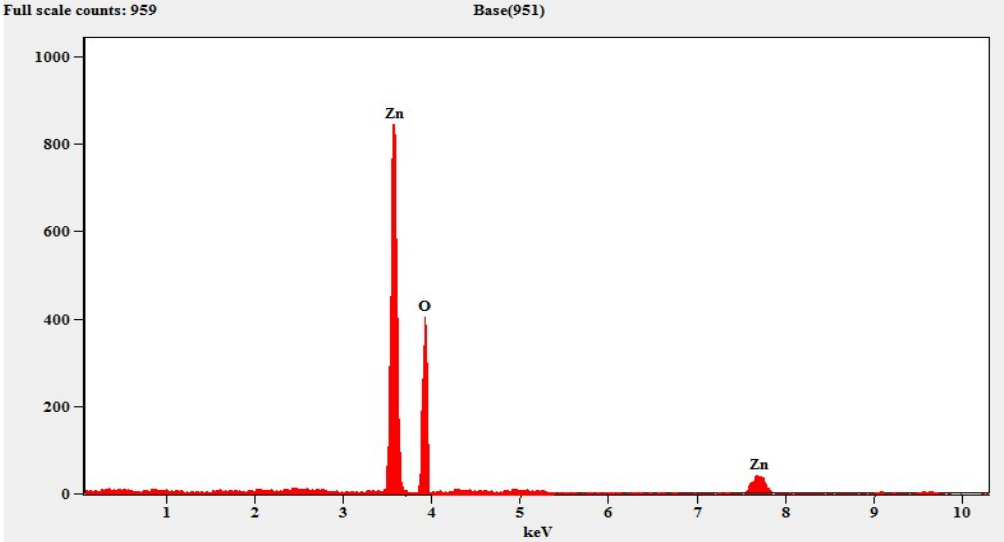

Figure 3: EDAX Spectra of Synthesized ZnO Nanoparticles

3.7 Anti- Oxidant Activity sing DPPH Method

The DPPH (2,2-diphenyl-1-picrylhydrazl) enzyme test reported that the maximum inhibition range from 40.70 to 71.68 % at concentration of 20-100 mg/ml **(Figure 4)**. However, the standard drug ascorbic acid is exhibited a maximum inhibition range from 55.75 to 80.30% at the same concentrations of 20 to 100mg/ml **(Table 3)**. The previous study **[27]** reported that leaves of *Mellingtonia hortensis* flower the maximum DPPH radical scavenging activity was 9.49±0.41 at 300µg/ml concentration. The IC50 value in leaves was found to be 40.97µg/ml concentration and was compared with standard ascorbic acid **(Figure 4a)**.

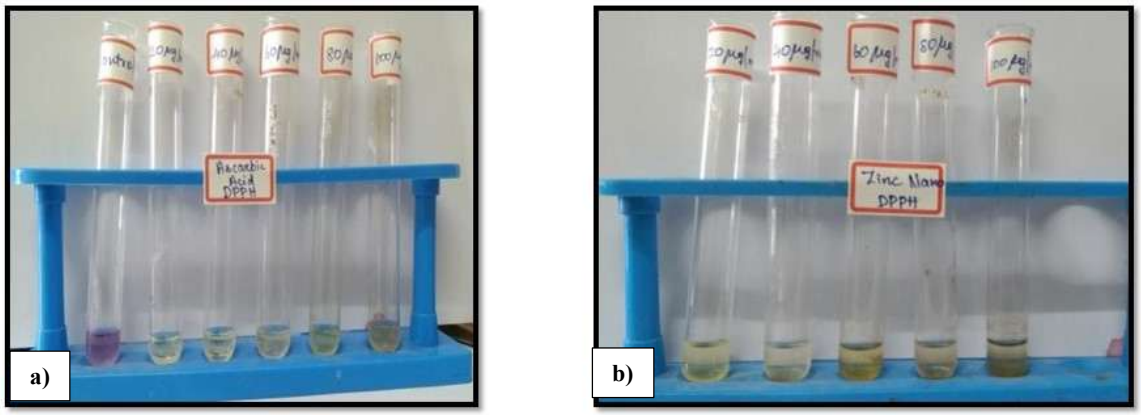

Figure 4: Anti-Oxidant Activity using a) Ascorbic Acid and b) ZnO NPs

Table 3: Antioxidant Activity of DPPH Method

S. No	Concentration (mg/ml)	Scavenging Effect (%)	
		Zinc nanoparticles of flower *Millingtonia hortensis*	Ascorbic acid
1.	20	40.70	55.75
2.	40	46.90	62.83
3.	60	52.21	70.79
4.	80	59.29	78.76
5.	100	71.68	82.30

3.8 Invitro Antiurolithiatic Activity of *Millingtonia hortensis*

The crystallation and nucleation of characteristics of *Millingtonia hortensis* crystals was determined by estimation of the crystal weight. The crystal growth was matured by the gel method. The gel method containing the control using the magnesium acetate at the end high yield of crystal growth has been observed then the nucleation took 24hrs **(Table 4)**. Following that the nucleation, addition of synthesized zinc nanoparticle is reduced the mass and delayed of nucleation of crystal observe in 96 hrs **(Figure 5)**. Few researchers reported that synthesis ZnNPs using *costusignus* flower the growth of crystal were gradually reduced from 2.44 (without any adutive) to 0.28 (with present of zinc nano particles) which shows the inhibition struvite crystal **(Figure 6) [28]**.

Table 4: Harvested Crystals Percentage Inhibition

Crystal	Class	Analysis	Harvested Crystals(gm)	Inhibition Percentage
Struvite	A	Control	2.26	0%
	B	Control+Distilled water	2.20	2.65 %
	C	Control+Ethanol	2.16	4.42%
	D	Control + 1% synthesized ZnO nanoparticles	0.80	64.60%
	E	Control +2%synthesized ZnO nanoparticles	0.72	68.14%
	F	Control+3%synthesized ZnO nanoparticles	0.63	72.12%
	G	Control+4%synthesized ZnO nanoparticles	0.54	76.10%
	H	Control+5%synthesized ZnO nanoparticles	0.38	83.18%

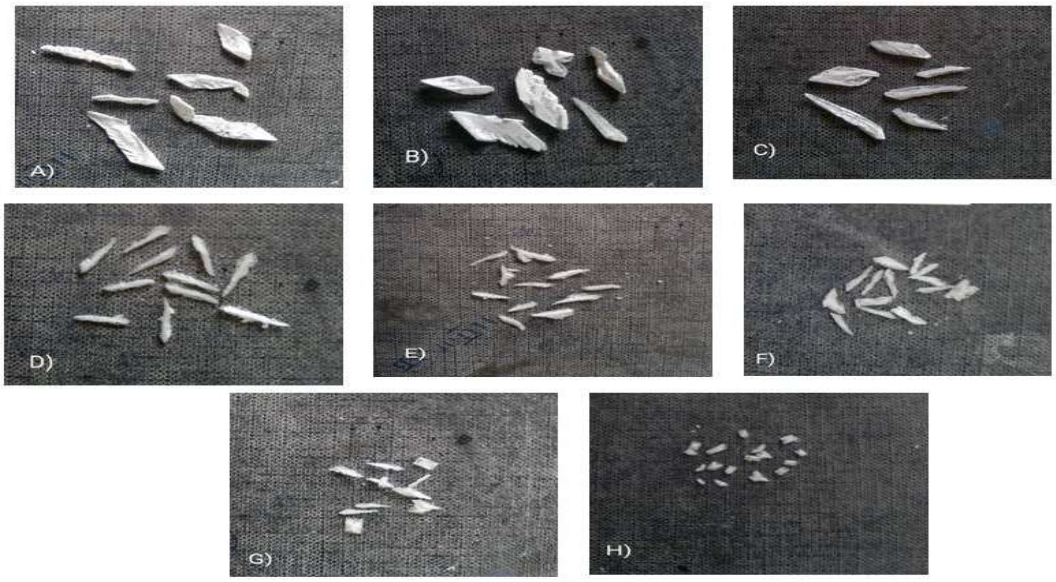

Figure 5: The harvested crystals of struvite obtained from *Millingtonia Hortensis* flower in the gel method (A) without any additive, (B) control with distilled water, (C) Control with ethanol, (D) control with 1% ethanol extract (E) Control with 2% of ethanol extract, (F) Control with 3% ethanol extract (G) Control with 4% ethanol extract (H) Control with 5% ethanol extract

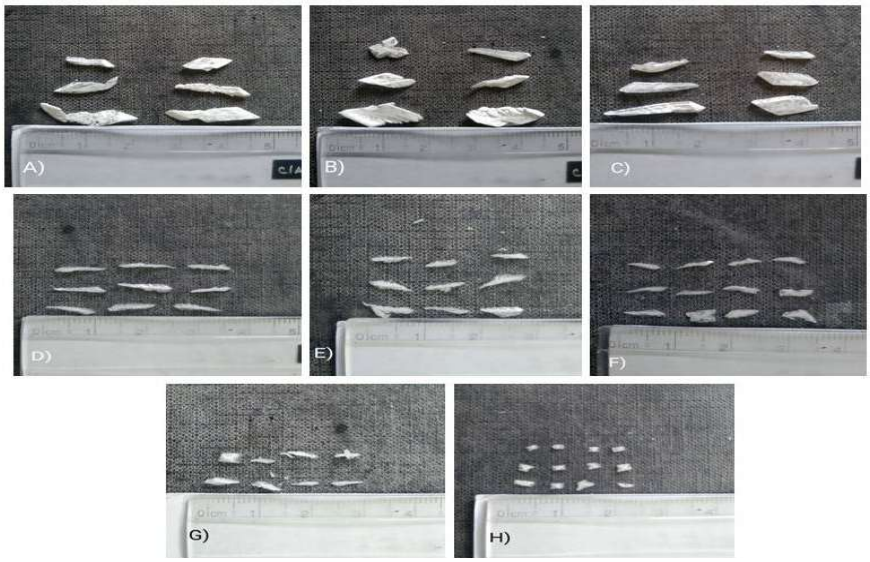

Figure 6: The measurement of struvite obtained from *Millingtonia hortensis* flower in the gel method (A) Without any addictive (B) Control with the distilled water (C) Control with the Ethanol, (D) Control with 1% ethanol extract (E) Control with 2% of ethanol extract, (F) Control with 3% ethanol extract (G) Control with 4% ethanol extract (H) Control with 5% ethanol extract

3.9 Antimicrobial Activity of Synthesis Zinc Nanoparticles from *Millingtonia hortensis*

The effect of Antibacterial activity of ethanolic extract of ZnNPs synthesized from *Millingtonia hortensis* were exposed to *Enterococcus aerogenes, Pseudomonas aeruginosa, Escherichia coli, Enterococcis faecalis* at concentration 100mg/ml. At the concentration of 80mg/ml the essence showed the antibacterial activity of all the bacteria.

But is addition of susceptible against *Enterococcus aerogenes* (5mm) and *Pseudomonas aeruginosa* (4mm) **(Table 5)**. Different methods applied to synthesis, chemical modification and addition with other nanomaterials affects the physical and morphological characteristics of nanoparticles. It leads to change in antibacterial properties. ZnO NPs is one of the most widely used Nano particulate materials are due to their antimicrobial properties **(Figure 7a)**. Using a *Millingtonia hortensis* flower ethanol extract *of E.coli, Klebsiella pneumonia, Entercoccus faecalis, Staphylococcus aureus* and the fungal species *Candida albicans, Candida vulgaris* **[29]**. The essence revealed that the effective inhibitory action against pathogen at concentration 60, 80and 100µg/ml. *Millingtonia hortensis* is revealed that the maximum inhibition of 13 mm against *E.coli, Klebsiella pneumonia* showing the minimum inhibition from 10µg/ml to 25µg/ml.

Table 5: Antibacterial Activity of Synthesis Zinc Nanoparticles from *Millingtonia hortensis*

Conc.	B1 *E. coli (mm)*	B2 *Klebsiella pneumoniae (mm)*	B3 *Enterococcus faecalis (mm)*	B4 *Staphylococcus aureus (mm)*
S	15	7	6	13
60	8	1	2	6
80	9	2	3	7
100	10	3	4	8
E	0	0	0	0

3.10 Antifungal Activity of Zinc Nanoparticles from *Millingtonia hortensis*

The antifungal activity using the concentration of ethanolic extract of synthesis zinc nanoparticles from *Millingtonia hortensis* against test organism. The results revealed that additional potent and highest activity against *Candida albicans* (8mm zone of inhibition) at 100mg/ml, developed by a highest activity against *Candida vulgaris* (7mm zone of inhibition) and *Candida albicans* (8mm zone of inhibition) at a 100mg/ml. As a concentration of essence enlarged from 20-100mg/ml. A minimum fungal concentration of zinc oxide is establishing to 0.1mg/ml **(Table 6)**. This concentration causesan inhibition over a 95% in the growth of *C.albicans.* A zinc nanoparticles are inhibited the growth of *C.albicans* **[30]**.

Table 6: *Invitro* Antifungal Activity of Synthesis Zinc Nanoparticles From *Millingtonia hortensis*

Samples	Concentrations(µg/ml)	Organisms Zone of inhibition(mm)	
		F1 *Candida albicans*	F2 *Candida vulgaris*
Samples	60	5	3
	80	6	4
	100	7	5
Standard (Std)	10µl/disc	10	7
Ethanol	10µl/disc	0	0

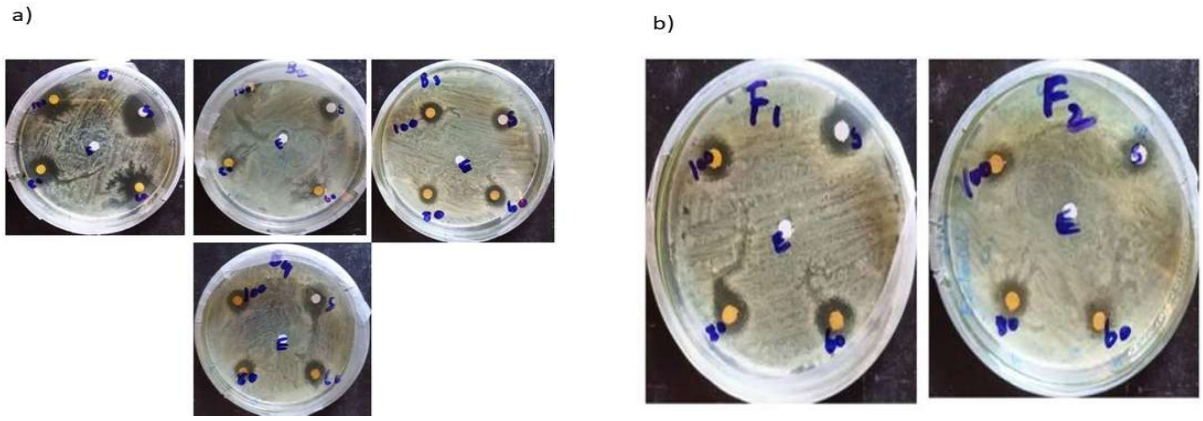

Figure 7: ZnNPs synthesized from *Millingtonia hortenis* a) Antibacterial Activity and b) Antifungal Activity

4. CONCLUSION

The major goal of green synthesis of zinc nanoparticles has to be provided an alternate flower for limiting the negative impacts brought on by physical processes and chemical techniques. The synthesized ZnNPs were examined using UV - VIS and peak intensity at 277.4nm was noted. FT- IR analysis showed functional groups and characteristics of synthesized zinc nanoparticles. According to SEM analysis, the synthesized ZnNPs have a spherical form. By using EDAX analysis the elemental signals were located. XRD analysis revealed ZnNPs were crystalline nature. The appropriate length and weight of the crystals were calculated. The average weight and size of the developed struvite crystals gradually lowered by gradually increasing the proportion of ZnNPs mediated via *Millingtonia hortensis* flower extract. The highest level of inhabitation was discovered to be 88.3%. According to an analysis of FT-IR Spectra, shifting of the bond revealed that the inhibitory activity of synthesized zinc nanoparticles. This study also examined the Antibacterial and Antifungal activity of ZnNPs on clinically relevant uropathogens and their ability to suppress struvite crystal formation. This result to shed light on the possibility of plant-based Nano medicine for UTI therapy.

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