
ORIGINAL ARTICLE

Synthesis of Black cardamom & Black pepper mediated Zinc oxide Nanoparticles and Their Antibacterial activities

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ABSTRACT

In the present work, black cardamom (*Amomum subulatum*) and black pepper (*Piper nigrum*) extract mediated zinc oxide nanoparticles have been synthesized. These nanoparticles are characterized by ultraviolet-visible spectroscopy and dynamic light scattering (DLS). The size of the synthesized zinc oxide nanoparticles found between 10-40 nm. These nanoparticles show a very good bacteriocidal influence on *E. coli* according to concentration of these nanoparticles used. In this work three different concentration of zinc nanoparticles were used to evaluate antibacterial activities.

Keywords: Zinc oxide, Phytosynthesis, spice extract, UV-Vis. spectroscopy, Antibacterial

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INTRODUCTION

As the biologically synthesised nanoparticles are eco-friendly and have remarkable properties over bulk materials, this field is attracting more attention of researchers [1, 2]. Biological methods are more beneficial than chemical and other methods [3]. In these biological methods plant extracts work as reducing as well as capping agents for the synthesis of nanoparticles [4, 5].

Plant preparations are the best choice now-a-days to use as medicine for various diseases as they are safe and more effective than allopathic because having no side effects [6]. Spices i.e. plant products are used in many medicinal applications and also used to impart aroma and taste to the dishes.

From ancient time black pepper has been used as all purpose medicine by general population [7, 8]. The alkaloids like 1-peperoyl piperidine are responsible for its pharmacological properties by obstructing various metabolising enzymes [9]. According to Ayurveda, the pungency and heating properties of black pepper help in metabolizing food and its heat works as a stimulant like lighting a fire [10].

Black cardamom (*Amomum subulatum*) is a very famous spice in India and other countries also. It is derived from seed pods of the black cardamom plant of Zingiberaceae family. Black cardamom is commonly known as "Bari ilaichi". This plant is very commonly used in Ayurvedic medicine [11] to treat various diseases like gastric ulcers. This plant has ability to enhance gastrointestinal health, guard the respiratory system, improve the immune system and also inhibit various cancers. It also works as an expectorant and prevent from cough, cold and sore throat etc. by relieving the mucous membrane and normalizing the flow of mucous through the respiratory tract [12].

Zinc oxide nanoparticles (ZnO NPs) are environment friendly, non toxic and biocompatible. ZnO is assimilated into the packaging of the food materials proposing preservative properties. ZnO nanoparticles reported for their antimicrobial potential. They are active against gram-positive as well as gram-negative bacteria and also show extensive activity against more resistant bacterial spores. As per the knowledge of author, this is the first study reporting comparison of antibacterial activities of zinc oxide nanoparticles synthesised from black cardamom and black pepper.

MATERIAL AND METHODS

Collection of the material

The black pepper and black cardamom were collected from local market of Mathura city, India. All the chemicals used were of AR grade, purchased from CDH and used without further purification.

Preparation of Plant extracts

The samples were thoroughly washed with triple distilled water. 25 gram of black pepper and 25 gram of black cardamom were crushed and boiled in 250ml of triple distilled water for 20 minutes. These both solutions were filtered with the Whatmann filter paper and stored at 4°C until further use for present investigation.

Synthesis and Characterization of Zinc oxide Nanoparticles

The 100 ml of extract of black pepper or black cardamom was added to the 200 ml of 0.1M zinc chloride solution drop wise with stirring. Then 100 ml of 1M NaOH solution was added with stirring at room temperature. This mixture is left overnight, then filter, wash and dry to obtain zinc oxide nanoparticles. The synthesis of nanoparticles was confirmed by the change in color as shown in Figure 1 & 2. The surface plasma resonance (SPR) vibration is responsible for this color change.



(a)



(b)

Figure 1: Zinc oxide nanoparticles with black pepper (a) before synthesis (b) after synthesis



(a)



(b)

Figure 2: Zinc oxide nanoparticles with black cardamom (a) before synthesis (b) after synthesis

Preparation of Media

The media was prepared as per the guidelines given in Bacteriology Manual(13). All the dry ingredients given in the manual were taken in beaker and dissolved in distilled water. The so prepared medium was sterilized by keeping this in autoclave at 121°C for 30 minutes. 15 ml of this medium was poured in petri plate and this plate was incubated for 24 - 48 hours at 37°C.

Composition

peptone - 0.5%
NaCl - 0.5%
agar - 1.5%
beef extract - 0.3%

distilled water
pH - (6.8) at room temperature.

Collection of Bacteria

Both the bacteria were procured from CSIR-IMTECH, Chandigarh

Characterization

Initially, the synthesis of ZnO-NPs was confirmed by color change of solution and by absorption spectrum produced by UV-Vis spectrophotometer at 200-700 nm wavelength. Dynamic light scattering (DLS) was employed to determine the size using Zetasizer Nano ZS (Malvern Instruments, UK). All the analysis was carried out in an automatic mode.

Antibacterial assay

Antibacterial activity of black pepper and black cardamom mediated nanoparticles was tested by the disc diffusion method against gram-negative *E. coli* bacterial culture, prepared by the standard process. Before the use, petri plates and media were autoclaved. 10 µL of pure bacterial culture was uniformly spread on nutrient agar media in petri plates using L-rod. 10 µL of each sample with different concentration of NPs was poured on a sterile disc. Three sterile discs of different concentration were placed on the bacterial culture in both petri plates. These plates were incubated for 48 hours at 37°C. After 48 hours results were observed. The zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

Physicochemical characterization

UV-Visible absorption spectra of the ZnO particles synthesized from the black pepper and black cardamom are presented in Fig.3. Both the samples exhibit strong UV absorption spectra with the absorption peak ranging from 320 to 350 nm due to its surface Plasmon resonance. The position and width of absorption peak depend upon the size, shape of metal nanoparticles and also upon the dielectric constant of the medium (14). In the spectra of spherical nanoparticles only a single SPR band is observed while in the anisotropic particles two or more SPR bands may observe depending on the shape of the particles. The number of SPR peaks would vary with the symmetry of the nanoparticle (15-17).

The free electrons are responsible to produce an SPR absorption band (18-21). These free electrons in metal nanoparticles jump freely between the conduction and valence band which are close to each other. The highest absorbance peak was observed at 320nm and 350 nm for black pepper and black cardamom mediated zinc oxide nanoparticles respectively (Figure 2). The size of the obtained zinc oxide nanoparticles was in the range of 10-40 nm (Figure 4).

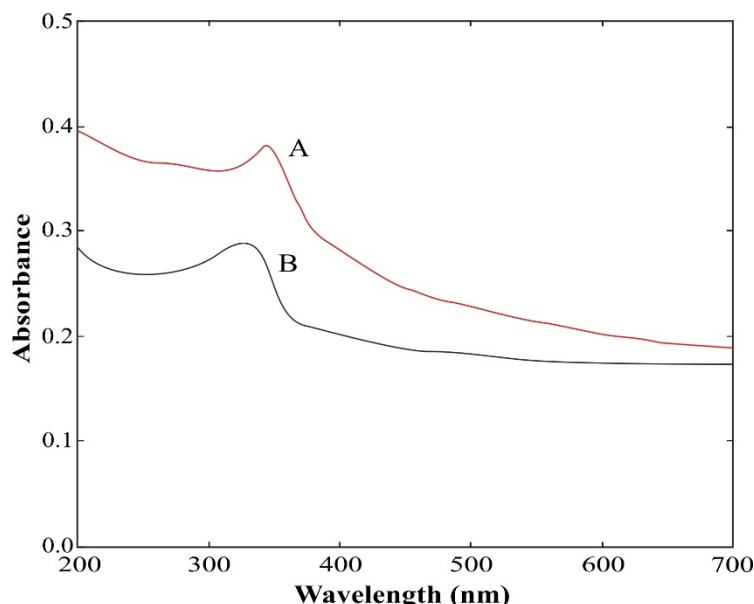


Figure3: UV-Vis spectra of Zinc oxide nanoparticles
(A) Black pepper extract mediated
(B) Black cardamom extract mediated

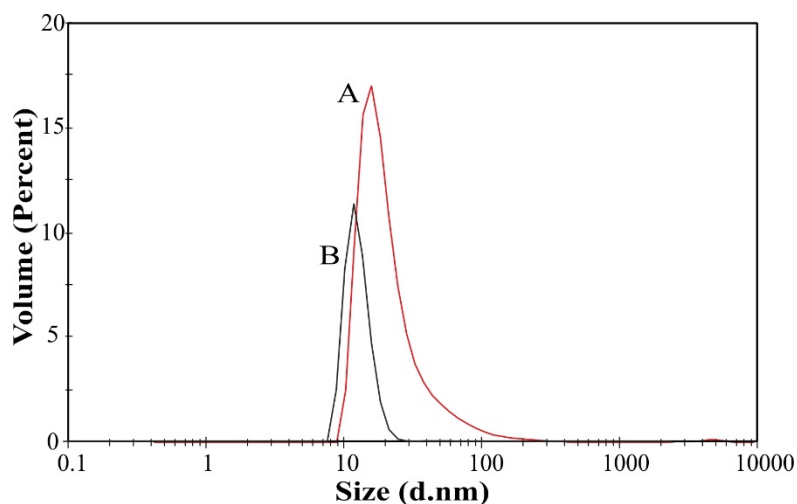


Figure 4: Size analysis of Zinc oxide nanoparticles
 (A) Black pepper extract mediated
 (B) Black cardamom extract mediated

Antibacterial Activities

Because of the enhanced surface area of nanoparticles than atomic size, nanoparticles can easily interact with bacterial cells. This is clear from the results shown in Fig.5 that zinc oxide NPs synthesized by the black cardamom extract are better bactericidal agents than black pepper extract nanoparticles. The zone of inhibition was measured in mm (Table 1). NPs have better antibacterial activity than the free metal ions.



Figure 5: Zone of inhibition against E.coli
 (a) Black cardamom (b) Black pepper

Table 1: Antibacterial Assay- Zone of inhibition (in mm)

S.NO	Different Conc. of Spice Extract (gm)	Zone of Inhibition By Zn Nanoparticles from Black pepper (mm)	Zone of Inhibition By Zn Nanoparticles from Black Cardamom (mm)
1.	1	12	13
2.	5	13	15
3.	10	15	17

CONCLUSION

The green synthesis of nanoparticles has been successfully carried out using the extract of *black pepper* and *black cardamom*. The size of the obtained zinc oxide nanoparticles was in the range of 10-40 nm. The phytochemicals present in *black pepper* and *black cardamom* extract are responsible for the bioreduction of metal ions into metal nanoparticles. Zinc oxide NPs synthesized by the black cardamom extract are better bactericidal agents than black pepper extract nanoparticles against gram-ve bacteria *E.coli*. Thus green approach of synthesizing nanoparticles can be utilized in antibacterial action.

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