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NANOPARTICLES OF ZINGIBER OFFICINALE AS POTENTIAL NANOMEDICINE IN FOOD POISONING

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Abstract:

The present research focused on the utilization of traditional herbal pathway for the treatment of food poisoning by considering *Zingiber officinale* as a model drug, since herbal treatment could be more powerful for fast recovery with no harmful side effects. One of the most common health issues encountered during travelling is food poisoning in Asian countries including India, Nepal, Bangladesh and Pakistan which is mainly due to poor sanitation and lack of awareness about proper hygiene. Travelling through railways is more prone to food borne infections that normally results from ingestion of food contaminated by bacteria or toxin produced by bacterial growth. As a preservative measures, there is a need of a broad spectrum antibacterial drugs for bacterial growth inhibition as well as for initiation of strong immune response against bacterial infections. For this purpose, *Zingiber officinale* derived nanoparticles were examined against species of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella abony* that are mainly responsible for food poisoning in humans. The particles were characterised by different analytical techniques. The results were interpreted on the basis of minimum inhibitory concentration and agar well diffusion processes.

Keywords: *Zingiber officinale*; Food poisoning; Nanoparticles; Bacterial infections; Antibacterial drug

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1. Introduction

One of the most common problems that arise while travelling is the food poisoning **[1]**. The reason behind it is the lack of fresh supply of food material. Many times it has been observed that due to ingestion of packed food or previously stored drinks, they get contaminated easily by bacterial growth. The most commonly reported bacterial species like *Salmonella abony or Escherichia coli* cause food poisoning by eating raw or uncooked foods like sprouts, or previously chopped salads, chicken and eggs **[2]**; *Bacillus cereus* causes food poisoning by eating poorly cooked food like meat or mutton **[3]**; *Staphylococcus aureus* causes food poisoning by eating food like unpasteurized milk, contaminated by some toxin **[4]** whereas *Klebsiella pneumoniae* mainly causes food poisoning by eating contaminated pork, fish **[5]**, poultry products and street foods **[6]**. The most visible symptoms of food poisoning are diarrhea, fever, vomiting, dehydration and nausea.

Herbal treatment has gained importance in our modern clinical science; from last so many decade herbal medicines have overtaken the use of synthetic drugs **[7, 8]**. Every single part of a plant such as barks, roots, rhizomes, leaves, buds and even stem have shown their vital role to cure food poisoning that may be caused by any of the microorganisms such as virus, bacteria and fungus **[9]**. These food borne infections can be cured by drugs like sulphonamides, trimethoprim, nitrofurantoin and many more, but due to their adverse side effects such as diarrhoea, fever, nausea, skin rashes, itching etc. they are now being replaced by herbal medicines **[10-12]**.

Herbal treatment includes use of plant extracts containing vital biologically active components. One of the most frequently used herbal plants is ginger (*Zingiber officinale*) which is a very common spice around Asian countries including India, Nepal, China and Japan. From ancient time, it has been used as a natural antibiotic to control bacterial and viral infections, common cold, cough, nausea, headache, digestion, fatigue and pain. It is also added in processed food to add flavour and in drinks like tea as a refreshing agent in countries like India. It boosts up the immune system of the patient to fight against the disease for fast recovery. It also enhances the self-healing capacity in the patient. Ginger can work as an effective therapeutic agent if taken in a small quantity, since its excess consumption may lead stomach burning, heartburn, throat irritation and may also cause skin irritation. Hence, it is necessary to evaluate its minimum amount (mg/mL) that can show active therapeutic effects on infected areas. In the present study, the capacity of ginger to act as an antibacterial agent has been evaluated by inhibiting bacterial growth when applied in very lesser quantity.

2. Materials and methods

2.1. Materials

For the synthesis of nanoparticles, the fresh rhizomes of *Zingiber officinale* were collected from local market. The bacterial culture used for the experiments were *Escherichia coli* (E. coli) NCIM 2256, *Staphylococcus aureus* (S. aureus) NCIM 2079, *Bacillus cereus* (B. cereus) NCIM 2217,

Klebsiella pneumonia (K. pneumonia) NCIM 2957 and *Salmonella abony* (S. abony) NCIM 2257. Media used during the experiments were nutrient agar media, Muller Hinton agar, Tryptone soya broth, Muller Hinton broth all were purchased from HiMedia laboratories private limited, India.

2.2. Methods

2.2.1. Identification of *Zingiber officinale*

The plant sample was identified with authentication as *Zingiber officinale* Roscoe by State Forest Research Institution (SFRI), Jabalpur, India. The form of plant used for identification was rhizome. The method of authentication was herb soft developed by SFRI.

2.2.2. Preparation of Nanoparticles

The fresh rhizomes of *Zingiber officinale* (250 g) were dipped in deionised sterile water for around 30 minutes in order to remove all the unwanted mud and impurities and then they were peeled off and extracted to get the fresh juice using an electronic juicer **[13, 14]**. The juice was stored in refrigerator for 2 h in order to settle down all the fine particles. Then the juice was centrifuged using a Metzer optic table Centrifuge Modelm Melz-4044 at 3000 rpm (revolutions per minute) for 45 minutes. After centrifugation the obtained supernatant liquid was removed through filtration in order to eliminate fibrous residue and fine particles settle at the bottom of the centrifuge tubes were collected for thin layer chromatography (TLC). The compounds isolated from TLC plates were washed with HPLC grade acetonitrile and dried using Buckner Rotavapour at RT. Particles were collected and dried at a low temperature (4 ^{0}C) **[15]** since the low temperature might preserve the medicinal properties of ginger (Zingiber officinale). The particles were collected inside air tight poly (ethylene) packets for further studies.

3. Analysis

3.1. ^{1}H Nuclear Magnetic Resonance (^{1}H NMR)

For quality assessment of the TLC treated ginger compounds, the samples were indentified spetrophotometrically using Bruker NMR spectrometer (500 MHz) by IISER Bhopal (M.P).

3.2. Scanning Electron Microscopy (SEM)

The surface morphological information of the prepared nanoparticles was sought using Scanning Electron Microscopy (SEM) Quanta 200 (Field Electron and Ion Company) provided by IIITDM Jabalpur (M.P), India. High magnification images were obtained at an accelerating voltage of 5.00 kV.

3.3. Transmission electron microscopy (TEM)

TEM analysis was performed to confirm the average particle size ginger particles using microscopic images provided by Transmission electron microscope of 200 kV in the UGC-DAE Consortium for Scientific Research, Indore (M.P), India.

3.4. Thermo gravimetric analysis (TGA)

TGA characterisations were performed to measure the thermal stability. The analysis was conducted using a Shimadzu Thermo-gravimetric analyser model no. 50 and the facility was provided by Dr. APJ Abdul Kalam Central Instrumentation Facility at Jiwaji University, Gwalior (M.P) India. The experiments were performed in nitrogen gas environment with a flow rate of 100 milli litre/minute.

4. Experimental

4.1. Collection of Micro-organisms

The bacteria E. coli, S. aureus, B. cereus, K. pneumoniae and S. abony used for the antibacterial experiments were procured from ATCC (American Type Culture Collection) through Microbiologics® USA. These bacteria were collected from Excellent Bio Research Solutions Pvt. Ltd., a NABL and FSSAI (Government of India) accredited laboratory, Jabalpur, India.

4.2. Preparation of Media

During the study, the pure cultures were maintained in nutrient agar media. Media was prepared by suspending 28.0 g dehydrated powder in 1 L double distilled water and the whole content was heated to boiling in order to dissolve the medium completely. The content was dispensed as required and sterilized by autoclaving at 15 lbs pressure and 121 ^{0}C temperature for 15 minutes. The suspension was mixed well before using **[16]**.

4.3. Preparation of Inoculums

The nanoparticles of ginger were screened for antibacterial activities against the selected bacterial colonies. The agar well diffusion method was followed during the experiments to determine zone of inhibition, based on Bauer-Kirby method **[17, 18]**.

The yield from the bacterial suspension was about 10^4-10^5 cells/mL which was further used to determine the minimum inhibitory concentration (MIC) of the nanoparticles.

4.4. Preparation of Antibacterial Drug Extracts

Powdered samples were added to a sterile conical flasks containing hot double distilled water and the resultant mixture content were stirred for 30 min using a magnetic stirrer plate. The obtained extract was used as stock samples which were further diluted for the determination of MIC.

4.5. Determination of Minimum Inhibitory Concentration (MIC)

The lowest concentration of the synthesized antibacterial drugs that can work effectively to inhibit the growth of the pathogen is coined as MIC. The experiment was performed using the Muller Hinton agar **[19]**. The experiment for negative controls was also performed in order to check the presence or absence of the pathogens, so that false results could be eliminated.

4.6. Preparation of Agar Plates

For the antibacterial susceptibility experiments the agar plates were was prepared using Muller Hinton agar. The prepared media plates were stored at 12 to 15 ^{0}C in order to protect the media from moisture.

4.7. Preparation of Inoculated Plates

The selected test organisms were used as inoculums were spread over the entire surface of agar Petri plates using the streak plate technique and the process was repeated thrice. All the inoculated plates were then left undisturbed and allowed to dry for 10 to 15 min at ambient temperature **[20]**.

4.8. Antibiotic Susceptibility Test

The agar well diffusion method was used to conduct the antibacterial sensitivity test against each test organism. The experiment was conducted and after 48 hours the results were expressed in the form of clear zone around each well which were termed as zone of inhibitions. Presence of zones on the plates indicated the effectiveness of the antibacterial drug against pathogens which was measured in millimetres (mm) using a zone measurement scale (HiMedia, India).

Positive control was also set up using gentamycin disc of 10 µg which was incubated at 37 ± 1°C for 48 h. Media control was set using Mueller Hinton agar plate which was also incubated under similar conditions.

5. Results

5.1. Analysis

5.1.1. ^{1}H NMR

^{1}H NMR of the ginger particles is depicted in **Fig. 1**. The chemical structures are predicted by focusing on the values of chemical shifts. With the help of provided spectrum, it is clear that ginger is a polyphenol derivative which consists of phenolic compounds like gingerol which may get converted into paradol, shogaol, zingerone and in lesser extent terpene derived zingiberene and phellandrene are also recognized in the spectrum **[21, 22]**.

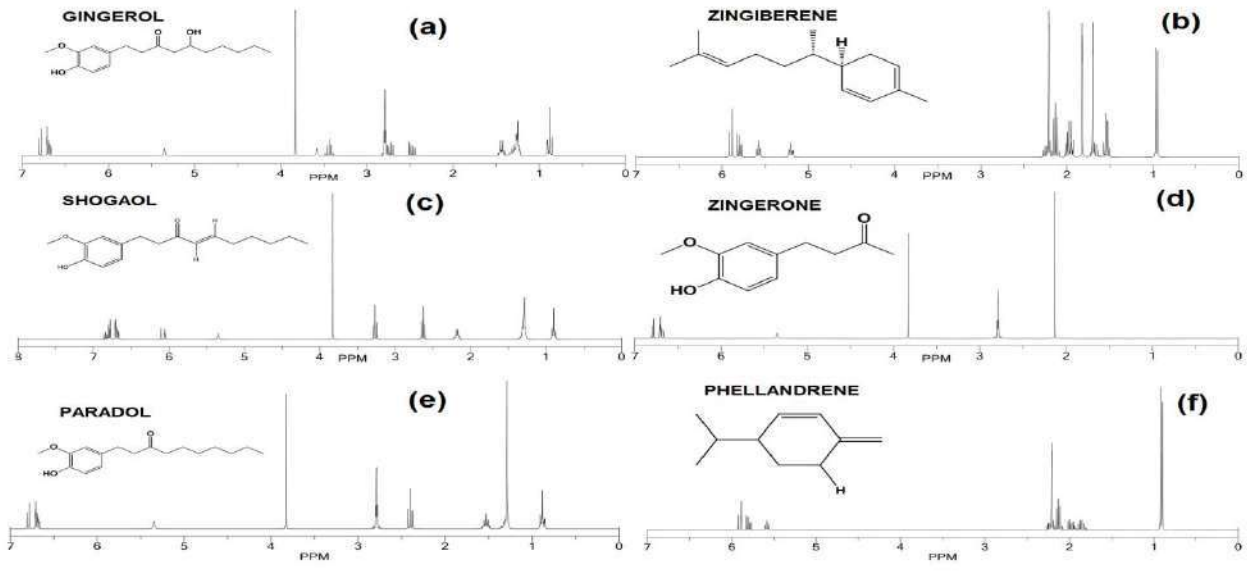

Figure 1 The ^{1}H NMR of some of the ginger based biologically active chemical compounds identified from fresh ginger extract.

5.1.2. SEM

The results of SEM analysis are depicted in **Fig. 2**. The analysis has provided the surface morphological details that states that the ginger particles had uniform, smooth and spherical boundaries. In figure, particles are irregularly distributed throughout the image and are appearing to be beyond the nano-scale which is regarded due to the association of the nanoparticles during sample preparation for the analysis.

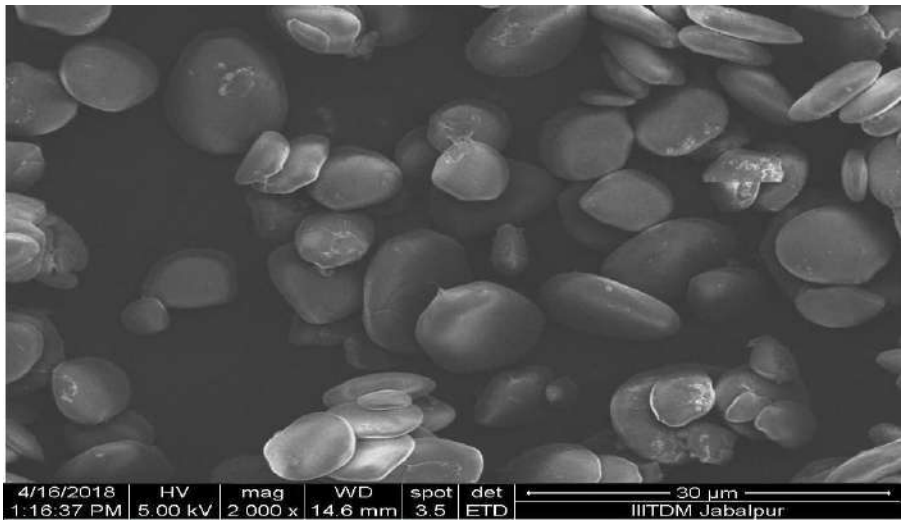

Figure 2 SEM image for ginger nanoparticles

5.1.3. TEM

TEM analysis has predicted the average particle size as 30 nm as shown in **Fig. 3**. The analysis reported that the ginger particle falls under the range of nano scale; therefore it can be termed as nanoparticle.

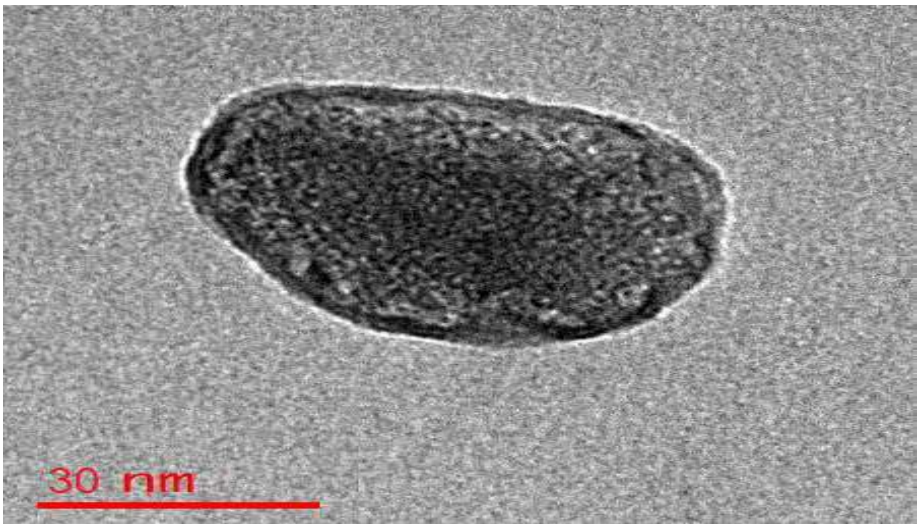

Figure 3 TEM image for ginger nanoparticles

5.1.4. TGA

Result of TGA for ginger nanoparticles is depicted in **Fig. 4**, which revealed that particles can resist temperature up to 50±5 ^{0}C and the further increase in temperature causes a weight loss resulting in decomposition of the nanomaterial. Hence the nanoparticles are capable to withstand human body temperature which is just 37±2 ^{0}C and can be used against food poisoning.

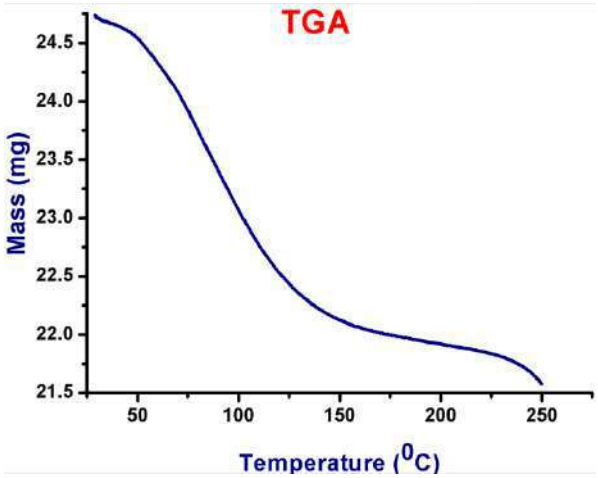

Figure 4 TGA report for ginger nanoparticles

5.2. Minimum Inhibitory Concentration

The results of MIC of the extracts which were incubated with Muller Hinton broth and cultures of *E. coli*, *S. aureus*, *B. cereus*, *K. pneumoniae* and *S. abony* in different sterile test tubes are summarized in **Table 1**. The presence of bacterial growth was signified by the presence of turbidity in the incubated tubes. The incubated tube with lowest drug concentration that completely inhibited the bacterial growth by showing no turbidity was considered to be the MIC for that specific test organism.

Results of MIC play an important role in determining the antibiotic doses for the susceptibility test.

Table 1. Results of MIC of various concentration of ginger extract

Bacteria	**Zone size in mm**					
	Stock	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
E. coli	25	21	18	13	-	-
S. aureus	26	22	16	10	-	-
B. cereus	23	18	14	-	-	-
K. pneumonia	25	22	14	-	-	-
S. abony	26	23	17	-	-	-

5.3. Antibiotic Sensitivity Test

The antibiotic sensitivity tests were conducted to determine the antibacterial properties of ginger nanoparticles toward positive and negative strains of test organisms. After 48 h of incubation period, the results were obtained, showing clear zone of inhibitions for different concentration of test extracts. The obtained results were demonstrated in form of images as shown in **Fig. 5**.

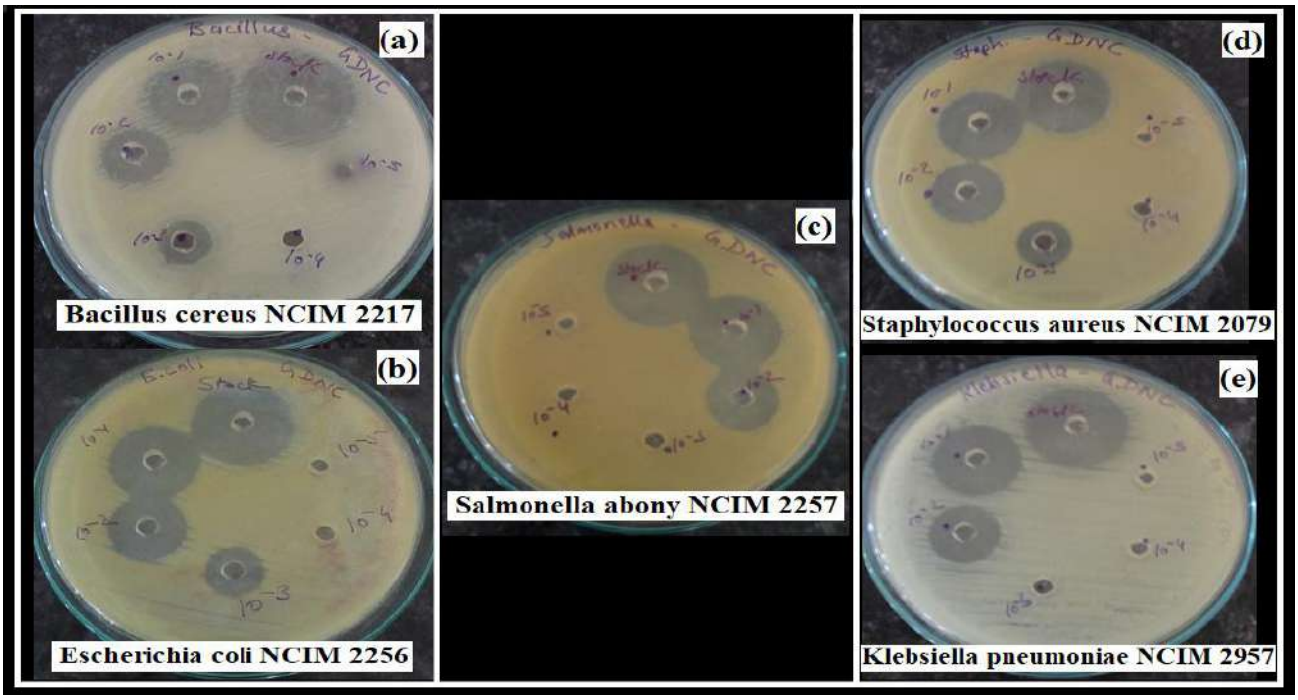

Figure 5 Antibacterial sensitivity test of ginger extract for **(a)** *Bacillus cereus* **(b)** *Escherichia coli* **(c)** *Salmonella abony* **(d)** *Staphylococcus aureus* and **(e)** *Klebsiella pneumoniae*

6. Discussion

In the present study, the synthesis of ginger nanoparticles was very simple and cost effective. Nanotechnology is emerging as an excellent technique to produce naturally derived bactericidal agents. Ginger nanoparticles were found to be in nano range that provided larger surface area for the antibacterial activities. Due to the larger surface area, the nanoparticles can closely and greatly interact with micro-organisms. During the course of interaction, nanoparticles raptured the bacterial cell membrane made up of peptidoglycon that caused leakage of cytoplasmic constituents resulting in death of the bacterial cell. In such a way, they inhibit bacterial growth and a clear zone of inhibition is obtained during the experiments.

The antibacterial susceptibility experiments were conducted on various concentrations of ginger extracts against food poisoning pathogens such as *B. cereus*, *E. coli*, *S. abony*, *S. aureus* and *K. pneumoniae*. Results were concluded based on MIC and agar diffusion experiments which state that all the test cultures were susceptible to the ginger extracts. Hence the non-toxic and biodegradable

ginger derived nanoparticles can be used in medical practices for the treatment of food poisoning in human.

7. Conclusions

The results of ^{1}H NMR, SEM, TEM and TGA well characterize the prepared nanomaterials. Ginger nanoparticles can be easily obtained by simple extraction method. Particles are polysaccharide based biologically active nanoparticles and therefore can be considered for herbal medications. In this research work, the largest inhibition zones were obtained against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative). The obtained results for antibacterial susceptibility test for nanoparticles confirmed its promising antibacterial activities against both gram positive and gram negative bacterial colonies and, therefore, can be considered as effective bactericidal drug for controlling and curing food poisoning.

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Conflicts of Interest

The authors do not have any conflicts of interest.

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