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Rice bagasse extract-based green synthesis of zinc oxide nanoparticles: characterisation, assessment of anti-skin cancer, antibacterial, and antioxidant properties

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ABSTRACT

In the present study, an eco-friendly, sustainable, simple, and cost-effective method was employed for the synthesis of ZnO NPs, using the ethanolic extract of rice bagasse as a capping and stabilizing agent. The obtained ZnO NPs were characterized using different analytical techniques. The UV–Vis spectra of the biosynthesized ZnO NPs showed a strong absorption peak at 374.23 nm. XRD and electron microscopy both verified that ZnO NPs, which have an average size of 28 nm, have a hexagonal/cubic crystalline structure. Fourier-transform infrared (FT-IR) spectroscopy identified absorption bands that suggest the presence of phytoconstituents functioning as capping agents. The zeta potential study revealed that the ZnO nanoparticles had a negative charge of -27.6 mV, suggesting enhanced electrostatic repulsion among particles, which contributes to improved colloidal stability. It is important to note that the ZnO NPs' cytotoxic impact on the cancer cells under investigation is dose-dependent. Skin cancer IC_{50} was found to be 415.46 μg/ml ZnO NPs. Additionally, the NPs showed strong antibacterial and antifungal action, especially against the pathogenic fungus *Candida albicans* (ATCC 10,221), Gram-negative bacteria *Escherichia coli* (ATCC 8739), and Gram-positive *Staphylococcus aureus* (29,213). They also showed a great deal of antioxidant potential. These results imply that ZnO NPs synthesised from rice bagasse provide a viable platform for pharmacological and therapeutic applications.

1. Introduction

The anticipated worldwide population surge, predicted to surpass 9 billion by 2050 and approach 11 billion by 2100, has become a critical issue of a sufficient food supply a critical issue. Researchers are devising techniques to reduce food deterioration and extend shelf life, while simultaneously investigating advantageous microbial uses in the food industry [1]. By Sustainable Development Goal 2 of Zero Hunger, significant advancements have been made in enhancing the production of poultry, cattle, and crops. Nonetheless, these developments have concurrently increased food and agricultural waste [2]. Food waste is progressively being transformed into commercially viable items. They function as feedstock for the synthesis of biofuels, bioplastics, and the extraction of valuable chemicals. Agro-industrial leftovers are reused for energy generation, mushroom growing, and the manufacturing of paper and cardboard [3]. Notwithstanding these uses, the existing utilisation capacity is inadequate in comparison to the substantial quantities of waste produced. In the inadequate mechanisms for managing garbage, several areas resort to open incineration or land burial of garbage, which leads to environmental damage, including air and water contamination, and intensifies global warming [4]. Open combustion emits fine particulate matter (PM), which presents considerable health hazards. The 2019 Global Burden of Disease (GBD) research identified PM 2.5 exposure as the sixth leading risk factor for worldwide death. In an agricultural centre in Southeast Asia, the incineration of agricultural residues has been recognised as the sixth leading cause of death [5].

This scenario underscores an immediate need for creative, sustainable approaches to manage and enhance agricultural leftovers, guaranteeing environmental sustainability as well as Food as well as health stability [6]. Remaining crop material and agro-industrial waste, mostly

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consisting of lignocellulosic biomass, namely cellulose, hemicellulose, and lignin, constitute a significant resource for generating bio-based energy and high-value goods. Utilising a biorefinery technique, these components may be hydrolysed by chemical or enzymatic methods to provide fermentable sugars, including glucose, xylose, and l-arabinose [7]. The subsequent processes of anaerobic digestion, fermentation, and composting facilitate the generation of diverse bio-based products, such as organic acids and bioenergy, enzymes, bioplastics, and biofertilizers. Ample lignocellulosic biomass sources such as sweet sorghum bagasse, corncobs, rice straw, and sugarcane bagasse are exceptionally conducive to biorefinery processes owing to their extensive availability and compatibility for transformation into value-added products [8]. A new toxicity study of twenty-four metal oxide nanoparticles has produced a thorough dataset, providing significant insights for the formulation of nano-structure–activity relationships (n-SAR). This dataset comprises multiparametric toxicity profiles evaluated across two separate cell lines, measured across different exposure periods and concentration ranges, hence enabling the determination of application areas and decision thresholds [9].

In light of the increasing need for renewable and sustainable energy sources, bioethanol has surfaced as a viable substitute for fossil fuels. The microbial fermentation of lignocellulosic biomass is regarded as an efficient approach for bioethanol production, especially because these feedstocks are non-edible and do not threaten food security. A full biorefinery architecture may be developed to achieve zero-waste production by concurrently producing bioethanol and high-value chemicals, thereby enhancing process economics. Pyruvate decarboxylase (PDC) is a crucial enzyme in the bioethanol pathway that catalyses the decarboxylation of pyruvate, producing acetaldehyde and carbon dioxide [10]. PDC catalyses a carboligation reaction whereby active acetaldehyde interacts with benzaldehyde to produce R–phenylacetylcarbinol (PAC), an essential step in the manufacture of medicinal compounds like ephedrine and pseudoephedrine. These chemicals are used therapeutically for asthma, hypotension, nasal congestion, and as vasopressors in emergency medicine [11]. Additionally, ephedrine has adrenergic properties and has been investigated for its potential uses in sports medicine, weight management, and as a neuroprotective agent for disorders including cerebral ischaemia and brain damage. Their usefulness in the management of chronic obstructive pulmonary disease and life-threatening hypotensive situations has been confirmed. Due to its therapeutic significance, ephedrine is included on the World Health Organization's 21st essential drugs List [12].

The synthesis of commercial PAC generally entails the decarboxylation of pyruvate, followed by the carboligation of benzaldehyde by PDC-mediated biotransformation, resulting in a final product valued at roughly USD 146 per kilogram [13]. Diverse microbial species, such as yeasts, fungi, and bacteria, have been evaluated for their potential in this biotransformation. Rosche et al. examined 105 yeast strains and found certain *Candida* species as proficient PAC makers with little vulnerability to the harmful effects of benzaldehyde and acetaldehyde [14]. *Rhizopus javanicus* was identified as a prolific generator of PAC among 14 fungi that generate ethanol. Our prior research indicated that *C. tropicalis* outperformed 50 evaluated strains in terms of ethanol and PAC production [15].

This research evaluates the green approach used to synthesize ZnO NPs using rice bagasse ethanolic extract. Standard protocol used to evaluate the structure and morphological characterization of synthesised ZnO NPs. In addition, biological activities like skin-cancer, antimicrobial, and antioxidant properties were evaluated to show a sustainable approach for pharmacological and medical applications.

2. Materials and methods

2.1. Collection and preparation of plant specimens

Rice bagasse originates from *Oryza sativa L.* (family *Poaceae*), a fundamental cereal crop grown in tropical and subtropical areas across the globe. It is important to specify the particular cultivar or variety, as well as its geographical origin (Kashipur, Udham Singh Nagar, Uttarakhand, 29.2104°N, 78.9819° E). Rice bagasse is a lignocellulosic agricultural waste generated as a by-product of local rice mills in India. The bagasse produced was sieved post-rice extraction to provide the final husk and fibrous product, and thereafter chosen to ensure the exclusion of undesirable foreign items such as stones or debris from other materials. The bagasse was washed with double-distilled water to remove surface contaminants and dust. To prevent the breakdown of phytochemicals due to sunshine exposure, the washed material was shade-dried at ambient temperatures (25–30 °C) for 7-10 days. Subsequently, the desiccated rice bagasse was subjected to an oven for drying at 40 °C for 24 hours to eliminate all moisture. The dried sample was then sieved through a 60-mesh screen and coarsely ground with a grinder, which was then stored in an airtight container at room temperature until extraction was performed [16].

2.2. Preparation of rice bagasse ethanol extract

Using 100 mL of 70 % ethanol and constant stirring, 10 g of the powder was extracted over the course of 24 hours at room temperature. Whatman No 1 filter paper was then used to filter the mixture, and the final product was stored for later use at room temperature or 4 °C [17].

2.3. Synthesis of ZnO nanoparticles (ZnO NPs)

The green synthesis of zinc oxide (ZnO) nanoparticles was conducted with an ethanolic extract of rice bagasse as a natural reducing and stabilising agent. The extract was filtered and preserved at 4 °C for prompt utilisation. For nanoparticle synthesis, 50 mL of 0.1 M zinc acetate dihydrate [$Zn(CH_3COO)_2 2H_2O$] solution was combined with 50 mL of the produced rice bagasse extract while maintaining continual magnetic stirring at 600 rpm. The pH of the reaction mixture was modified to 10 using 0.1 M NaOH to facilitate the precipitation of zinc hydroxide. The reaction was sustained at 75 °C for 2 hours, during which a colour shift and the emergence of a white precipitate signified the synthesis of ZnO nanoparticles. The mixture was subsequently cooled to ambient temperature and allowed to mature for 24 hours to guarantee the complete production of nanoparticles. The precipitate was obtained using centrifugation at 10,000 rpm for 15 min, subsequently washed with distilled water and ethanol to eliminate unreacted substances, and finally dried at 60 °C for 12 hours. The dry powder was further calcined at 400 °C for 2 hours in a muffle furnace to improve crystallinity and eliminate any remaining organic material [18,19].

2.4. Characterization of ZnO NPs

Characterisation of biosynthesised ZnO NPs using rice bagasse extract. Certain analytical methods were used to ascertain the structural and morphological surface aspects of the nanoparticles [20].

2.4.1. UV–Visible spectroscopy

The optical properties of the synthesised ZnO NPs were characterised using UV–Visible spectroscopy. A specified amount of ZnO NPs powder was dissolved in ethanol and subjected to ultrasound for 15 min to produce a homogeneous colloidal suspension [21]. A UV–Visible spectrophotometer (such as the Shimadzu UV-1800 or a comparable model) was used to examine the suspension over a wavelength range of 200–800 nm. The blank reference consisted of distilled water, used to calibrate the spectrophotometer before sample analysis. All spectra recordings were conducted under identical circumstances at ambient temperature (21 °C).

2.4.2. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to ascertain the functional groups in synthesised ZnO NPs to detect the presence of phytochemicals involved in the creation and stabilisation of nanoparticles [22]. Dry powder of ZnO NPs was mixed with KBr at a 1:100 ratio. A hydraulic press was then used to compress the powder into a pellet. An FTIR spectrophotometer was used to get FTIR spectra in the 4000–400 cm-1 range.

2.4.3. X-Ray diffraction (XRD)

XRD analysis (Bruker D8 Advance or equivalent) with Cu Kα radiation (λ 1.5406 Å), 40 kV; 30 mA, was used to evaluate the crystal structure of the synthesised ZnO nanopowder. The data were obtained in the 2θ range of 20° to 80°, and the average crystallite size was calculated applying the Scherrer equation [23].

2.4.4. Scanning electron microscopy (SEM)

The surface appearance and particle size of the synthesised ZnO NPs were analysed by SEM [24]. A minimal amount of dried ZnO NPs powder was evenly applied on a carbon-coated SEM stub and then thinly covered with gold sputter to improve conductivity. The microscope used for the sample analysis was a scanning electron microscope functioning within an accelerating range of 1020 kV. The morphology, distribution, and aggregation of the nanoparticles were documented at various magnifications using a microscope.

2.4.5. Dynamic light scattering (DLS)

To characterise the surface charge and colloidal stability, zeta potential was analyzed, and to evaluate the particle size distribution (PSD) of synthesised ZnO NPs, DLS was used [25]. In all experiments, a small quantity of ZnO NPs powder was introduced into deionised water, and after 15 min of sonication, a homogenous suspension devoid of aggregates was obtained. The sizing was conducted using a particle size analyser equipped with a zeta potential measurement station at ambient temperature. The polydispersity index (PDI) was measured at a scattering angle of 90 degrees to ascertain the hydrodynamic size of the particles to evaluate particle dispersion.

3. Biological activities

3.1. Anti-Skin cancer activity / cytotoxicity assay

The cytotoxic activity of biosynthesised nano-sized zinc oxide (ZnO NPs) was assessed to evaluate its anti-cancer efficacy on a human skin cancer cell line. The investigation was conducted by utilizing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test, a sophisticated colorimetric method used to assess cell viability by evaluating mitochondrial enzymatic potential [26].

3.1.1. Cell line and culture conditions

The malignant human melanoma (A375) cell line was procured from a recognised national cell bank (e.g., NCCS, Pune, India). The cells were maintained at 37 °C in a humidified environment with 5 % CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) foetal bovine serum (FBS) and 1 % penicillin-streptomycin solution.

3.1.2. Preparation of ZnO NP suspensions

The biosynthesised ZnO NPs were then distributed in sterile phosphate-buffered saline (PBS) solution and exposed to sonic vibration for 20 min to prevent particle aggregation. Cells were serially diluted at variable doses.

3.1.3. MTT cytotoxicity assay

After 24 hours of attachment, cells were seeded onto microtiter plates in A375 96-well plates with a total volume of about 100 μL, with approximately 1 × 10^4 cells per well. After that, the plates were placed in incubators at 37 °C in 95 % air and 5 % CO2 [27]. After incubation, new DMEM with varying concentrations of ZnO nanoparticles was added to change the medium. The positive control was an increase in doxorubicin (10 μg/mL), whereas the negative control was untreated cells. The plates were incubated for a further twenty-four hours at 37 °C. After treatment, each well received 20 microlitres of MTT stock solution (5 mg/mL in PBS), which was then allowed to sit at 37 °C for four hours. After the medium was evacuated and disposed of, the purple formazan crystals that had formed from the viable cell count were dissolved in 100 μL of DMSO. The Bio-Rad iMark microplate reader was used to measure the absorbance at 570 nm.

3.1.4. Morphological observation

The cellular morphological changes were seen using an inverted phase-contrast microscope. Observable symptoms of cytotoxicity, characterised by cellular shrinkage, membrane blebbing, and cell separation, were evident based on a concentration-dependent pattern.

3.2. Antimicrobial activity

The agar well diffusion and broth microdilution methods were applied to assess the antibacterial efficacy of ZnO NPs against certain bacterial and fungal pathogens [28]. Test organisms included *S. aureus, P. aeruginosa,* and *C. albicans*. The agar well diffusion assay was conducted using various concentrations of ZnO NPs (25, 100, 120 μg/mL) introduced into wells of an inoculated agar medium, and the zone of inhibition was calculated. The data suggested a concentration-dependent antibacterial action, with larger zones of inhibition reported at higher doses. The minimum inhibitory concentration (MIC) was determined using a broth dilution approach, with values ranging from 25 to 100 μg/ml, depending on the strain.

3.3. Antioxidant activity

The in vitro antioxidant potential of green-synthesized ZnO NPs was assessed using the DPPH free radical scavenging method [29]. Various concentrations of ZnO NPs (10–100 g/mL) were incubated in darkness with DPPH in methanol at 37 °C for 35 min. The absorbance was diminished and quantified using a UV–Vis spectrophotometer at a wavelength of 517 nm. The results indicated that the radical scavenging activity was dose-dependent, suggesting that ZnO NPs had significant antioxidant properties. This phenomenon is attributed to the presence of phytochemicals in the rice bagasse extract, which function as reducing and capping agents, hence enhancing the free radical scavenging ability of the nanoparticles in question.

4. Statistics

All experimental data were statistically represented as the mean and standard deviation (SD) of three separate trials. A one-way ANOVA was used to evaluate the significance of differences between the comparison and treatment groups. Non-linear regression analysis was used in dose-response curves. The statistically significant p-value was established at < 0.05. A graphical representation was created to elucidate the trends in cytotoxicity, antioxidant activity, and antibacterial efficacy of ZnO NPs across various test dosages.

5. Results and discussion

Phytochemical screening of bioactive compounds in rice bagasse extract was conducted to elucidate the mechanism of nanoparticle formation [30]. The findings indicated the presence of several critical functional phytoconstituents (Table 1) that are recognised for their ability to reduce metal ions and inhibit aggregation by stabilising the nanoparticle surface.

These phytochemicals possess functional groups, including –OH, –COOH, and aromatic rings, which serve as natural reducing agents for Zn^{2+} ions and stabilisers in the production of ZnO nanoparticles. Their

Table 1
Screening of the ethanol extract of rice bagasse for qualitative phytochemical properties.

Phytoconstituents	Ethanol extract
Phenol	+
Flavonoids	+
Tannins	–
Saponins	+
Alkaloids	–
Triterpenoids	+

robust antioxidant capacity underpins the documented radical scavenging activity of the synthesised ZnO nanoparticles.

5.1. UV–Visible spectroscopy

The UV–Vis absorption spectrum of the biosynthesized ZnO NPs showed a distinct and sharp absorption peak at approximately 374.23 nm, which is characteristic of electron transitions from the valence band to the conduction band, which causes ZnO's inherent band-gap absorption [31]. This study indicates the synthesis of ZnO NPs with a nanoscale dimension. The UV–Vis spectra of phytosynthesised ZnO NPs are shown in Fig. 1.

5.2. Fourier transform infrared (FTIR) spectroscopy

The FTIR study revealed many significant functional groups in the rice bagasse extract and ZnO NPs [32]. The extensive absorptions at 3472 cm^{-1} correspond to OH stretching vibrations of phenolic and hydroxyl functional groups. At 1640 cm^{-1}, there are peaks corresponding to $C = O$ stretching, showing the presence of carboxyl functional groups. A distinctive signal at 481 cm^{-1} was identified, corresponding to ZnO stretching vibrations, indicating the effective synthesis of ZnO NPs. These functional groups signify the participation of bioactive molecules in the reduction of nanoparticles and the stabilisation of inclusion.

The FTIR analysis of rice bagasse-mediated ZnO nanoparticles identified characteristic absorption bands associated with phenolic –OH groups, $C = O$ (carbonyl), and C–O–C (ether or ester) linkages, suggesting the adsorption of polyphenols, flavonoids, and potentially lignin derivatives onto the nanoparticle surface. These phytoconstituents augment the antioxidant and cytotoxic characteristics of ZnO nanoparticles [33]. The surface functionalisation of nanoparticles with phenolic –OH groups, indicated by broad FTIR peaks in the range of 3200–3500 cm^{-1}, enhances their radical scavenging capacity. The electron-donating ability of flavonoids and phenolic acids, including ferulic acid and p-coumaric acid, aids in the neutralisation of DPPH and ABTS radicals. Surface-bound phytochemicals synergise with the inherent ROS-generating capacity of ZnO to neutralise free radicals, therefore enhancing total antioxidant efficacy. Phytochemicals, including flavonoids and phenolic acids, attached to the nanoparticle surface, can be internalised by cancer cells. These secondary metabolites disrupt cellular redox balance, generate oxidative stress, cause mitochondrial malfunction, and activate apoptotic pathways. The ZnO core produces reactive oxygen species (ROS) in intracellular acidic environments, while surface-attached phytochemicals may specifically increase oxidative stress in cancer cells, resulting in heightened cytotoxicity. FTIR bands at around 1700 cm^{-1} ($C = O$) and 1230 cm^{-1} (C–O stretching) indicate the existence of esterified polyphenols, which may influence protein expression or signalling pathways related to cancer cell apoptosis [34]. FT-IR of phytosynthesized ZnO NPs is shown in Fig. 2.

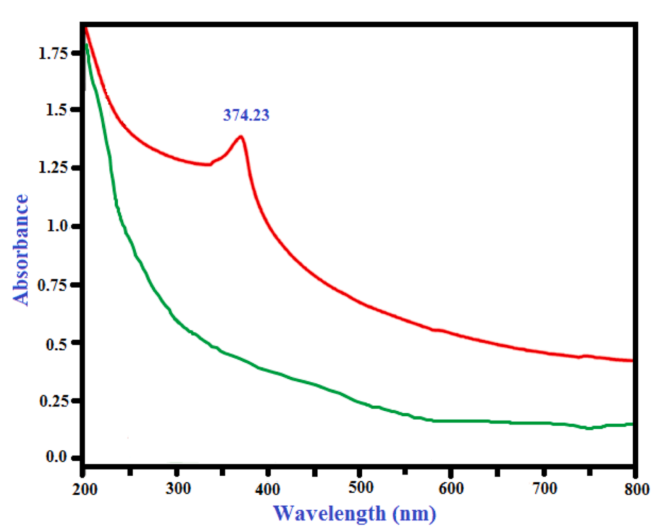

Fig. 1. UV–Vis spectrum of phytosynthesised ZnO NPs.

5.3. X-ray diffraction (XRD) analysis

XRD diffractograms exhibited strong and powerful peaks at 2θ indices of 31.7°, 34.4°, 36.2°, 47.5°, and 56.6°, corresponding to the planes of the hexagonal cubic crystalline structure of ZnO, specifically (100), (002), (101), (102), and (110). The crystallite size was ascertained using the Debye-Scherrer formula, indicating dimensions of around 28 nm, so confirming that the resultant particles are crystalline and nanometric [35]. Fig. 3 depicts the XRD pattern of phytosynthesized ZnO NPs. The observed Bragg peaks have been indexed according to the standard JCPDS file for ZnO (JCPDS card no 36–1451) [36].

5.4. Scanning electron microscopy (SEM)

The SEM images disclosed that the ZnO NPs displayed a mostly quasi-spherical to hexagonal morphology with little aggregation, a typical finding associated with green synthesis because of the presence of organic capping agents. The distribution of the particles was quite uniform over the field. SEM pictures of the photosynthesised ZnO NPs are shown in Fig. 4.

5.5. Zeta potential analysis

The findings indicated a surface charge of −27.6 mV, demonstrating moderate stability of the ZnO NPs in aqueous solution as assessed by the zeta potential method. The negative charge results in electrostatic repulsion among the particles, leading to less aggregation, so confirming the colloidal stability conferred by the capping phytochemicals. Fig. 5 represents the Zeta potential of zinc oxide nanoparticles synthesised from rice bagasse.

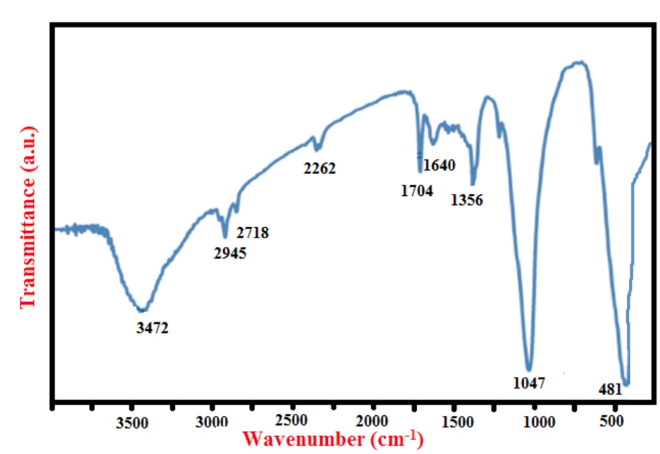

Fig. 2. FT-IR of phytosynthesized ZnO NPs.

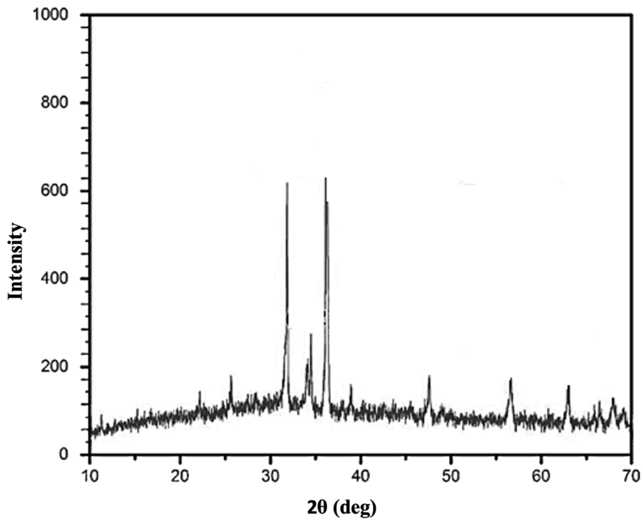

Fig. 3. XRD pattern of phytosynthesized ZnO NPs.

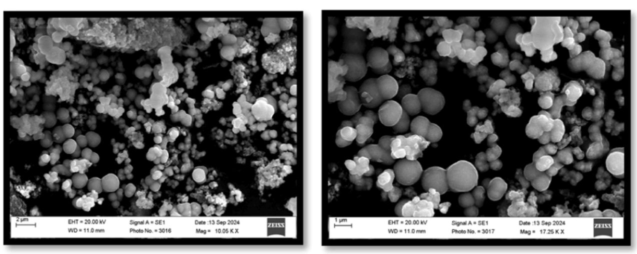

Fig. 4. SEM images of the photosynthesized ZnO NPs.

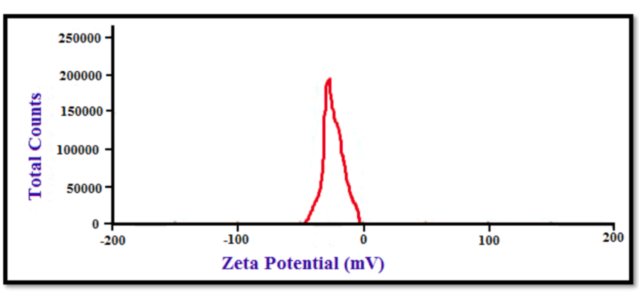

Fig. 5. Zeta potential of zinc oxide nanoparticles synthesised from rice bagasse.

5.6. Anti-Skin cancer activity / cytotoxicity assay

The biosynthesised ZnO NPs were evaluated for their cytotoxic effects on human skin cancer cells (A375 melanoma cell line) using the MTT assay technique, which represented a potent dependenton dosage reduction in cell viability [37]. The cells subjected to varying doses of ZnO NPs (10 to 1000 μg/mL) exhibited increased cytotoxicity, with the lowest viability recorded at concentrations above 50 μg/mL. The IC_{50}, defined as the concentration at which 50 % cell suppression occurs, was calculated to be around 415.46 μg/ml after 24 hours of incubation, indicating significant anti-proliferative effects of the nanoparticle. Morphological study using inverted optics revealed changes in the morphology of ZnO NPs treated cells, including shrinkage, cell dissociation, membrane blebbing, and rounding, indicative of apoptotic cell death. The untreated control cells seemed healthy and elongated. Furthermore, the augmented intracellular orange/red fluorescing nuclei were seen under the fluorescence microscope after cellular staining with Acridine Orange/Ethidium Bromide (AO/EB). Collectively, these findings demonstrated that ZnO NPs triggered apoptosis. The results suggest that oxidative stress, membrane rupture, and activation of the apoptotic pathway may constitute the harmful effects of ZnO NPs, maybe mediated via the surface of rice bagasse extract. In conclusion, the data demonstrate that ZnO NPs possess significant anti-skin cancer characteristics and should be considered a viable nature-derived nanotherapeutic treatment against melanoma. Fig. 6 provides the cell viability % efficiency of the ZnO NPs and Rice bagasse extract against cancerous cells (A-375).

5.7. Antimicrobial

The antimicrobial efficacy of phytosynthesized ZnO NPs against the pre-tested bacterial and fungal strains, with the impact contingent upon the concentration of the nanoparticle sample [38]. The most significant zone of inhibition was seen against *S. aureus* (21.4 ± 0.5 mm), followed by *P. aeruginosa* (17.2 ± 0.7 mm) and *C. albicans* (16.5 ± 0.4 mm) at the respective lower concentrations. The antibacterial efficacy of ZnO nanoparticles was validated by the Minimum Inhibitory Concentration (MIC) values obtained from the broth dilution experiment, which varied from 26 to 78 μg/mL depending on the microbial species. The MIC of *S. aureus* was the lowest at 26 μg/mL, indicating more susceptibility, whereas *C. albicans* exhibited a somewhat higher MIC of 78 μg/mL, suggesting some resistance. This discovery supports the prospective use of ZnO NPs as an effective natural antibacterial agent in both medicinal and environmental applications. Fig. 7 represents the zone of inhibition against bacterial stain, and Table 2 provides the minimum inhibitory concentration of ZnO Nanoparticles.

ZnO nanoparticles produce reactive oxygen species, including superoxide anions (O_2^-), hydroxyl radicals, and hydrogen peroxide (H_2O_2), particularly in the presence of light or in watery environments. These reactive oxygen species induce oxidative damage to bacterial cellular constituents, encompassing lipids, proteins, and nucleic acids. Reactive oxygen species disrupt the electron transport pathway, resulting in energy depletion and cellular apoptosis. ZnO nanoparticles engage with bacterial cell membranes by electrostatic attraction, mostly attributed to the negative zeta potential of the bacterial cell wall and the surface charges on ZnO. This interaction results in membrane destabilisation, enhanced permeability, and the leaking of intracellular constituents (e.g., ions, proteins, and nucleotides). Smaller nanoparticles (specifically 18–25 nm) possess an elevated surface area-to-volume ratio, facilitating enhanced membrane interaction and potential damage [39].

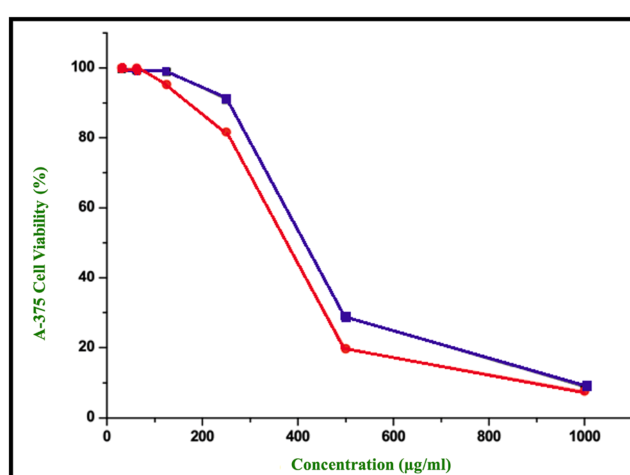

Fig. 6. Cell viability % efficiency of the ZnO NPs and Rice bagasse extract against cancerous cells (A-375).

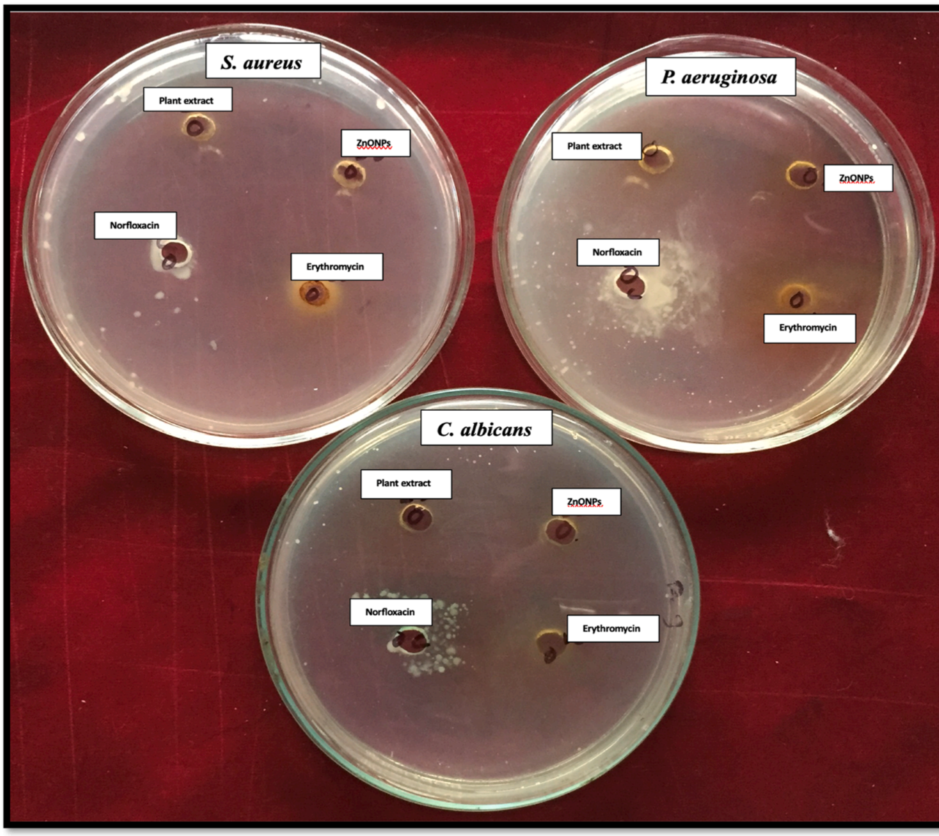

Fig. 7. Zone of inhibition against bacterial stain.

Table 2
Antimicrobial activity of ZnO nanoparticles.

Microorganism	Type	Zone of Inhibition (mm) at 100 μg/mL	MIC (μg/mL)
S. aureus	Gram-positive	21.4 ± 0.5	26
P. aeruginosa	Gram-negative	17.2 ± 0.7	34
C. albicans	Fungus	16.5 ± 0.4	78

5.8. Antioxidant activity

Green synthesised ZnO NPs were evaluated for their antioxidant potential using the DPPH radical scavenging assay, with increasing concentration [40]. The percentage of DPPH inhibition considerably increased with the quantity of ZnO NPs, namely from 10 to 100 μg/mL. At the maximum dosage (100, 200, and 300 for ZnO NPs and ascorbic acid, respectively), a peak scavenging activity of 76.3 % and 51.2 % was recorded, in contrast to 89.4 % and 10 % for the conventional antioxidant, ascorbic acid. The IC_{50} concentration of ZnO NPs, which scavenged 50 % of the DPPH radical, was around 43.7 ± 1.6 μg/mL, indicating significant antioxidant properties. Phytochemicals extracted

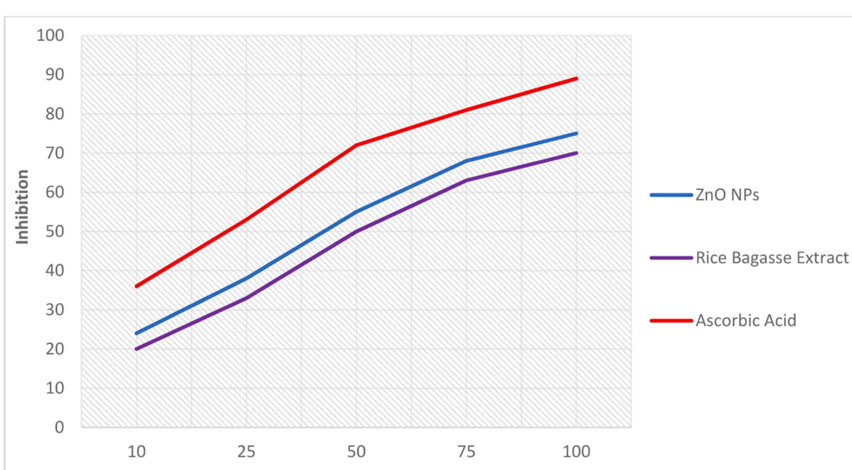

Fig. 8. DPPH Radical scavenging activity of photosynthesized ZnO NPs.

from rice bagasse have been identified to act as electron donors on the surface of ZnO NPs, neutralising free radicals and producing an antioxidant effect. The research suggests that synthesised ZnO NPs may serve as prominent antioxidants by significantly scavenging free radicals. Fig. 8 represents the DPPH radical scavenging activity of photosynthesized ZnO NPs.

Rice bagasse, a by-product derived from juice extraction of rice stalks or residues from parboiled rice milling, constitutes a significantly underutilised agro-industrial lignocellulosic waste. In contrast to more often studied biomass materials like banana peel, sugarcane bagasse, or neem leaves, rice bagasse has garnered very little focus in nanoparticle synthesis, notwithstanding its abundant phytochemical composition and widespread availability in rice-producing areas [41]. Rice bagasse is chemically comprised of substantial quantities of cellulose, hemicellulose, and lignin, in addition to polyphenols, flavonoids, and reducing sugars, which function as natural reducing, stabilising, and capping agents in the green production of metal nanoparticles. Research has shown that the antioxidant capacity of rice-derived by-products is significant owing to the presence of ferulic acid, coumaric acid, vanillic acid, and various other phenolic compounds. These chemicals not only enable the effective reduction of metal ions but may also confer bioactivity to the synthesised nanoparticles. The elevated silica content in rice bagasse differentiates it from other plant residues, potentially affecting the physicochemical properties of the ZnO nanoparticles, including particle size distribution, crystallinity, and surface functioning. From a sustainability standpoint, rice bagasse is plentiful in rice-producing nations and is generally disposed of or burnt, exacerbating environmental degradation. Employing this biomass for nanoparticle synthesis provides a value-added use while promoting waste reduction, green chemistry, and circular economy concepts. The selection of rice bagasse is warranted due to its chemical richness, reduction potential, innovative application, regional availability, and environmental importance, rendering it a desirable and unique candidate for the green synthesis of zinc oxide nanoparticles [42].

By reducing zinc ions (Zn^{+}) and producing and stabilising ZnO nanocrystals with rice bagasse extract, eco-friendly ZnO nanoparticle synthesis occurs. Rice bagasse contains phytochemicals such polyphenols, flavonoids, reducing sugars, tannins, and lignin-derived phenolic acids, making this technique environmentally friendly. Ferulic acid, vanillic acid, and p-coumaric acid are phenolic compounds with hydroxyl and carboxylic functional groups in rice bagasse. Reducing sugars and other organic acids in the extract influences ZnO nanoparticle nucleation and development, impacting size and form [43]. These biomolecules preferentially bind to crystal facets, stabilising the crystalline phase and enabling anisotropic growth. Polyphenols and lignin derivatives adsorb onto nanoparticle surfaces as natural capping agents, preventing agglomeration and improving dispersion. In general, FTIR and UV–Vis spectroscopy confirm the interaction between surface ZnO and –OH, –COOH, and aromatic compounds from the extract. The eco-friendly production of ZnO nanoparticles using rice bagasse has economic and environmental benefits. Rice bagasse, a common byproduct of rice production, is often burned or dumped in landfills, causing environmental damage and biomass underutilisation. Cost-effective, high-value nanoparticle synthesis from this waste material supports sustainable development. Traditional nanoparticle synthesis requires expensive and harmful chemical reagents. The green method eliminates these. The use of aqueous rice bagasse extract as a reducing and capping agent reduces synthesis steps, energy use, and production costs. Under mild conditions (ambient temperature, atmospheric pressure), the synthesis method is highly scalable and ideal for resource-limited industrial applications [44].

6. Conclusion

This research concluded that manufacturing costs might be reduced while offering more economical and less harmful techniques for synthesising ZnO NPs via green synthesis using rice bagasse ethanolic extract. The biosynthesised ZnO nanoparticles were thoroughly characterised as crystalline, stable, mostly spherical, and of nanoscale dimensions. The phytochemicals in rice bagasse facilitated the reduction, capping, and stabilisation of the nanoparticles. To evaluate the efficacy of ZnO NPs in anti-skin cancer assays, biological experiments were performed, revealing that the nanoparticles demonstrate significant anti-skin cancer activity by effectively reducing the viability of A-375 skin cancer cells in a dose-dependent manner. Moreover, the ZnO NPs exhibited significant antibacterial potential against both bacteria as well as a fungal strain, demonstrating their broad-spectrum efficacy. The antioxidant ability of the ZnO NPs is notably strong, exhibiting pronounced DPPH radical scavenging activity superior to that of raw rice bagasse extract. These findings demonstrate the potential of rice bagasse-derived ZnO NPs as effective, multifunctional bio-nanomaterials with promising applications in the biomedical, pharmaceutical, and cosmetics sectors. Unoptimized versions may be subjected to additional investigation in future research focused on in vivo activity and distribution into established systems.

CRediT authorship contribution statement

Amrish Kumar: Writing – original draft, Methodology. **Amanpreet Kaur:** Investigation, Conceptualization. **Man Vir Singh:** Writing – review & editing, Validation. **Vinod:** Validation, Methodology. **Soniya Dhiman:** Writing – review & editing, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The authors are unable or have chosen not to specify which data has been used.

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