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Zinc based Indian traditional drug (Yashad Bhasma): Preparation, characterization and its bacterial response

Richa Bhargava[1]*, Naresh Kumar[1], Ranu K.Dutta[2], Avinash C.Pandey[2], N.K.Singh[3], Sanjeev Kumar[4]

[1]Department of Physics, Motilal Nehru National Institute of Technology, Allahabad, (INDIA)

[2]Nanotechology Application Center, University of Allahabad, Allahabad, (INDIA)

[3]Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad, (INDIA)

[4]Shah-Schulman Centre for Surface Science and Nanotechnology, Dharmsinh Desai University, Nadiad, (INDIA)

E-mail : bhargavaricha28@gmail.com

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ABSTRACT

We report on the preparation of Zinc based 'Yashad Bhasma' that involves purification of pure Zinc metal using herbal juices followed by detoxification and calcinations at high temperature (~ 900°C). The structural, optical and elemental properties of the Yashad Bhasma have been investigated. The structural study of Yashad Bhasma revealed the existence of single phase ZnO with Hexagonal wurzite structure. Fourier transform- infra red (FT-IR), Photoluminescence (PL), Raman Spectroscopy, and Energy dispersive X-Ray analysis (EDX), confirms the presence of only ZnO without any other impurity in these Bhasmas. We also report on the effect of Yashad bhasma on the growth of *Escherichia coli* (*E. Coli*) DH5α and *Listeria monocytogenes (L. monocytogenes)* bacteria. Preliminary results show that Yashad Bhasma is less toxic as in comparison to the earlier studied ZnO Nanoparticles. Even at very high concentration i.e. 4500 μg/ml, the inhibitory effect is very mild. *In vitro* study suggests that the particles of Yashad Bhasma are not toxic to the microorganism present in the environment and the growth pattern does not change in the presence of these particles.

KEYWORDS

Yashad Bhasma;
Zinc oxide;
XRD;
Raman;
Bacterial activity;
Growth rate.

INTRODUCTION

Bhasmas of various metals have been used very extensively in the Indian tradition as an 'Ayurvedic medicine' since last several centuries[1]. Bhasmas are basically mixed oxides converted from their metal counterparts. According to the Ayurveda, a unique herbo-mineral procedure may be adopted for the preparation of the Bhasmas[1-4]. This unique herbo-mineral preparation of Bhasmas involves different processes as such as Sodhana (Purification), Maran (detoxification), and Putta (Calcination). These processes are reported to be useful for the removal of the harmful contents of the metal and make these Bhasma suitable for the possible medical applications[1-3]. For example, the metal zinc plays the vital role in the preparation of nucleic acid and different proteins[4], while the zinc based Bhasma 'Yashad Bhasma' is commonly used for dysentery, diuretic, hypoglycemic and as an astringent agent[1,4]. Recently, Wadekar et al.[2,3] has studied copper based

'Tamra Bhasma' and tin based 'Vang Bhasma'. Detailed structural analysis of Vang Bhasma was carried by the authors and composition of Bhasma was reported as SnO_2 whereas, in case of Tamra Bhasma the chemical composition was CuO. The physicochemical characteristics of gold based 'Swarna Bhasma', along with the study of gold nanoparticles on the arthritis rat is investigated by C. L. Brown et al.[5]. They have reported that Swarna Bhasma can be used as an anti arthritis agent. Kumar et al.[4], on the other hand, reported a detailed study of the elemental analysis of various Bhasmas extracted from various metals (Fe, Zn, Hg, Au, Ag, etc.) of the various Indian Ayurveda companies. Elemental analysis of 'Yashad Bhasma', obtained from the various Ayurveda firms has also been studied by Bhagwat et al.[6]. Until now only physical and chemical studies on different Bhasmas have been reported by the researchers. In order to establish its therapeutic properties it is necessary to perform the biological activities of these Bhasma.

Although, in Ayurveda there are several tests such as Rekhapuran (filling the crevices of fingers), Nischandrikaran (the loss of metallic property of shine), Apunarbhava (non- revivability) and Varitar (Floating on water) to control over the quality of these bhasmas[1,2], the scientific acceptance of such tests are under consideration.

In this paper, we report on the preparation of Zinc based 'Yashad Bhasma' and study of their physical and compositional properties. The Bhasma was characterized by using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy dispersive X-Ray analysis (EDX), Fourier-transform infrared spectroscopy (FT-IR), Photoluminescence (PL) and Raman Spectrometer. Preliminary tests have been performed to see the effect of this Yashad Bhasma on the microorganism present in the environment. We have studied the effect of Yashad Bhasma on gram negative as well as gram positive bacteria such as *E. Coli* DH5α and *L. monocytogenes* respectively.

EXPERIMENTAL

In the preparation of Yashad Bhasma, chunks of Zinc metal (> 99.9% purity) undergo a repeated calcinations cycle at the red hot condition (~900°C) and followed by an intermediate immersion of these red hot chunks into the room temperature kept sesame oil, and buttermilk successively (repeated seven times). The product, thus obtained, which remains in the solid form is called as 'Shodhit Zinc' and the process is called as Shodhana. It was then rinsed with water and dried under sunlight. The product thus obtained, was mixed well with the powder of dried Neem leaves (botanical name: Azadirachta Indica) and potassium nitrate in the mass ratio of 10:4:1 with the help of an iron spoon. This process is called as Maran, and is said to be responsible for detoxification of Bhasma[1]. In this process, the solid form of the Shodhit zinc started converting into powder form. Immediately, after converting the entire solid of Shodhit zinc into powder, a reversed iron plate was kept over it and was heated until the iron pan becomes red hot. Thereafter, it was allowed to cool at room temperature naturally. In the product, thus obtained, juice of aloe barbadensis miller (juice is extracted from the leaves and the mass ratio of the leaves to product was taken as 1:5) was added and mixed until a homogeneous paste was obtained. The paste was then heated in the furnace (~900°C) for 11 times. The heat treatment of the paste is referred as Puttan or calcination. The furnace was then allowed to cool naturally up to room temperature. The sample obtained was subjected to Ayurvedic tests such as: Rekhapuran (filling the crevices of fingers, which shows a well- prepared sample was so fine that it got settled in between the finger lines), Nischandrikaran (the loss of metallic property of shine), Apunarbhava (non- revivability) and Varitar (Floating on water).

The X-Ray Diffraction analyses of these samples were carried out by using Rigaku D/Max 2200 diffractometer with CuKα radiation at λ= 1.5406 Å. Fourier transform infrared spectroscopy (FT-IR) absorption was measured using a model Shimadzu FTIR 8400S over the frequency range of 4000-400 cm^{-1}. The sample was excited by 514.5 nm Argon laser to record the Raman Spectra using Renishaw in Via micro- Raman spectrometer. Photoluminescence (PL) measurements were recorded at 300 K by using a 300 W xenon arc lamp as the emission source and a Hamamatsu photo multiplier along with a 1/4 m monochromator as the detecting system. The spectra were recorded with excitation at 275 nm radiation. Micro-

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structural and chemical analysis of the samples was done using field emission scanning electron microscope (FESEM - FEI Quanta 200) equipped with an Oxford Inca energy dispersive x-ray (EDX) detector.

For studying the bacterial activity of Yashad Bhasma, gram negative and gram positive bacteria named as *Escherichia coli (E. Coli)* DH5α and *Listeria monocytogenes* respectively were used. Both these bacteria were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. These bacteria were maintained in the Luria Bertani (LB) media at 37°C in an incubator for all experiments. Approximately, 10^3 Colony Forming Units (CFU) of both the bacterial strains were cultured on LB agar plates with and without Yashad Bhasma. To measure the growth rate of bacteria, growth curves were recorded. In order to record the growth curve, these bacteria were grown in 50 ml of LB medium in the presence of different concentrations *viz.* 2500, 3000, 3500, 4000, and 4500 μg/ml, of Yashad Bhasma. A control growth of bacteria along with the different concentrations was also recorded for the Yashad Bhasma. The growth rates and bacterial concentration were determined by measuring optical density (OD) *i.e.* absorbance in every one hour. Double beam UV-visible spectrometer (electronic corporation of India make, UV 5704SS) was used at 600 nm for recording OD. To maintain the quality of the data, the bacterial tests have been repeated three times. The statistical analysis was done using *paired t*-test using OriginPro 8 SRO software. Each of the experimental values was in comparison to the control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.001.

RESULTS AND DISCUSSIONS

Physical properties

(1) Structural analysis (XRD)

The structural properties of the Yashad Bhasma have been studied by XRD. Figure 1, shows the XRD pattern of Yashad Bhasma having five clear diffraction peaks. This clearly, reveals the polycrystalline nature of the Yahsad Bhasma. An attempt has been made to index these peaks with the different zinc oxides. These peaks could be identified as arising due to reflections

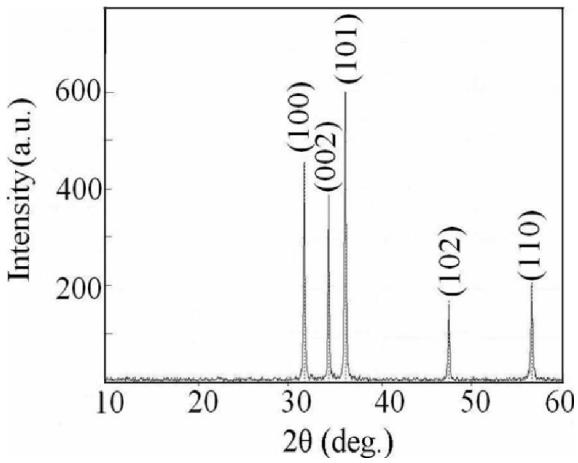

Figure 1 : XRD pattern of yashad bhasma prepared using ayurvedic procedure

from (100), (002), (101), (102) and (110) planes located at 2θ = 31.791, 34.460, 36.275, 47.578, and 56.637 respectively of wurtzite ZnO (JCPDS file no.890510)[7-10]. One may observe that races of other oxides of zinc were purely absent in this Yashad Bhasma. TABLE 1 depicts the d-values and corresponding (*hkl*) planes for the bulk ZnO and Yashad Bhasma. The lattice parameters 'a' and 'c' estimated using the data, have been presented in TABLE 2. It may be observed that the value of lattice parameters 'a' and 'c' of the Bhasma as determined above is close to the standard values of ZnO.

Optical properties

(1) Fourier transform-infrared spectroscopy

To investigate the organic and inorganic contaminations in the Yashad Bhasma, FT-IR spectroscopy was carried out. The FT-IR spectrum of Yashad Bhasma is shown in figure 2(a). In FT-IR transmittance spectrum of the Bhasma, a very strong and intense peak near 500 cm^{-1} has been observed, that corresponds to the Zn-O stretching mode for ZnO crystals[11,12], whereas the broad absorption peaks around 3400 cm^{-1} due to O-H stretching are observed. The peak due O-H bonds has been observed due to moisture present in the bhasma. Peaks corresponding to CO_2 and C=O near 2200 cm^{-1} and 1450 cm^{-1} respectively are also observed. Additional peak obtained around 1100 cm^{-1} could be identified as arising from O-O bond and the origin of this peak might be from the adsorbed oxygenates[2]. No other peak due to the organic impurity was observed in the spectrum.

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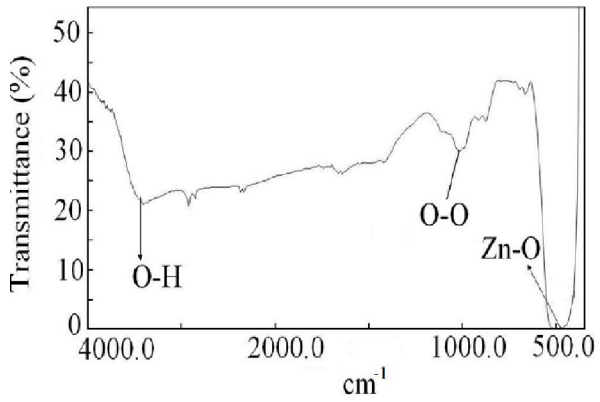
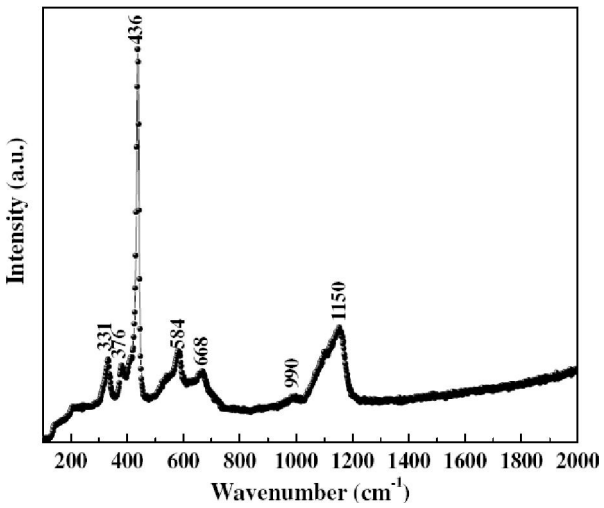


(a)



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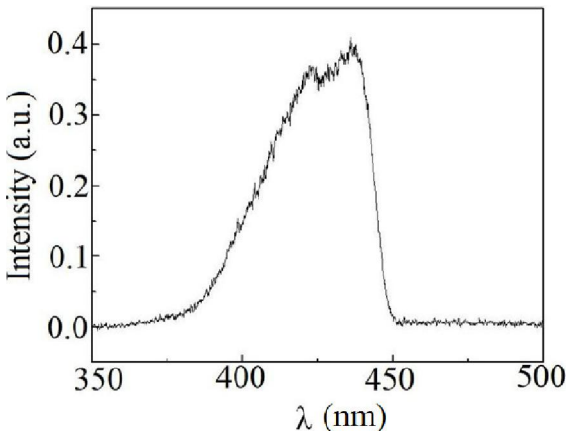
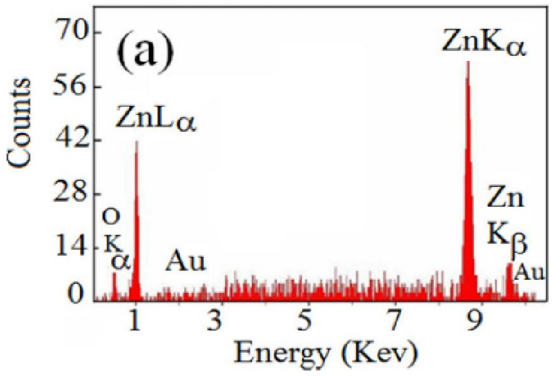


(c)

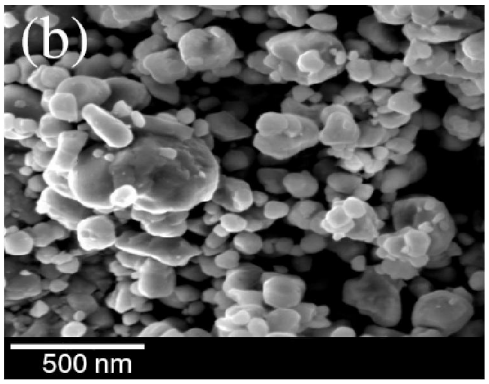
Figure 2 : (a) FT-IR spectra of yashad bhasma, (b) Raman spectra of Yashad Bhasma, (c) Photoluminescence of yashad bhasma

Raman spectroscopy

Bonding environment of the Yashad Bhasma was also studied by using Raman Spectroscopy. The Raman



(a)



(b)

Figure 3 : (a) Scanning electron micrograph of yashad bhasma.(Color on line), and (b) EDX of Yashad Bhasma

spectrum of Yashad Bhasma is shown in figure 2(b). A very strong peak at 435 cm^{-1} is seen to dominate the Raman spectra. The peak at ~437 cm^{-1} may be identified as E_{2H} mode of ZnO due to the high frequency phonons[8,9,13-17]. The broad and asymmetric nature of this peak is typical of the Raman active mode specially observed in wurtzite structure. A similar peak near or at 437 cm^{-1} has also been reported in the literature[13-17]. There are two peaks at 331 cm^{-1} and 379 cm^{-1} in the low wavelength number region. A broad band at 579 cm^{-1} with a shoulder at 538-540 cm^{-1} and a weak band at 652-665 cm^{-1} is also observed in the low wavelength number region. The 331 cm^{-1} peak arises due to the second order Raman due to the multiple scattering and corresponds to A_1 symmetry[8,13,17]. Raman active mode of vibrations observed at 579 cm^{-1} may be due to A_{1L}. The wave-number, symmetries of the modes found in Raman spectra of Yashad Bhasma is tabulated in TABLE 3.

Photoluminescence studies

Emission spectra of the Yashad Bhasma were stud-

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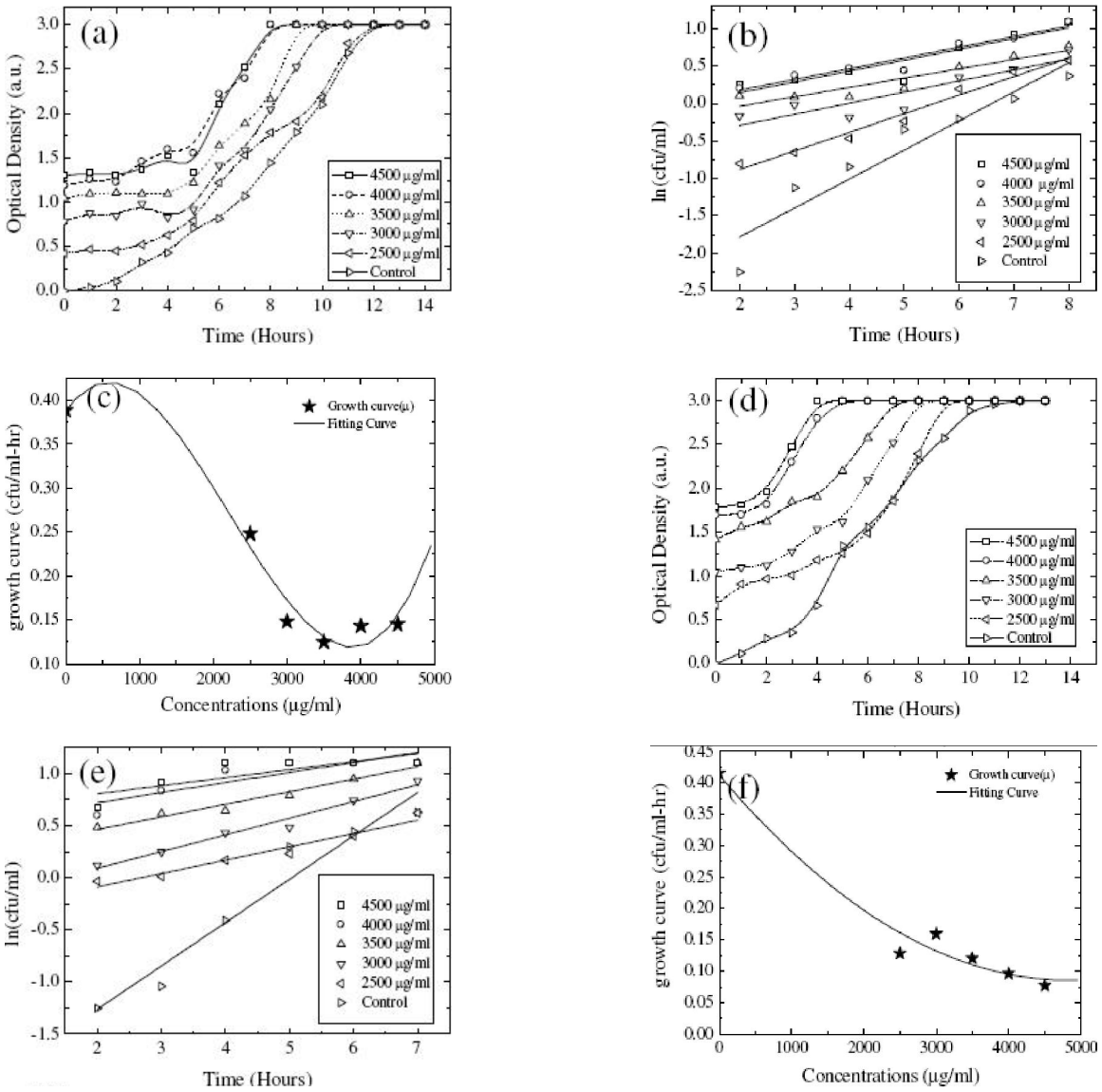

Figure 4 : (a) Growth curve of *E.coli*, (b) Logarithmic plot to calculate growth rate of *E.coli*, (c) Plot between growth rate and concentration for *E.coli*, (d) Growth curve of *L. Monocytogenes*, (e) Logarithmic plot to calculate growth rate of *L. Monocytogenes*, and (f) Plot between growth rate and concentration for *L. Monocytogenes*

ied to identify the surface and impurity levels of the Bhasma. The PL spectrum of Yashad Bhasma obtained is shown in figure 2(c). As is clear from PL spectra, there is an indication of near band edge emission. However, deep level emission in yellow and green range was observed ~430 nm. The origin of this emission may be due to the presence of structural defect and impurities[16,18,19]. The peak ~430 nm of Yashad Bhasma when deconvoluted, exhibited the presence of strong peaks at ~436 nm (2.84 eV) and at ~ 422 nm (~2.94 eV). The observed luminescence peak 422nm are due to transitions from zinc interstitial (Zn_i) while the peak at~2.84 eV are due to zinc acceptor defects located at 2.8 eV above the valance band. It is known that ZnO has a relatively open structure, with a hexagonal close packed lattice where Zn atoms occupy half of the tetrahedral sites and all the octahedral sites are empty, which are open for accommodating native defects. Zn interstitial and oxygen vacancies are known to be the predominant ionic defects with ionization energies varying from 0.05 to 2.8 eV[13].

Microscopic and elemental studies

(1) Field emission scanning electron microscopy (SEM)

Microstructural and chemical analysis of the

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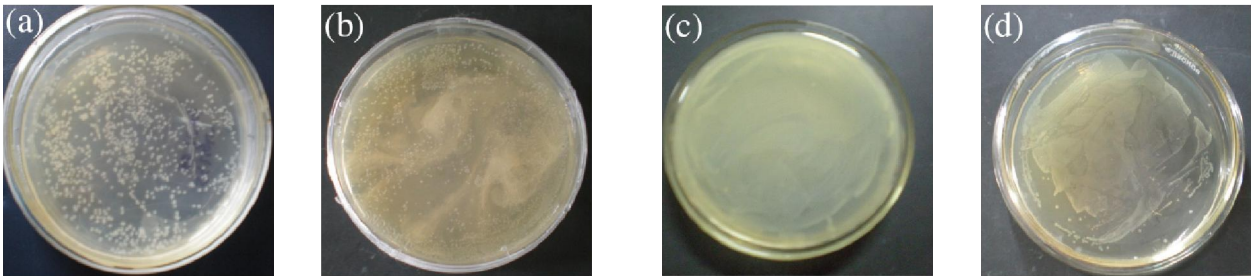

Figure 5 : (a) and (b) bacterial colonies of *E.coli* on agar plate for control (without Yashad Bhasma) and 4500mg/ml of Yashad Bhasma respectively, (c) and (d) Lawn of *L.Monocytogenes* on agar plate for control (without Yashad bhasma) and 4500mg/ml of Yashad Bhasma respectively

samples, were carried out by using field emission scanning electron microscope. The Field emission scanning electron micrographs (FESEM) micrographs as shown in figure 3(b) evidently show the microstructural heterogeneity. The shape and size of these particles were not uniform. In this sample more compact agglomerates prismatic ZnO particles of 300–500 nm range was observed.

(2) Energy dispersive X-ray spectroscopy (EDX)

An EDX spectrum normally displays the peaks corresponding to the energy levels for which the most X-ray had been received. An EDX spectrum plot not only identifies the element corresponding to each of its peak, but the type of X-ray to which it corresponds as well. For example, a peak corresponding to the amount of energy possessed by X-rays emitted by an electron in the L-shell going down to the K-shell is identified as K_{α} peak. The peak corresponding to X-rays emitted by M-shell electrons going to the K-shell is identified as K_{β}[12]. The EDX spectrum of the Yashad Bhasma is shown in figure 3(a). However, the elemental compositional data obtained using EDX is tabulated in TABLE 4. The results indicate that only Zinc, oxygen and gold elements are present in the Yashad Bhasma. This EDX spectrum confirms the chemical composition of ZnO. The gold peak arises from gold coating of the sample to minimize charging in the FESEM during the examination.

(3) Bacterial activity tests

In order to examine the bactericidal effect of Yashad Bhasma, the growth of *E. Coli* DH5α and *L. monocytogenes* were recorded in the presence of different concentrations of Yashad Bhasma i.e. 2500, 3000, 3500, 4000, and 4500 μg/ml in LB medium. The growth curves for the *E. Coli* and *L. monocytogenes* bacteria are as shown in figure 4a and 4d respectively. As seen in the growth curves, it is clear that both the bacteria follow the similar growth pattern when the concentration of Yashad Bhasma is the highest *i.e.* 4500 μg/ml. It has been observed that the steepness of the growth curve in the logarithmic phase lowers as we go from 2500 μg/ml to 4500 μg/ml concentrations for both the bacteria.

Recently, Zhang et al.[20] reported the bacterial activity of ZnO nanofluids against *E.Coli* DH5α bacteria. It has been reported that for the concentration of 2g/l of ZnO nanofluids, 100% killing of bacteria takes place due to the interaction between the bacteria cells and ZnO particles and the electrostatic forces between them have been made responsible for the same. Whereas, Brayner et al.[21] studied the biocidal effects and cellular internalization of ZnO nanoparticles on *E. Coli* bacteria. These nanoparticles were synthesized in di(ethylene glycol) (DEG) medium forced by hydrolysis of Zn^{2+} salts. The bacterial tests were performed in LB medium with different concentrations of ZnO nanoparticles. It has been observed that when *E.Coli* cells come in contact with ZnO nanoparticles having the concentration higher than 0.013 M in DEG medium, cellular internalization occurred. Similar results were also observed very recently by Huang et al.[22] on *Streptococcus agalactiae* and *Staphylococcus aureus*, in the presence of ZnO nanoparticles in an EG medium but at the concentration of 0.016 M. Tam et al.[23] also investigated the antibacterial activity of ZnO nanorod array prepared by the hydrothermal method against gram negative and positive *E.Coli* and *B. atrophaeus* respectively. They have observed that ZnO was more effective for gram-positive than the gram-negative

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TABLE 1 : XRD data for Yashad Bhasma and standard ZnO

Yashad Bhasma	Standard ZnO(JCPDS Card No. 890510)	
d(Å)	d(Å)	(hkl)
2.8125	2.8179	(100)
2.6005	2.6049	(002)
2.4744	2.4786	(101)
1.9096	1.9128	(102)
1.6238	1.6269	(110)

TABLE 2 : Parameters obtained using XRD data

Parameters	Yashad Bhasma	Standard ZnO (JCPDS Card No. 890510)
a(Å)	3.253	3.248
c(Å)	5.198	5.204
ρ(gm/cm3)	5.68	5.61

TABLE 3 : Comparison of raman spectra of Yashad Bhasma and ZnO

Yashad Bhasma(ν cm-1)	ZnO	Symmetry
331	329	A_1
379	379	A_{1T}
410	410	E_{1T}
435	436	E_{2H}
579		A_{1L} or E_{1L}

bacteria because they have simpler membrane structure. In our case, although the different characterization of Yashad Bhasma as discussed in section 3.1, revealed that the Bhasma is purely a ZnO with wurtzite structures, but bacterial tests are somewhat different from the reported ZnO. The bacterial test of Yashad Bhasma on gram-negative and gram-positive bacteria, does not show any inhibition even in the presence of very high concentration of Yashad Bhasma i.e. 4500 μg/ml.

Effect of different metal oxide nanoparticles on different Bacteria have been studied by different groups. Hu et al.[24] have reported the effect of ZnO, CuO, Al_2O_3, La_2O_3, Fe_2O_3, SnO_2, and TiO_2 nanoparticles on *E.Coli*. It has been observed that the cytotoxicity decreases with the increase in cation charge in nano size metal oxides. Sadiq et al.[25] have shown that the Alumina nanoparticles have mild growth inhibitory effect only at high concentration of 1000 μg/ml against *E. Coli*. They have established a linear relationship between nanoparticles concentration (x) and growth rate (μ) by fitting the growth rate and concentration. And fitting parameters resulted that the there is a negligible dependence of growth rate on the concentration of nanoparticles.

Similarly, we also attempted to establish a relationship between Yashad Bhasma concentration and growth rate for both gram-negative and gram-positive bacteria. As seen in the figure 4a and 4d, for all the concentrations of Yashad Bhasma, both the bacteria followed the traditional growth curve with proper logarithmic phases and the nature of the growth curve was similar to the one recorded for the control. The equation that describes the growth rate of the bacterial growth in the logarithmic phase may be expressed as:

$$N = N_o e^{\mu t} \quad (1)$$

where N = bacterial cell counts at time t, N_0 = Initial cell count, and μ = growth rate constant for bacteria.

The logarithmic phases of the growth curves for or all the concentrations of *E. Coli* bacteria and *L. monocytogenes* were identified to be 2 to 8 hours and 2 to 7 hours from figure 4a and 4b respectively. The natural logarithm of the growth data in these time intervals for *E. Coli* bacteria and *L. monocytogenes* were plotted against time and shown in figures 4b and 4d respectively. For the calculation of growth rate (μ) of the bacteria for the corresponding concentrations, all the data was linearly fitted and the slope of each fit gives us the growth rate corresponding to that concentration. The growth rate obtained from the liner fit for both these bacteria is shown in TABLE 5. It is clear from the table that the growth rate decreases with the increase in concentration. [The fluctuations in growth rate for different concentration may arise because of human error]. Also even at higher concentrations bacterial cells grow with small rate, and show that growth is not completely inhibited.

To obtain the relationship between concentration of Yashad Bhasma (x) and growth rate of bacteria (μ), growth rate was plotted against the Yashad Bhasma concentrations for both the bacteria and shown in figure 4c and 4f for *E. Coli* and *L. monocytogenes* respectively. Using polynomial fitting, a relationship between x and μ was derived for both the bacteria and follows the following equation.

$$\mu = a + b1 \times x + b2 \times x^2 + b3 \times x^3 \quad (2)$$

The values of coefficients a, b1, b2, and b3 as determined from the polynomial fitting, have been pre-

TABLE 4 : Elemental composition data from EDX

Element	Wt%	At%*
Carbon (C)	00.30	01.96
Oxygen (O)	00.86	04.18
Zinc (Zn)	68.88	82.02
Gold (Au)	29.95	11.84

***Based on ZAF Quantification (standard less)**

TABLE 5: Growth rate of *E.Coli* and *L. Monocytogenes* for different concentrations of Yashad Bhasma

Concentration (x) of Yashad Bhasma (μg/ml))	Growth rate (μ)	
	E. Coli	*L. Monocytogenes*
0	0.38853	0.41552
2500	0.24803	0.1279
3000	0.14818	0.15992
3500	0.12476	0.12038
4000	0.14307	0.09617
4500	0.14489	0.07702

TABLE 6 : Fitting parameters for growth rate vs concentration curve

Fitting parameters	*E. Coli*	*L. Monocytogenes*
a1	0.3889	0.4126
b1	1.384×10^{-4}	-1.3587×10^{-4}
b2	-1.1257×10^{-7}	$+ 1.4101\times10^{-8}$
b3	$+ 1.6821\times10^{-11}$	0

sented in TABLE 6. As depicted in the table, the value of b1 is of the order of 10^{-4} for both the bacteria, indicates that there is a significant dependence of growth rate on the concentration of Yashad Bhasma. Other b2 and b3 coefficients, however, are comparably very small ($<10^{-7}$) and their effect may be ignored.

The bacterial tests on Yashad Bhasma thus show the dependence of growth rate for both gram-negative (*E. Coli*) and gram-positive (*L. monocytogenes*) bacteria on concentration of the Bhasma. It has been seen that even the higher concentration of Yashad Bhasma *i.e.* 4500 μg/ml does not inhibit the growth of bacterial cells. The image of bacteriological tests of *E. Coli* and *L. monocytogenes* on solid agar plates without Yashad Bhasma (Control growth) and with 4500 μg/ml concentration of Bhasma is shown in figure 5a-5d. The figure 5a and 5b clearly show colonies in the case of E. Coli for control as well as 4500 μg/ml concentration of Yashad Bhasma respectively. Whereas, in case of *L. monocytogenes,* figures 5c and 5d show that there is a dense lawn for control and lawn for control as well as 4500 μg/ml concentration of Yashad Bhasma respectively. Thus, indicating the growth of both the bacteria even at a higher concentration of Yashad Bhasma.

CONCLUSIONS

Zinc based 'Yashad Bhasma' was successfully synthesized by using unique herbo-mineral method, that includes repeated calcinations of pure zinc metal. The Bhasma, thus produced, was confirmed by subjecting it to various Ayurvedic tests. Structural, optical, and elemental analysis of the Bhasma has also been carried out by various analytical techniques, such as XRD, FT-IR, Raman, FE-SEM and EDX. The FT-IR spectrum and XRD of this Yashad Bhasma predict that its main constituent is Zinc Oxide with single phase hexagonal Zinc Oxide phase. The average particle size of the Bhasma, were approximately 200-300 nm. As ZnO belongs to $\mathbf{C_{6v}^4}$ the space group with two formula units per primitive cell and with all atoms occupying C_{3v} sites, and for the Yashad Bhasma we obtain the same Raman Active modes, we can conclude that Yashad Bhasma also belongs to $\mathbf{C_{6v}^4}$ space group, and it is also having C_{3v} sites occupying all its atoms. Preliminary studies were done to see the toxicological impact of Yashad Bhasma on gram-negative (*Escherichia coli* DH5α, *E. Coli)* and gram-positive (*Listeria monocytogenes, L. monocytogenes*) bacteria. The bacterial tests on Yashad Bhasma show the dependence of growth rate for both bacteria on concentration of the Bhasma and decrease with the increase in concentration. The results shows that very high concentration of Yashad Bhasma (4500 μg/ml) does not induce any damage to them in contrast to the chemically synthesized ZnO nanoparticles that induces the damage to the bacterial cells at low concentrations only. This study, therefore, shows that although the Yashad Bhasma being ZnO as concluded by different characterization, but it is less toxic to the environmental microorganisms in comparison to the chemically synthesized ZnO. We feel that the herbal preparation of Yashad Bhasma is mainly responsible for these bacterial results. The results may be used further to establish a therapeutic behavior of Zinc metal based Yashad Bhasma.

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