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EXPLORATION OF THREE INDIAN SPICES FOR ZINC OXIDE NANOPARTICLES SYNTHESIS AND ASSESSMENT OF THE THERAPEUTIC POTENTIAL OF THESE SYNTHESIZED NANOPARTICLES

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Keywords:

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ABSTRACT: Nanotechnology has revolutionized multiple scientific domains, especially biomedical sciences. Zinc Oxide (ZnO) nanoparticles (NPs) due to their unique properties find a wide application in biomedicine, food, agriculture, cosmetics, pharmaceutical, textile, rubber and electronic industries. Green synthesis of ZnO NPs employing plant-based extracts is an eco-friendly and sustainable alternative to conventional chemical synthesis. Present study explores the potential of Indian Spices *viz. Brassica hirta* (Yellow Mustard), *Piper cubeba* (Kabab Chini), and *Amomum subulatum* (Black Cardamom) as reducing and stabilizing agents in green synthesis of ZnO NPs and its detailed characterization with respect to UV-Visible Spectroscopy, X-Ray Diffraction (XRD), and Field Emission Scanning Electron Microscopy (FE-SEM). The UV-Visible spectra of the synthesized ZnO nanoparticles revealed the absorption maxima in the range between 290-350 nm which confirms the synthesis of ZnO nanoparticles. The XRD pattern confirmed the crystalline nature and hexagonal wurtzite structure of the ZnO NPs. The FESEM analysis revealed the cylindrical shape in case of *Brassica hirta* ZnO NPs, hexagonal shape in case of *Piper cubeba* ZnO NPs and irregular shape in case of *Amomum subulatum* ZnO NPs. These biogenically produced ZnO NPs were then used to evaluate their possible antibacterial, antioxidant and anti-inflammatory properties. ZnO NPs demonstrated significant antimicrobial activity against *Staphylococcus aureus*, *Bacillus*, *Escherichia coli*, *Proteus*, *Aspergillus niger*, and *Candida albicans*, while their antioxidant and anti-inflammatory properties were also comparable to the respective standards use in the assays. This research highlights the potential biomedical applications of Indian Spice-mediated ZnO NPs, demonstrating their efficacy in various therapeutic interventions.

INTRODUCTION: There is tremendous demand of nanoparticles in the field of chemistry, medicine as well as biotechnology [1]. Different metals and metal oxides are used to produce nanoparticles such as silver, copper, gold, zinc, titanium, magnesium etc. Out of which, ZnO NPs are particularly significant due to their unique properties like high electron mobility, wide band gap, high piezoelectric property, Biocompatibility and environmental sustainability [2].

Along with these properties, ZnO NPs are reported for their antimicrobial, anticancer, antidiabetic, antioxidant, antifungal, antiparasitic, anti-inflammatory activity and wound healing properties and thus have potential biomedical applications. ZnO NPs have been listed by US FDA as “Generally Recognized as Safe” (GRAS)

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due to their non-toxic nature at low concentrations [3]. Traditional methods for ZnO NPs synthesis involve physical and chemical processes that require hazardous chemicals and high energy input [4]. Green synthesis of ZnO NPs utilizes biological agents such as plant extracts, bacteria, fungi, algae etc. for nanoparticle synthesis which has many advantages *viz*. cost-effective, non-toxic nature, biodegradability, non-hazardous to environment and use of natural resources like plants and microorganisms [5].

Indian spices such as *Brassica hirta* (Yellow Mustard seeds), *Piper cubeba* (Kabab Chini), and *Amomum subulatum* (Black cardamom) possess notable antimicrobial, antioxidant properties and anti-inflammatory properties and thus have long been used in traditional Indian medicine. The bioactive compounds like Phenolic acids, flavonoids, terpenes etc. found in the aqueous extract of these plants can act as an oxidizing, reducing, and capping agent for the synthesis of biogenic ZnO NPs. Various researchers have demonstrated the diverse biomedical applications of ZnO NPs, including: Antimicrobial Activity [6], Antioxidant Properties [7], Anti-inflammatory Effects [8].

Although plant-mediated green synthesis of ZnO NPs has been widely explored using extracts from various plant species, including some species of *Brassicai* [9] and *Piper* [10] genus, the authors did not identify any scientific reports stating, the use of *Brassica hirta* or *Piper cubeba* extracts for ZnO NP synthesis in the databases searched. Accordingly, this study explores these species as novel biogenic agents for ZnO NP synthesis.

Thus, current study is aimed synthesize ZnO NPs using Indian spices like *Brassica hirta*, *Piper cubeba* and *Amomum subulatum* and its detailed characterization with respect to UV-Visible spectroscopy, X- ray Diffraction studies and Field Emission Scanning Electron Microscopy. Furthermore, the antimicrobial, antioxidant and anti-inflammatory properties of ZnO NPs were studied.

MATERIALS AND METHODS:

Sample Collection: Spices such as *Brassica hirta* (Yellow Mustard seeds), *Piper cubeba* (Kabab Chini) and *Amomum subulatum* (Black Cardamom) were procured from local markets in Pimpri, Pune, Maharashtra, and authenticated based on morphological characteristics.

Chemicals and Reagents: All chemicals and reagents used in the present study were of analytical grades, and Reagent-grade (purity ≥ 98%) in particular Zinc acetate dihydrate [Zn $(CH_3CO_2).2H_2O$], Sodium hydroxide, Nutrient Agar, Mueller-Hinton Agar, Potato dextrose Agar, Bovine serum albumin, Potassium ferricyanide, Ferric chloride, Trichloroacetic acid, Ascorbic acid and Dimethyl Sulfoxide (DMSO) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Ciprofloxacin (500mg), Ketoconazole and Diclofenac sodium tablet were purchased from Local medical store.

Preparation of Aqueous Extracts of *Brassica hirta, Piper cubeba and Amomum subulatum* by Decoction Method [11]: The decoction method was used for aqueous extraction of three spices viz. *Brassica hirta, Piper cubeba* and *Amomum subulatum.*

The seeds of above three spices were washed with distilled water to remove dust particles and completely air-dried to remove the moisture contents and were crushed using mortar and pestle to prepare the fine powder. 10g powder of each spice was soaked in 150 mL distilled water separately and boiled for 20 minutes.

The extracts were filtered through Whatman filter paper No. 1 and the obtained extract of each spice was used for phytochemical analysis and ZnO NPs synthesis.

Phytochemical Analysis of Aqueous Extract of *Brassica hirta*, *Piper cubeba*, *Amomum subulatum*: The aqueous extract of *Brassica hirta*, *Piper cubeba*, *Amomum subulatum* was tested for presence or absence of phytochemicals like flavonoids, saponins, carbohydrates, proteins, alkaloids, tannins, steroids, glycosides, coumarin and amino acids which acts as a reducing and capping agent for the synthesis of ZnO NPs. Qualitative phytochemical analyses for the extracts were performed according to a study [12].

TABLE 1: PROTOCOLS FOR PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACTS OF ALL THREE SPICES

Phytochemical Test	Protocol	Observation
Flavonoids (Alkaline reagent test)	2 mL of 2 % (w/v) NaOH + 2 mL aqueous extract + 2 drops of diluted HCl	Yellow to colourless, indicates the presence of flavonoids
Saponins (Foam Test)	500µL aqueous extract + 500µL H_2O + shaken for 15 minutes	Stable foam indicates the presence of saponins
Quinone	1mL aqueous extract + 1mL of concentrated H_2SO_4	Red colour indicates the presence of Quinone
Carbohydrates (Benedict reagent test)	500 µL aqueous extract + 500 µL Benedict reagent + heated for 2 minutes	Brown to red colour indicates presence of reducing sugars
Alkaloids (Wagner's reagent test)	2 mL aqueous extract + 1mL of diluted HCl + 1 mL of Wagner's reagent + shake well	Reddish-brown precipitate indicates the presence of alkaloids
Proteins (Xanthoproteic Test)	2 mL aqueous extract + few drops of concentrated Nitric acid solution	Yellow colour indicates the presence of protein
Tannins (Ferric chloride test)	1 mL aqueous extract + 1mL distilled water + 2 drops of ferric chloride.	Transient greenish colour indicates the presence of tannins
Steroids (Salkowski test)	1mL aqueous extract + 10 mL chloroform + 10 mL concentrated sulphuric acid	Upper layer turns red and sulphuric acid layer yellow with green fluorescence
Cardial Glycosides (Keller-Kellani test)	2 ml aqueous extract + 2 ml glacial acetic acid + few drops of $FeCl_3$	Brown colour ring indicated presence Cardial Glycosides
Coumarin	3 mL of 10 % (w/v) NaOH solution + 2 ml aqueous extract	Yellow colour indicates presence of coumarin
Amino acid (Ninhydrin test)	2 mL aqueous extract + 2 mL of ninhydrin reagent + boiled for few minutes	Blue colour indicates the presence of amino acid

Green Synthesis of ZnO NPs using aqueous extracts of B*rassica hirta*, P*iper cubeba*, A*momum subulatum*: For green synthesis of ZnO NPs, 10 mL aqueous extract of all three spices *viz. Brassica hirta, Piper cubeba, Amomum subulatum* was added separately drop wise to the 50 mL, 0.25 M zinc acetate dihydratesolution under continuous stirring at 60°C for 2 hours on a hot plate magnetic stirrer. While stirring, 0.1M NaOH was added dropwise in the above solution to maintain the pH at 12. There was formation of off-white precipitate in *Brassica hirta* aqueous extract, brown precipitate in *Piper cubeba* aqueous extract and light brown precipitate in *Amomum subulatum* aqueous extract at the bottom of the beaker. All three solutions were centrifuged at 8000 rpm for 20 minutes to obtain the pellet. The white, brown and light brown precipitate of ZnO NPs was washed with distilled water thrice and driedthem overnight at 60°C [13].

Characterization of ZnO NPs: Characterization of synthesized ZnO NPs was performed using different techniques like; UV-Visible spectroscopy, X-ray Diffraction analysis and Field emission Scanning Electron microscopy (FESEM). The obtained ZnO NPs were dissolved in 1mL DMSO and the UV-spectra for these samples were scanned between the range of 200-800 nm on SHIMADZU UV-Spectrophotometer (SerialNo-A11455009292) to observe the characteristic peak confirming ZnO NPs formation [14]. The crystallinity of the ZnO NPs was analysed using Single crystal X-Ray Diffractometer (Bruker D8 Venture), using Cu/40k V/40mAas the X-Ray source with K-beta filter [15]. To analyse the surface morphology of the ZnO NPs, FESEM was performed using FEI Nova Nano SEM 450 and EDS: Bruker X Flash 6I30 used SEM grids were prepared by placing a small amount of sample powder on a copper coated grid and drying under lamp [16].

Antimicrobial Activity of Synthesized ZnO NPs [11]: The antibacterial activity of synthesized ZnO NPs was evaluated by agar well diffusion method. Two Gram Positive bacteria *viz. Staphylococcus aureus* and *Bacillus sp. and* two Gram Negative bacteria viz. *Escherichia coli* and *Proteus sp.* were used for the assay. Overnight grown broth culture (100 µL) of the test bacteria was spread on the sterile Muller-Hinton agar plate. Wells were punched on the spread plate using sterile 8 mm cork borer. 100 µL of each ZnO NPs having concentration of 1mg/ml was added into respective well using sterile micropipette. DMSO was used as negative control. Antibiotic Ciprofloxacin having concentration 500 mg/mL (Leeford) was taken as positive control.

The plates were first incubated at 4°C for 10 minutes for pre-diffusion and then at 37°C for 24 hours. Anti-bacterial activity was determined by measuring the diameter of zone of inhibition (mm).

The antifungal activity of synthesized ZnO NPs was also evaluated by agar well diffusion method using the above protocol with few modifications. *Candida albicans* and *Aspergillus niger*, sterile Potato Dextrose Agar plates and Ketoconazole (100 mg/mL) was used for the antifungal assay. The plates with *Candida albicans* were incubated at 37°C and those with *Aspergillus niger* were incubated at 30°C.

Anti-Inflammatory Activity of Synthesized ZnO NPs: The *in-vitro* anti-inflammatory activity of synthesized ZnO NPs was checked Bovine Serum Albumin (BSA) denaturation assay [17]. Briefly, 10, 20, 30, 40 and 50 µg of ZnONPs and diclofenac sodium was taken from a stock of 1 mg/ml solution in tubes labelled respectively.

To each test tube, 2 mL of 1% BSA and 390, 380, 370, 360 and 350 µL of distilled water was added. The tubes were incubated at room temperature for 10 minutes at 50°C in water bath for 10 minutes. Absorbance was measured at 660 nm in UV Spectrophotometer. The percentage of protein denaturation was determined using the following equation:

% inhibition = (Absorbance of control - Absorbance of sample × 100) / Absorbance of control

Antioxidant Activity of Synthesized ZnO NPs [14]: The antioxidant property of ZnO NPs was checked by reducing power assay using Potassium Ferricyanide by measuring their capacity to reduce ferric to ferrous ions. Different concentrations of ZnO NPs and Ascorbic acid (standard) ranged from 2 mg/mL to 8 mg/mL each was added in respectively labelled tubes. 1mL of absolute ethanol was added and mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (10 g/L); mixture was then incubated at 50°C for 20 min; 2.5 mL of trichloro acetic acid (100 g/L) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride (1 g/L). Absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Increased absorbance of the reaction mixture indicates stronger reducing power.

RESULTS:

Phytochemical Analysis of Aqueous Extract of *Brassica hirta*, *Piper cubeba* and *Amomum subulatum*: To find out the active components in the aqueous extracts of *Brassica hirta*, *Piper cubeba* and *Amomum subulatum*, phytochemical analysis ofthese extracts was performed.

It was observed that aqueous extract of *Brassica hirta* contains flavonoids, saponins, reducing sugars, alkaloids, proteins, tannins, steroids, glycosides, coumarins, and amino acids, but it was devoid of quinones, the aqueous extract of *Piper cubeba* showed the presence of saponins, quinone, alkaloids, proteins, steroids, and amino acids, but was devoid of flavonoids, reducing sugars, tannins, glycosides, and coumarins while the aqueous extract of *Amomum subulatum* contained saponins, alkaloids, proteins, steroids, coumarins, and amino acids, but was devoid of flavonoids, quinones, reducing sugars, tannins, and glycosides, the results are depicted in **Table 2**.

TABLE 2: PHYTOCHEMICAL ANALYSIS FOR *BRASSICA HIRTA, PIPER CUBEBA, AMOMUM SUBULATUM*

Phytochemical Test	*Brassica hirta*	*Piper cubeba*	*Amomum subulatum*
Flavonoid	Positive	Negative	Negative
Saponin	Positive	Positive	Positive
Quinone	Negative	Positive	Negative
Reducing sugar	Positive	Negative	Negative
Alkaloid	Positive	Positive	Positive
Protein	Positive	Positive	Positive
Tannin	Positive	Negative	Negative
Steroid	Positive	Positive	Positive
Glycoside	Positive	Negative	Negative
Coumarin	Positive	Negative	Positive
Amino acid	Positive	Positive	Positive

Green Synthesis of ZnO NPs Synthesized using Aqueous Extracts of *Brassica hirta, Piper cubeba, Amomum subulatum*: As the phytochemical analysis of aqueous extract of *Brassica hirta, Piper cubeba, Amomum subulatum* revealed that it contains Flavonoid, saponins, reducing sugars, proteins, alkaloids, tannins, steroids etc. which can act as an oxidizing, reducing, and capping agent for green synthesis of ZnO NPs, aqueous extract of Indian spices like *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* were used for green synthesis of ZnO NPs.

White precipitate of ZnO NPs was observed in the case of *Brassica hirta* and dark brown and brown precipitate of ZnO NPs was formed in case of *Piper cubeba*, and *Amomum subulatum*. The above ZnO NPs were characterized in detail with respect to its morphology, UV-visible spectrum, size, antimicrobial property, anti-inflammatory and antioxidant property.

Characterization of the ZnO NPs: The formed ZnO NPs were characterized with respect to UV-Visible spectroscopy, X-Ray Diffraction, and Scanning Electron Microscopy.

UV-Visible Spectroscopy of ZnO NPs: In order to study the optical absorption property of the synthesized ZnO NPs using, *Brassica hirta, Piper Cubeba, Amomum subulatum*, the UV–visible absorbance spectrum was monitored at room temperature in the wavelengths of 200 to 700 nm. The spectrum showed a peak at 350 nm in case of *Brassica hirta* ZnO NPs **Fig. 1A**, 300 nm in case of *Piper cubeba* ZnO NPs **Fig. 1B** and 290 nm in case of *Amomum subulatum* ZnO NPs which is specific for ZnO NPs **Fig. 1C**.

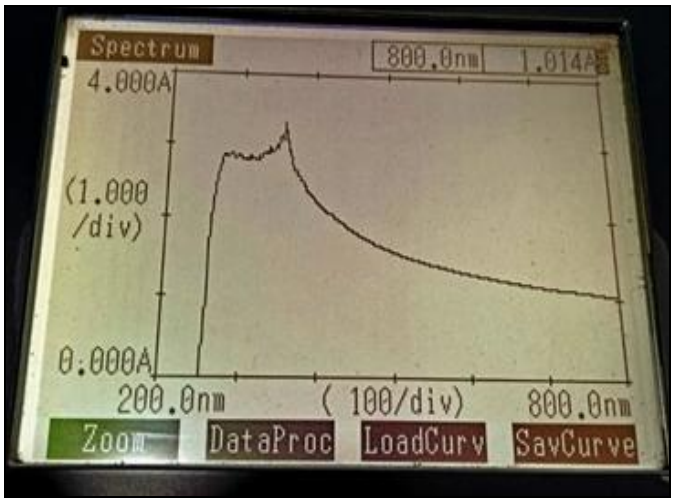


FIG. 1A: UV-VISIBLE SPECTRUM OF *BRASSICA HIRTA* ZNO NPs

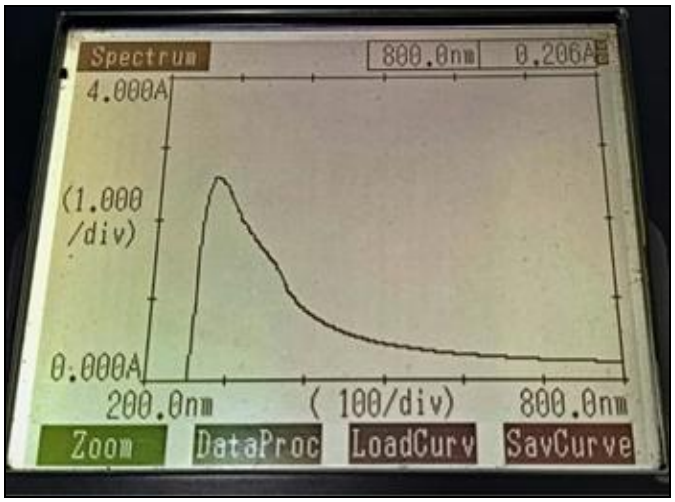


FIG. 1B: UV-VISIBLE SPECTRUM OF *PIPER CUBEBA* ZNO NPs

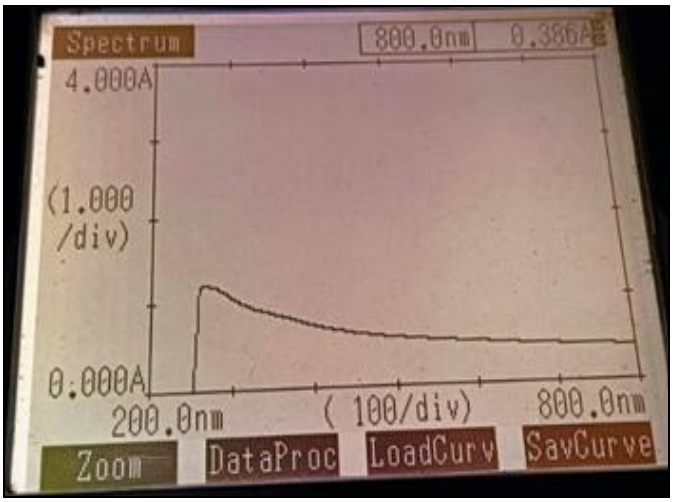


FIG. 1C: UV-VISIBLE SPECTRUM OF *AMOMUM SUBULATUM* ZNO NPs

FESEM Analysis of ZnO NPs: The surface morphology of the green synthesized ZnO NPs using Indian spices *Brassica hirta*, *Piper cubeba,* and *Amomum subulatum* was determined through Field Emission Scanning Electron Microscopy. The FESEM images provided insights into the structural characteristics of the synthesized NPs **Fig. 2A, B, C**. *Brassica hirta* ZnO NPs exhibited a morphology resembling cylindrical with the average sizes ranging from 18 to 35 nm **Fig. 2A**. *Piper cubeba* ZnO NPs exhibited a morphology showing irregular cylindrical shapes with the average sizes ranging from 27 to 48 nm **Fig. 2B**. *Amomum subulatum* ZnO NPs exhibited a morphology resembling irregular shape with the average sizes ranging from 30 to 113 nm **Fig. 2C**.

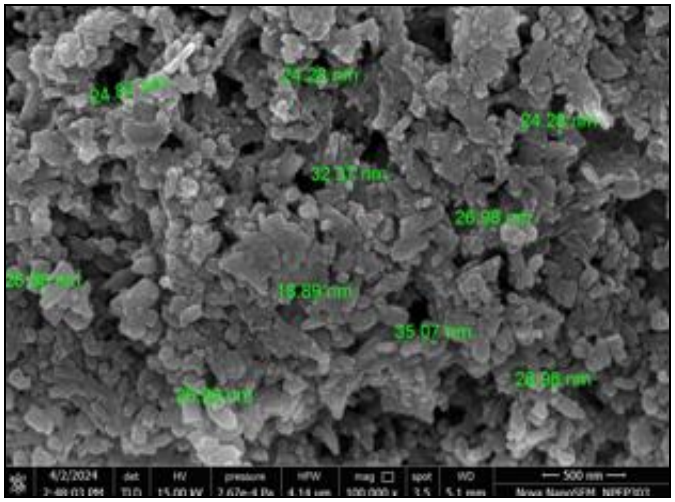


FIG. 2A: FE-SEM IMAGE OF *BRASSICA HIRTA* ZNO NPs

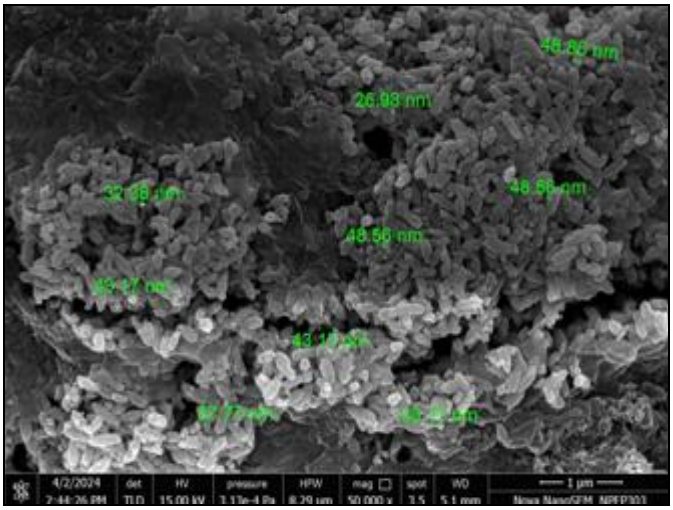


FIG. 2B: FE-SEM IMAGE OF *PIPER CUBEBA* ZNO NPs

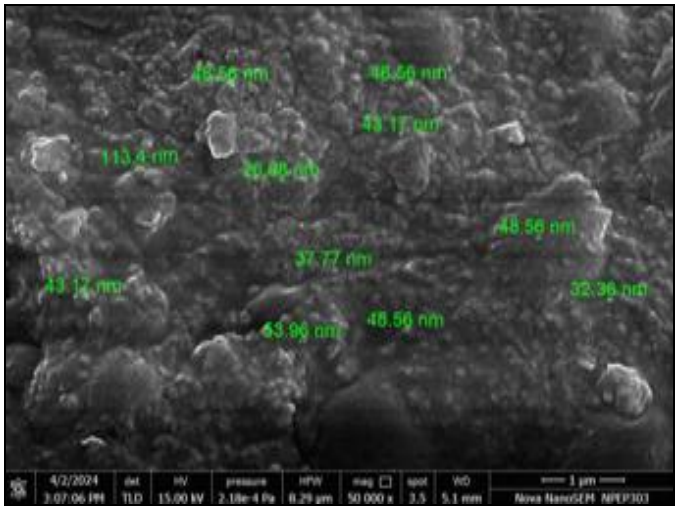
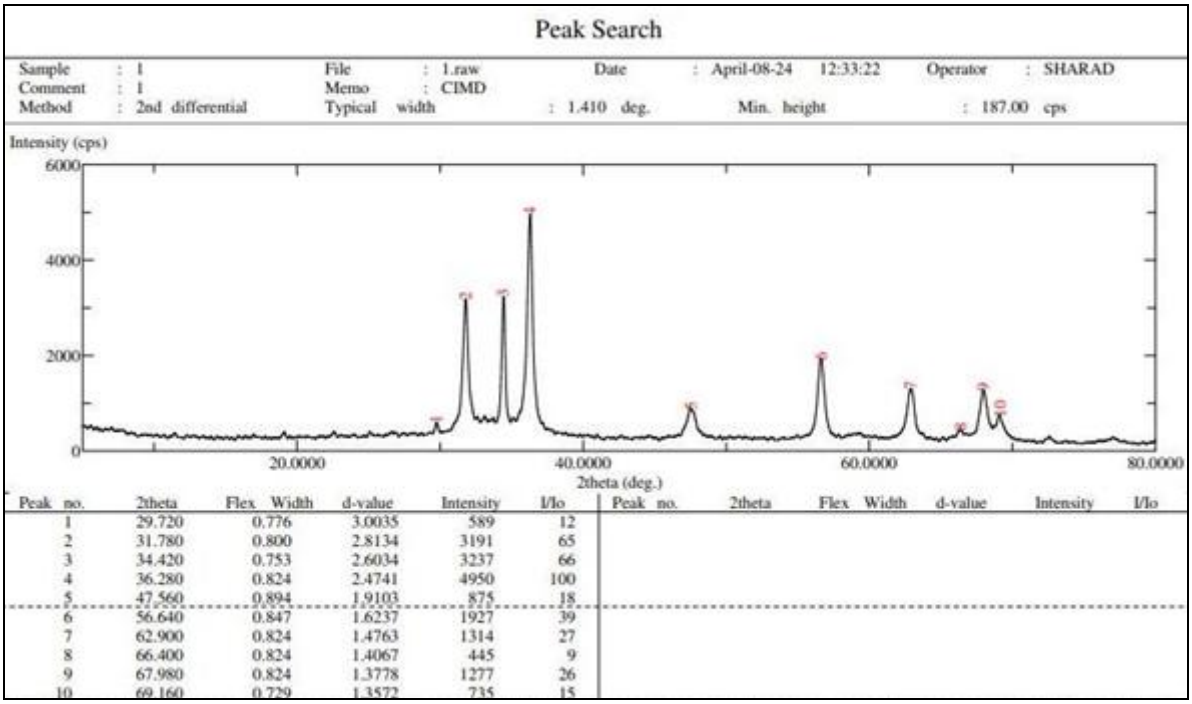


FIG. 2C: FE-SEM IMAGE OF *AMOMUM SUBULATUM* ZNO NPs

X-Ray Diffraction Analysis of ZnO NPs: The samples of ZnO NPs formed using aqueous extracts of *Brassica hirta, Piper Cubeba, Amomum subulatum* were analysed for XRD using Single crystal X-Ray Diffractometer.

XRD Analysis of *Brassica hirta* ZnO NPs: The X-ray diffraction pattern of ZnO NPs synthesized by using *Brassica hirta* is shown in **Fig. 3A**. The XRD pattern showed ten intense peaks in the whole spectrum of 2θ values ranging from 09 to 100 (29.72, 31.78, 34.42, 36.28, 47.56, 56.64, 62.90, 66.40, 67.98, and 69.76), which could be attribute to the 12, 65, 66, 100, 18, 39, 27, 09, 26, and 15 planes for zinc, respectively.

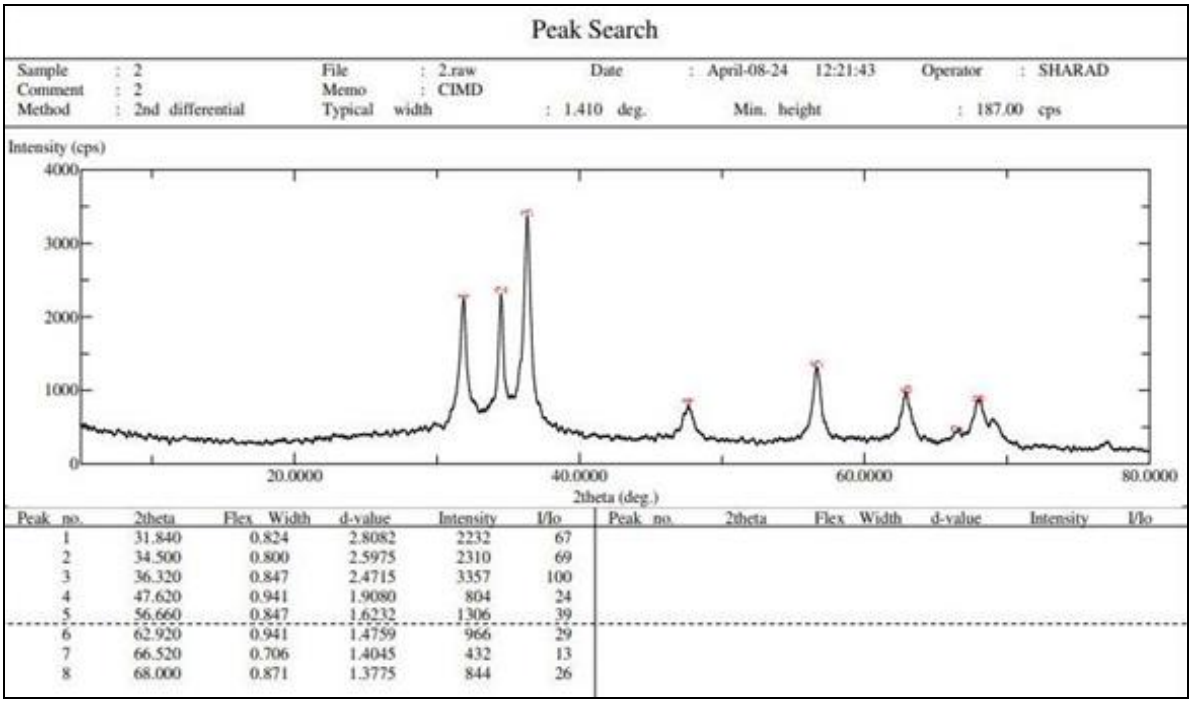
All the diffraction peaks confirmed the hexagonal wurtzite structure of ZnO NPs. The strong and narrow diffraction peaks confirmed the crystalline nature of synthesized ZnO NPs.



Peak no.	2theta	Flex Width	d-value	Intensity	I/Io
1	29.720	0.776	3.0035	589	12
2	31.780	0.800	2.8134	3191	65
3	34.420	0.753	2.6034	3237	66
4	36.280	0.824	2.4741	4950	100
5	47.560	0.894	1.9103	875	18
6	56.640	0.847	1.6237	1927	39
7	62.900	0.824	1.4763	1314	27
8	66.400	0.824	1.4067	445	9
9	67.980	0.824	1.3778	1277	26
10	69.160	0.729	1.3572	735	15

FIG. 3A: XRD ANALYSIS OF *BRASSICA HIRTA* ZNO NPs

XRD Analysis of *Piper cubeba* ZnO NPs: The X-ray diffraction pattern of ZnO NPs synthesized by using *Piper cubeba* is shown in **Fig. 3B.** The XRD pattern showed ten intense peaks in the whole spectrum of 2θ values ranging from 23 to 100 (27.14, 29.94, 32.00, 34.66, 36.48, 47.82, 56.80, 63.00, 68.12, and 69.36), which could be attribute to 28, 42, 72, 70, 100, 29, 45, 31, 33, and 23 planes for zinc, respectively. All the diffraction peaks confirmed the hexagonal wurtzite structure of ZnO NPs. The strong and narrow diffraction peaks confirmed the crystalline nature of synthesized ZnO NPs.

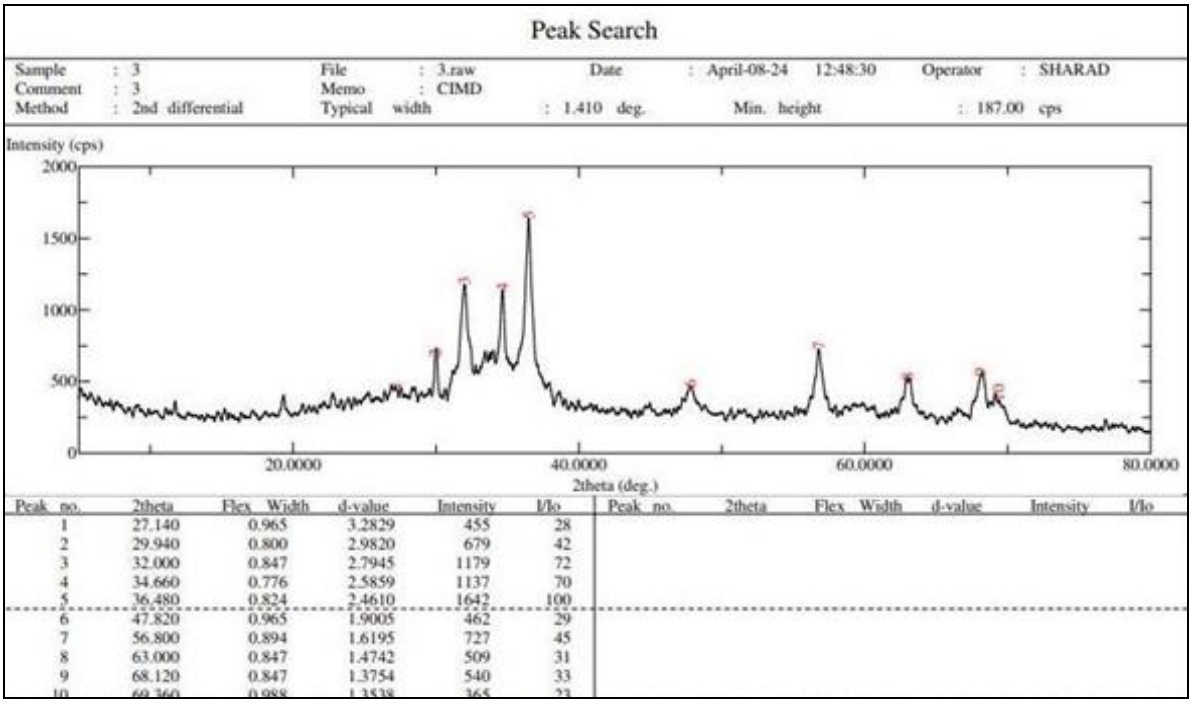


Peak no.	2theta	Flex Width	d-value	Intensity	I/Io
1	31.840	0.824	2.8082	2232	67
2	34.500	0.800	2.5975	2310	69
3	36.320	0.847	2.4715	3357	100
4	47.620	0.941	1.9080	804	24
5	56.660	0.847	1.6232	1306	39
6	62.920	0.941	1.4759	966	29
7	66.520	0.706	1.4045	432	13
8	68.000	0.871	1.3775	844	26

FIG. 3B: XRD ANALYSIS OF BIOSYNTHESIZED *PIPER CUBEBA* ZNO NPs

XRD Analysis of *Amomum subulatum* ZnO NPs: The X-ray diffraction pattern of ZnO NPs synthesized by using *Amomum subulatum* is shown in **Fig. 3C.** The XRD pattern showed eight intense

peaks in the whole spectrum of 2θ values ranging from 13 to 100 (31.84, 34.50, 36.32, 47.62, 56.66, 62.92, 66.52, and 68.00), which could be attribute to 67, 69, 100, 24, 39. 29, 13 and 26 planes for zinc, respectively. All the diffraction peaks confirmed the hexagonal wurtzite structure of ZnO NPs. The strong and narrow diffraction peaks confirmed the crystalline nature of synthesized ZnO NPs.



Peak no.	2theta	Flex Width	d-value	Intensity	I/Io	Peak no.	2theta	Flex Width	d-value	Intensity	I/Io
1	27.140	0.965	3.2829	455	28						
2	29.940	0.800	2.9820	679	42						
3	32.000	0.847	2.7945	1179	72						
4	34.660	0.776	2.5859	1137	70						
5	36.480	0.824	2.4610	1642	100						
6	47.820	0.965	1.9005	462	29						
7	56.800	0.894	1.6195	727	45						
8	63.000	0.847	1.4742	509	31						
9	68.120	0.847	1.3754	540	33						
10	69.360	0.988	1.3538	365	23						

FIG. 3C: XRD ANALYSIS OF BIOSYNTHESIZED *AMOMUM SUBULATUM* ZNO NPs

Antibacterial Activity of ZnO NPs: The antibacterial activity of synthesized ZnO NPs (1 mg/ml) using *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* extract was against two Gram positive (*Staphylococcus aureus* and *Bacillus sp.)* and two Gram negative (*Escherichia coli* and *Proteus sp.)* bacteria. The ciprofloxacin antibiotic (500 mg/ml) was used as a positive control and DMSO as negative control.

The data from **Table 3** illustrates the antibacterial efficacy of green-synthesized ZnO Nanoparticles (ZnO NPs) against four bacterial strains: two Gram-positive (*Staphylococcus aureus*, *Bacillus sp.*), and two Gram-negative (*Escherichia coli*, *Proteus sp.*). *Piper cubeba* derived ZnO NPs demonstrated the strongest overall performance among the three variants, particularly against Gram-positive bacteria. However, all ZnO NPs showed moderate activity compared to the standard antibiotic Ciprofloxacin. This trend is consistent with extensive literature on green-synthesized ZnO NPs, which report them as viable, eco-friendly antibacterials.

TABLE 3: ANTIBACTERIAL ACTIVITY OF ZNO NPs

ZnO NPs of	Zone of inhibition (mm)			
	Staphylococcus aureus	***Bacillus sp.***	***Escherichia coli***	***Proteus sp.***
Brassica hirta	17±0.312	17±0.231	20±0.254	17±0.246
Piper cubeba	20±0.217	20±0.272	19±0.191	17±0.227
Amomumsubulatum	17±0.251	07±0.248	15±0.218	15±0.235
Ciprofloxacin	38±0.166	30±0.203	34±0.177	30±0.212

Antifungal Activity of ZnO Nanopaticles: The antifungal activity of ZnO NPs synthesized by using Indian spices like *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* at a concentration of 1 mg/ml was tested against *Aspergillus niger* and *Candida albicans*, with *Piper cubeba* derived nanoparticles showing the highest efficacy among the three samples.

It was determined that all the ZnO NPs possessed same antifungal capacity at the tested concentration. Antifungal Ketoconazole (100 mg/ml) was used as a positive control and DMSO as a negative control. The measured diameters of zones of inhibition are depicted in **Table 4**. The synthesized ZnO NPs showed intermediate antifungal activity at 1 mg/ml concentration but at higher concentrations they can exhibit significant antifungal activity many against fungi. While the ZnO NPs are effective, they currently show lower potency than the standard antifungal Ketoconazole, which produced the largest zones of inhibition. This aligns with existing literature highlighting ZnO NPs as a promising, low-toxicity alternative to traditional azole-based treatments. *Aspergillus niger* was consistently more sensitive to all tested ZnO NP variants compared to *Candida albicans*, a trend supported by studies identifying *A. niger* as highly susceptible to green-synthesized metal oxides.

TABLE 4: ANTIFUNGAL ACTIVITY OF ZNO NPs

ZnO NPs of	Zone of inhibition (mm)	
	Aspergillus niger	***Candida albicans***
Brassica hirta	16±0.211	15±0.337
Piper cubeba	19±0.184	16±0.242
Amomum subulatum	18±0.198	14±0.216
Ketoconazole	30±0.236	22±0.179

Anti-Inflammatory Activity of Synthesized ZnO NPs using *Brassica hirta, Piper cubeba, Amomum subulatum*: The anti-inflammatory activity of ZnO nanoparticles synthesized using *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* was evaluated in comparison with diclofenac sodium **Fig. 5** and **Table 5**. All experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

Although ZnO nanoparticles exhibited slightly higher mean inhibition values than diclofenac sodium at higher concentrations, the observed differences were modest. Given that diclofenac is a clinically established anti-inflammatory drug, the present findings suggest that ZnO NPs demonstrate comparable efficacy under *in-vitro* conditions, rather than definitive superiority.

The enhanced activity may be attributed to nanoscale effects and phytochemical capping from the spice extracts. Further *in-vivo* and mechanistic studies are required to confirm their therapeutic relevance.

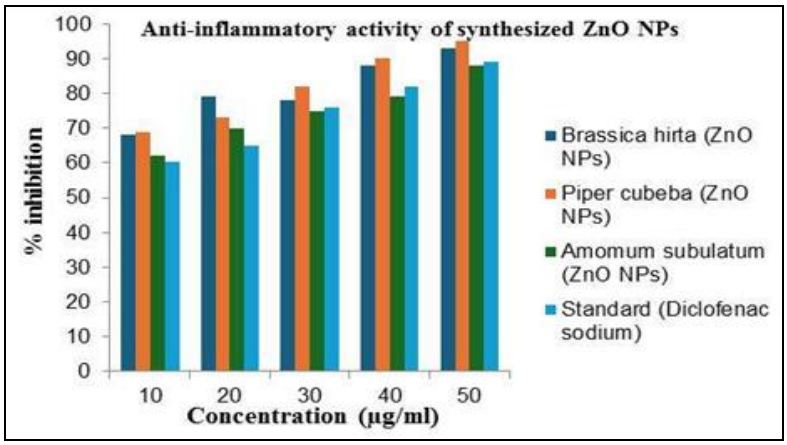


FIG. 4: ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED NPs

TABLE 5: ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED ZNO NPs

	% Inhibition									
	10ug		20ug		30ug		40ug		50ug	
	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
Brassica hirta (ZnO NPs)	69.8	1.0583	71.5	0.7	73.2	0.2	81.2	0.7211	92.566	0.5859
Piper cubeba	62.5	0.5567	66.1	0.8544	79.866	0.5131	90.366	0.8504	94.633	0.5507

(ZnO NPs) *Amomum subulatum* (ZnO NPs)	60.566	0.7767	71.233	0.6806	74.933	1.10151	77.233	0.6806	88.133	0.8082
Standard (Diclofenac sodium)	61.533	0.5033	65.4	0.7810	75.3	1.1269	81.766	1.0969	91.133	0.6110

Antioxidant Activity of Synthesized ZnO NPs: The ZnO NPs synthesized using Indian spices *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* was found possess antioxidant property. In this study, the reducing capacity of the ZnO NPs was assessed using the Fe^{3+} to Fe^{2+} reduction assay, where a colour changes from yellow to blue indicating the reduction of Fe. The antioxidant property of ZnO NPs increased with the increase in the concentration of ZnO NPs and the activity was comparable with the standard antioxidant, Ascorbic acid. The results are demonstrated in the **Table 6.**

TABLE 6: ANTIOXIDANT ACTIVITY OF SYNTHESIZED ZNO NPs

Concentration (mg/ml)	*Brassica hirta* (ZnO NPs)	*Piper cubeba* (ZnO NPs)	*Amomum subulatum* (ZnO NPs)	Standard (Ascorbic acid)
4	64±0.683	62±0.881	61±0.734	68±0.606
6	76±0.457	74±0.413	72±0.398	78±0.487
8	83±0.615	80±0.565	80±0.603	85±0.499

DISCUSSION: The present study supported the earlier findings that aqueous extracts of *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum* contain various phytochemicals, including alkaloids, flavonoids, tannins, saponins, glycosides, coumarins, steroids, and phenols [18].

The bioactive compounds like phenolic acids, flavonoids, saponins, alkaloids, tannins, terpenes and steroids etc. found in the aqueous extract of *Brassica hirta*, *Piper cubeba* and *Amomum subulatum* might act as an oxidizing, reducing, and capping agent for the synthesis of biogenic ZnO NPs. These compounds are responsible for the reducing properties of the spice extract, which are crucial in the formation of ZnO NPs, as well as for their capping and stabilizing effects. They enable the production by providing electrons to reduce metal ions (Zn^{2+}) into stable ZnO NPs and then attach to the surface to avoid aggregation.

There are very few reports of use of Indian spices for synthesis of ZnO NPs, which include Black cardamom and Black Pepper [19], Curry leaf [20], nutmeg [21], Zinger and Garlic Bulb [11], Tejpatta [22], Black cumin or Kalonji [23]. The present study also confirmed the successful synthesis of ZnO NPs using three spice extracts independently, as evidenced by UV-Vis spectroscopy, with absorption peaks at 350 nm, 300 nm, and 290 nm for *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum*, respectively and confirmed by XRD and SEM. Several researchers studied the UV-visible spectroscopic analysis of ZnO NPs and reported that the absorbance peak of ZnO NPs ranges between 310 nm and 360 nm of wavelength [14, 1]. Thus, the results of current study correlate with the earlier reports. In the present work, peaks at 2θ values and the corresponding planes confirmed the synthesis of ZnO NPs using *Brassica hirta* and similar peaks were earlier reported for ZnO NPs synthesized by using other spices [24, 15].

The agglomeration of smaller ZnO NPs particles resulted in the formation of larger particles, which imparts irregular forms to their structures and the same was reported by a similar study [25]. The hexagonal form and uneven distribution pattern of the produced ZnO NPs were also demonstrated in prior investigations [26].

The antimicrobial properties of the synthesized ZnO NPs were evaluated against *Staphylococcus aureus, Bacillus, Escherichia coli,* and *Proteus sp.*, demonstrating significant antibacterial activity at the tested concentration. The ZnO NPs assessed for their antibacterial properties demonstrated better results than obtained by another group with the ZnO NPs synthesized by using garlic bulb and *Z. officinale* root extract [11]. According to a recent study [27], ZnO NPs synthesized utilizing *Beta vulgaris*, had antifungal activity against *Aspergillus niger*, but ZnO NPs prepared from *Cinnamomum tamala* were active against *Candida albicans*.

Furthermore, ZnO NPs synthesized using *Brassica oleracea* var. italica extract demonstrated efficacy against both strains of fungus. Similarly, ZnO NPs synthesized from three different spice extracts in this present study, possessed significant antifungal activity against both these fungi.

Further, ZnO NPs exhibited potent antioxidant and anti-inflammatory properties, as confirmed by Fe^{3+} to Fe^{2+} reduction assays, indicating their ability to scavenge free radicals. The observed activity is likely attributed to their small size and high surface reactivity [14]. The findings highlight the potential of ZnO NPs synthesized from Indian spices for antimicrobial, antifungal, and therapeutic applications.

CONCLUSION: This study successfully synthesized ZnO NPs using *Brassica hirta*, *Piper cubeba*, and *Amomum subulatum*. To the best of our knowledge, this is the first report of the synthesis of ZnO NPs using *Piper cubeba* and *Brassica hirta*. These ZnO NPs were spherical and/ irregular in shape and average particle size was 61.90 nm. The biosynthesized ZnO NPs exhibited significant antimicrobial, anti-inflammatory, and antioxidant properties, highlighting their potential in nanomedicine, pharmaceuticals, and biotechnology. ZnO nanoparticles synthesized using *Piper cubeba*, exhibited higher medicinal potential in concentration-dependent manner as compared to the other two plants studied here. The antiviral and anti-cancer properties of these NPs should be investigated since they may play a significant role in modern medical care. Green synthesis provides an eco-friendly, cost-effective alternative to chemical methods, promoting sustainable nanotechnology applications.

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CONFLICTS OF INTEREST: Nil

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