



# Preparation and Exploration of Physical Properties of Calcium based Indian Origin Ayurvedic Medicine-Shankh Bhasma (Marine Drug) as Nanomaterials for its Applications

Sweta Sinha<sup>1</sup>, Rakesh Kr. Singh<sup>1\*</sup>, Nishant Kumar<sup>1</sup>, Subhash Pd. Singh<sup>2</sup>, Prabhat Kr. Dwivedi<sup>1</sup> and Rekha Kumari<sup>3</sup>

<sup>1</sup>Aryabhatta Centre for Nano Science and Nanotechnology, School of Engineering and Technology, Aryabhatta Knowledge University, Patna – 800001, Bihar, India; rakeshsinghp@gmail.com

<sup>2</sup>Department of Chemistry, AN College, Patna, Patliputra University, Patna – 800001, Bihar, India

<sup>3</sup>Department of Zoology, AN College, Patna and Department of Education, Govt. of Bihar, Patna – 800001, Bihar, India

## Abstract

The objective of the present research is to explore the Physical properties of a marine origin of Indian Ayurvedic medicine (Shankh Bhasma) as nanomaterials for its applications. *Shankh Bhasma* has been prepared by using the method as mentioned in Ayurvedic text which includes aloe-vera and buttermilk as ingredient. The preparation method was ecofriendly and hazardous chemicals are not used or emitted during preparation. The bhasma are characterized by the modern scientific techniques. X-ray diffraction measurement and Scanning electron microscopy analysis showed that *Bhasma* exists as agglomerated nano crystalline materials. Fourier transform infrared spectroscopy measurement reveals the bond stretching during the incineration process and found peaks of carboxylic group, calcium carbonate and calcium hydroxide. The photoluminescence measurement shows a broad spectrum in UV region and one prominent emission peak in the visible region at 405nm. The prepared shankh bhasma was examined on *E. coli* and found to have no effect. In present research, physical property measurement of shankh bhasma using state of the art techniques of 21<sup>st</sup> century not only support a foundation for the development of low cost ayurvedic natural marine based materials as nanomedicine but also used in other sectors of science and technology.

**Keywords:** Ayurveda, Marine, Nanomaterials, Physical Properties, Shankh Bhasma

## 1. Introduction

The word "*Bhasma*" in Ayurveda stands for holy ash, which indicates superfine nanocrystalline powder. The word Ayurveda stands as a knowledge system for long life and considered as a medicinal portion obtained from nature, capable of human life simulation. In Bhasmikaran process minerals, metals and marine products are transformed into tiny size particles using herbs, fruit juice as an ingredient with quantum vibration of sound and together with heat treatment. These processes produce superfine powder, called holy ash as nanomaterial<sup>1-3</sup>. The nanomaterials represent the crystalline size ranging

between 1nm-100nm<sup>4,5</sup>. The nanomaterials have found different properties as compared to bulk size materials. Generally, at the nanoscale surface structure causes discrete energy levels that yield drastic properties as compared to bulk size materials. Natural resources and marine based materials like conches have been used by traditional ayurvedic practitioners for a long time ago<sup>5</sup>. In Rasashashtra shankh bhasma is mentioned in the category of Sudhaverga. Bhasmas are found to be more efficacious than any other healing system due to superfine small particles and can show their efficacy at the molecular level, biocompatibility as compared to any chemically produced nanoparticles<sup>6</sup>. The metals are combined

\*Author for correspondence

with herbs which help in assimilation and delivery of the ingredients in to human body<sup>7</sup>. Shankh Bhasma is an Ayurvedic Bhasma of the category Sudhaavarga. In *Ayurveda science* calcium containing *dravyas* (Sudhavarga), ie. *Shankha, Shukti, Kapardika, pravala, Godanti, Khatika* etc were incorporated in later period. But the knowledge of Indian origin medicine under this group is available since Vedic period<sup>8</sup>. These drugs mainly contain calcium carbonate. Shankha as a shell is very hard and dense structure and is commonly prepared and is mentioned in several Ayurvedic literatures. In the present research crystal structure of shankh bhasma was measured using X-ray diffractometer, scanning electron microscopy and found nanocrystalline. This is one of the reason, why seashell (CaCO<sub>3</sub>) are hard materials as compared to other Ca based materials. Shankh Bhasma derived from conch shell is an Ayurvedic formulation popularly used for the treatment of dyspepsia and indigestion<sup>9</sup>.

Classically, Shankha Bhasma is alkaline in nature and causes significant reduction in ulcer index in both indomethacin and cold resistant models<sup>9</sup>. It is used for indigestion, flatulence, abdominal pain, vomiting belching, diarrhoea, belching and gastritis. The one of the objective of this research is to evaluate and explore the physical properties of shankh bhasma e.g. superfine structure, functional group, interaction nature among particles, surface morphology, and electronic structure using modern scientific tools of 21<sup>st</sup> century. The modern scientific tools include- X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Photoluminescence spectroscopy (PL). Calcinations is important process for size reduction and used to obtain percentage purity of bhasma materials. Therefore thermal analysis treatment using TGA-DTA like modern scientific equipments was also reported in this present work. Also, antimicrobial studies were reported on *E. coli* bacteria.

## 2. Material and Method

### 2.1 Raw Materials

The conch shells were collected from Bodh Gaya market of Gaya-Bihar. The juice of aloe vera and buttermilk was used for decoction. The main purpose of using aloe vera and buttermilk were to prepare the formulation in the alkaline medium for better action and easily absorbed in the body as nanomedicine. The Shankh bhasma prepared using aloe vera as ingredient is denoted as SBA, while Shankha bhasma prepare using buttermilk is denoted as SBB.

### 2.2 Nutrient Agar Media (NAM)

NAM media is a complex media containing nutrients including extracts from yeasts, meat, or plants, or digests of proteins and other sources.

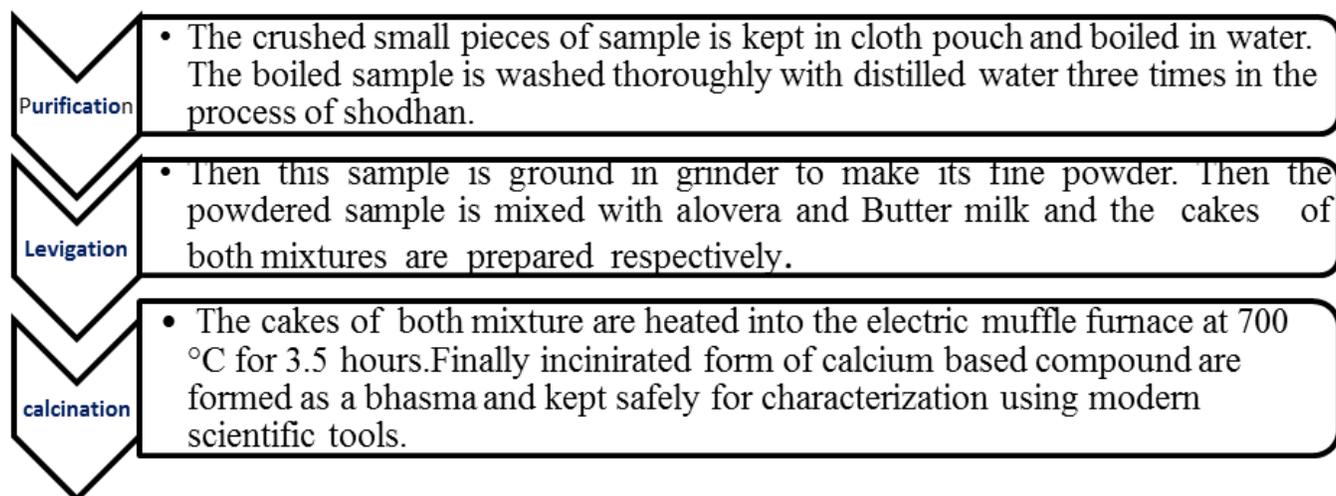
### 2.3 Micro-organisms

Gram negative bacteria, *Escherichia Coli* was used for microbial test. It is the most common inhabitant of lower intestine of warm blooded animals. It is chemoheterotroph that required carbon as a source of energy in medium.

### 2.4 Synthesis of Shankh Bhasma as Nano materials

The procedure for synthesis is shown in Figure 1. The method of synthesis is followed aa per the literature review<sup>10</sup>. Bhasmikaran or Calcination technique was used to make bhasma in naometric size. Finally calcined form of calcium oxides are formed as a bhasma and kept in air tight container at room temperature and the characterization using modern scientific techniques e.g. XRD, TGA-DTA, SEM, FTIR, PL. There are three major steps of Ayurvedic bhasma preparation which was followed, and are mentioned as follow:

1. Purification: Five conch shells (sample) of about 1.5 kg were crushed into small pieces and tied with cloth. Then these cloth packets were boiled in water and washed thoroughly with distilled water three times and this process is known as Shodhan.
2. Levigation: All crushed conch shells (sample) were ground using grinder to make it in powder form. 500 gm powder of coch shells was mixed with aloe vera juice, which is represented as SBA (shankh bhasma preparation using Aloe vera). Another 500 gm powder was mixed with buttermilk, represented by SBB (shankh bhasma preparation using buttermilk) simultaneously to make their dough. The resultant the cakes of both doughs were air dried for 2 hours.
3. Calcination: Cakes of SBA and SBB were kept in electric muffle furnace at 700°C for 3.5 hours. Again cakes were mixed with aloe vera juice and buttermilk and kept in electric muffle furnace at 700°C. Thus this process was repeated three times at regular interval of 3 hours during bhasmikaran process. Finally bhasma was prepared as a superfine white colour powder. The preparation details of shankh bhasma is shown as schametic diagram in Figure 1. During preparation, multiple heating and quenching in liquid media causes reduction in size of the particles, which is known as ecofriendly produced nanomaterials.



**Figure 1.** Synthesis procedure of Shankh Bhasmas (SBA and SBB).

## 2.5 Preparation of Shankh Bhasma as Hand Washes

Firstly the dirty hand was washed with normal distilled water and water was collected in test tube. Again the dirty hand was washed with Shankh Bhasma nanoparticles such as SBA and water was collected in test tube. The collected water was used for antimicrobial test in media. Few drops of water was taken from each tube and mixed with media in petri plates respectively. These plates were left overnight for incubation of temperature at 32°C and 70% humidity and microbial growth was checked. One of the main objective of this experiment was to obtain the hand wash effect as compared to other calcium based bhasma.

## 3. Results and Discussion

### 3.1 Thermal Analysis

Thermogravimetric Analysis (TGA) results of shankh bhasma prepared using aloe vera and buttermilk are shown in Figure 2 (a–b). First significant weight loss of sample found to be at approximately 300–500 °C due to decomposition of moisture and organic compounds, whereas the most significant weight loss occurs between 600°C and below 700°C, which is due to loss of CO<sub>2</sub> from carbonate. The sample converted as CaO phase was similar to earlier reported work<sup>11</sup>. In addition, the DTA curve, attributed to an exothermic peak at around 674°C, confirmed the decomposition to Ca(OH)<sub>2</sub>. The temperature of material should be above 725°C to form CaO completely which must be comparable from TGA curve, since there is no weight loss after 700°C. This

shows it is saturated after this temperature and the varied material should be hydroxide form. But on the other hand, the material SBB, the first significant drop is comparable with the SBA sample. But the major weight loss occurs in the range of after 680–750°C and there is also gradual shift in the exothermic peak in DTA curve to 743°C and this might be due to the formation of CaO or Calcite form of material present in the bhasma. The difference in thermal analysis measurement of SBA and SBB is due to interaction force between precursor powder of materials, which were prepared using Alovera and buttermilk media. This thermal analysis measurement indicates that during synthesis these herbal based ingredients play an important role in the desired form as functional materials and prepared at a particular temperature. This thermal analysis measurement also highlights the necessity of ancient time ayurvedic physicians were used to prepared such materials at certain temperature range.

### 3.2 Structural Measurement

The crystal structure was analyzed using X-ray diffractometer for their structural parametric analysis. The result shows that the prepared materials SBA and SBB have peaks corresponding to CaO as well as Ca (OH)<sub>2</sub> (Figure 3). The observation of Ca(OH)<sub>2</sub> phases might be because of the sample exposed to humidity in the air<sup>12</sup>. The sharp peaks in the XRD pattern indicate the crystalline nature of CaO nanoparticles. The present study shows that shankh bhasma mainly contains CaO and Ca(OH)<sub>2</sub> materials. Further crystallite size was evaluated using W-H plot and it was found that crystallite size for SBA is 73.71 nm and 86.78 nm for SSA respectively<sup>13</sup> (Figures 4a and

4b). Strain was found to be in agreement with crystallite size as it is found to decrease with increase in crystallite size, strain for SBA and SBB are 0.00116 to 0.00089. Thus XRD measurement shows that marine origin shankh bhasma prepared using aloevera and buttermilk are nanocrystalline materials and depends on the ingredient used. One of the characteristics of nanomedicine is smaller the crystalline size, larger it remains in the body and better chance of being distributed in the target sites<sup>14</sup>.

At global level, commercially, various metal oxides are being produced, which are chemicals in nature, toxic and large energy is required for its preparation. But in this present research, calcium based nanomaterials are prepared using low cost ecofriendly approach in which no harmful gases are emitted. Such medicine could be reached to people due to its low price. Therefore this methodology of production of nanomedicine may be an alternative method of production of calcium based materials at nanoscale.

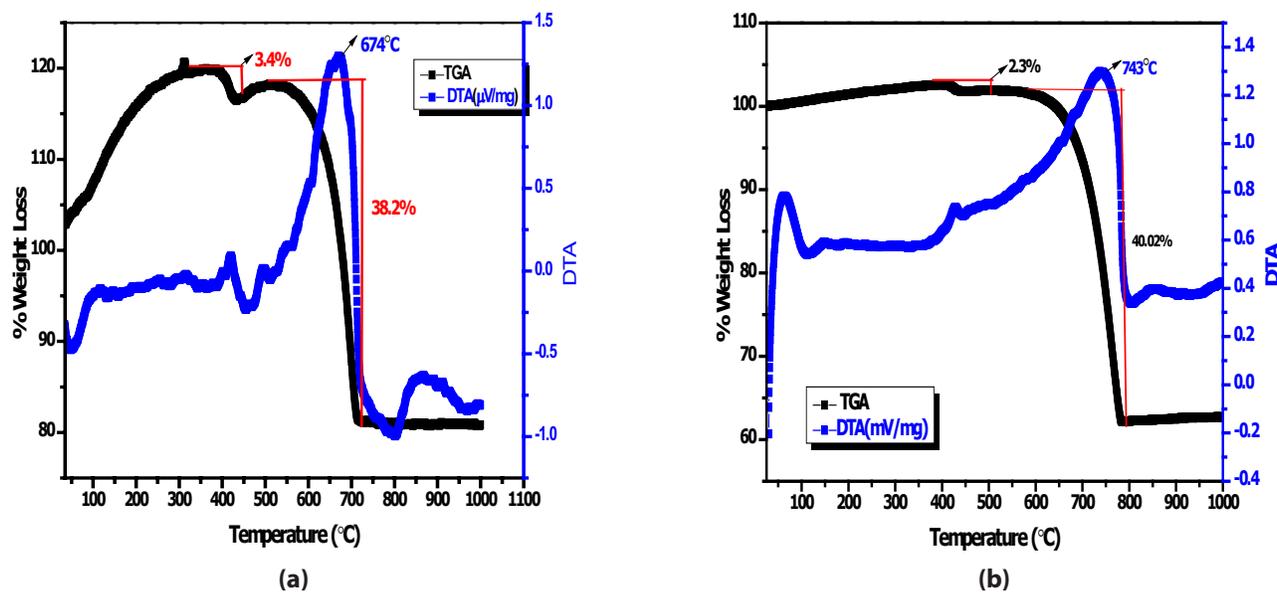


Figure 2. (a) TGA-DTA plot of SBA (b) TGA-DTA plot of SBB.

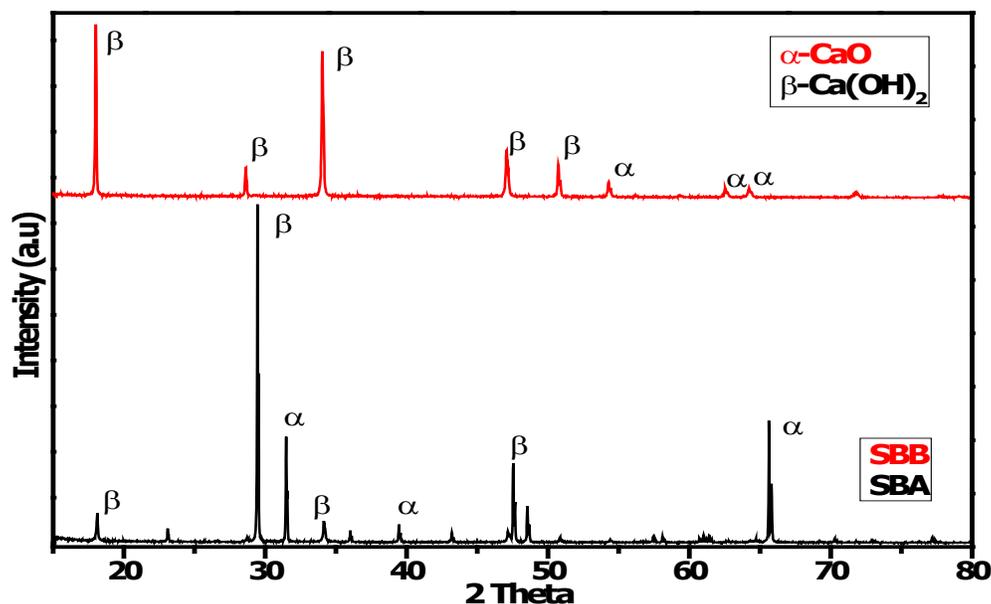
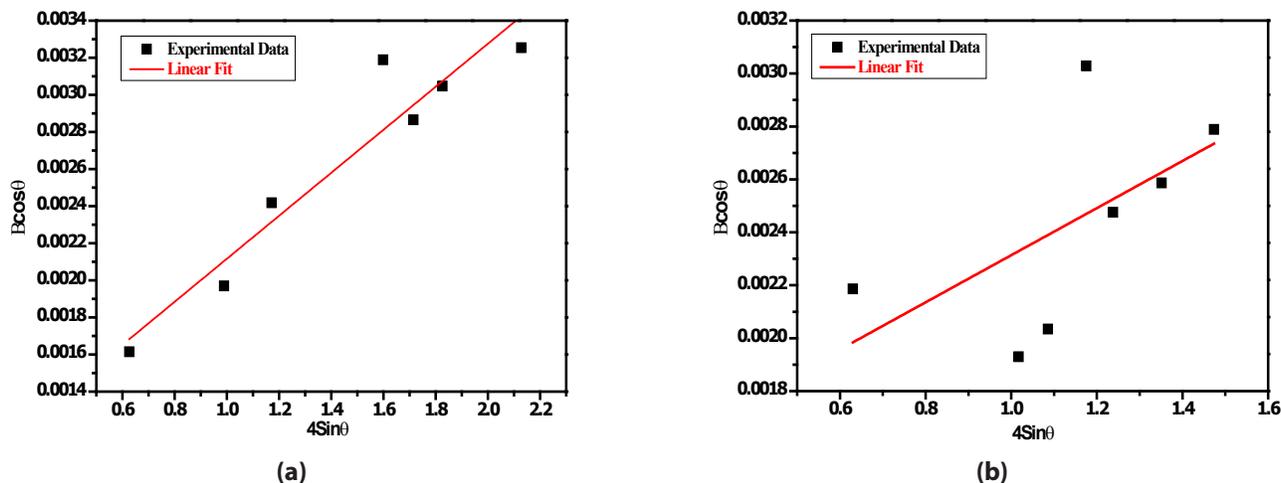


Figure 3. XRD pattern of SBA and SBB.

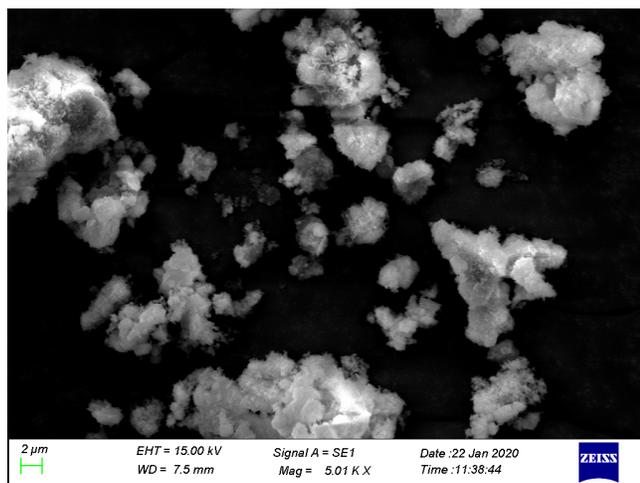


**Figure 4.** (a) W-H of SBA (b) W-H of SBB.

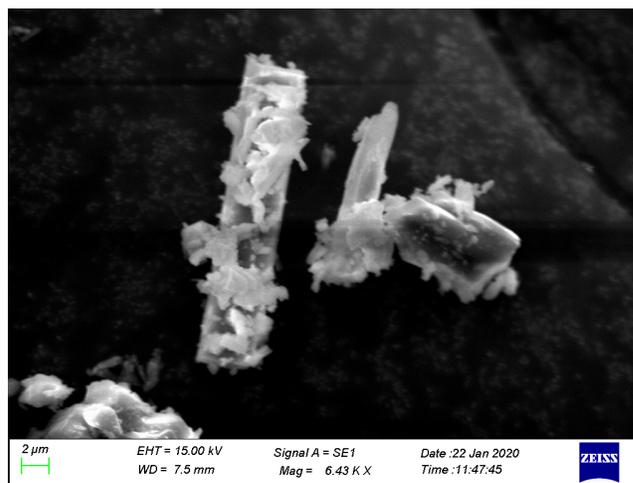
### 3.3 Surface Morphology Measurement

The morphology of the materials were studied using scanning electron microscope and are shown in Figure 5(a,b). The morphology of the nanoparticle was found to be oval and rod shaped with large agglomerated nanocrystalline particles. Multiple calcinations using aloe vera and buttermilk and their interactional forces shows different agglomerated size particles. In the field

of medicine, surface morphology dictates functional behaviors like bio distribution, cell internalization and cellular level. Hence, this ayurvedic bhasma is called targeted nanomedicine<sup>15,16</sup>. Agglomeration is a phenomenon at electromagnetic forces scale, in which crystals do not appear as single discrete unit but they appear in big clusters. Such agglomeration may be due to crystal-crystal interaction, and defects in crystal growth.



(a)



(b)

**Figure 5.** (a) SEM image of SBA (b) SEM images of SBB.

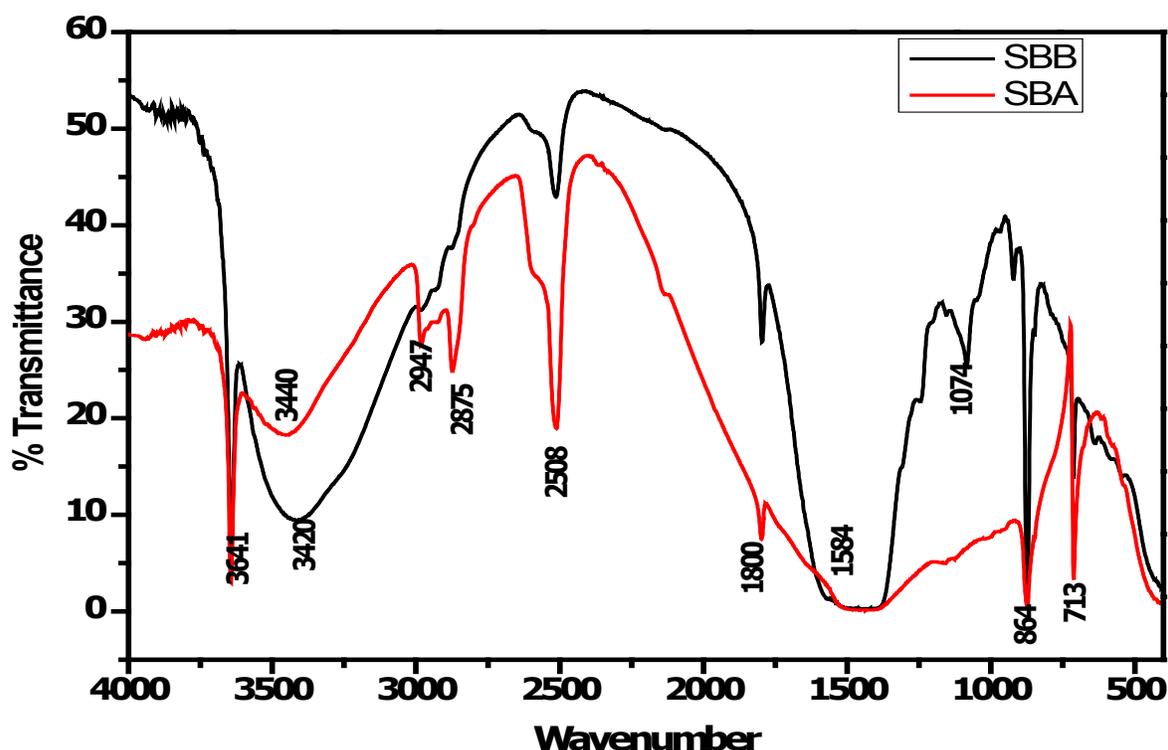
### 3.4 Functional Group Measurement (FTIR Spectroscopy Measurement)

The FTIR analyses materials functional group present in the prepared samples, and are shown in Figure 6

with details of functional group. The FTIR result of SBA showed broadband centering at  $1415.52\text{ cm}^{-1}$  which is a characteristic of C–O bond showing a bond between the oxygen atom of carbonate and calcium atom<sup>17</sup>. In

addition, there were two sharp bands at  $711.62\text{ cm}^{-1}$  and  $875.54\text{ cm}^{-1}$  showing characteristics of C-O bond mainly calcite form which is relevant with our thermal analysis. Other broad band around  $1500\text{ cm}^{-1}$  indicates the presence of Ca ion in the prepared material. The absorption band at  $3641\text{ cm}^{-1}$  correspond to OH bond which might be the reason for formation of hydroxide ion. On the other hand, SBB nanoparticles showed mostly similar peaks as

SBB but a characteristic peak around  $1074\text{ cm}^{-1}$  was found which are responsible for the formation of calcium oxide nanoparticles by carbonation process<sup>18</sup>. The presence of functional group depends on the type of ingredients used for preparation and in this present research aloe vera and buttermilk were used. Thus FTIR measurement highlights importance of Indian based traditional medicine, which are ecofriendly produced low cost Ca based materials of their origin from plant/natural ingredients.



**Figure 6.** SFTIR spectra of Shankh Bhasma (SBA) and Shankh Bhasma (SBB).

Peak position ( $\text{Cm}^{-1}$ )	Assigned functional group
3641	OH bond
3420	$\text{CaCO}_3/\text{Ca(OH)}_2$
2947	Organic Matter
2875	Organic Matter
1800	$\text{H}_2\text{O}$
1584	Ca Region
1074	CaO
864	Carbonate Ion
713	C-O

### 3.5 Photoluminescence Measurement

Photoluminescence Spectroscopy is an important tool to study the optical property of materials. The excitations were recorded in the range up to 800 nm. The emission spectrum in UV range having broad spectrum was obtained. The peak around 405 nm shows blue region confirmation and this might be originated from the recombination of free excitons due to exciton-exciton collision or might be due to intrinsic defects as oxygen and calcium interstitials. Since some groups also found such luminescence in metal oxides Like ZnO etc.<sup>19</sup>. Thus luminescence measurement shows that the prepared

materials may be useful in other science and technology field . The only difference is that the materials are prepared using ecofriendly approach in which no harmful chemicals and gases are emitted. While other calcium

based and metal oxide also shows such luminescence but are prepared using other physical and chemical method<sup>20</sup>, which are prepared using chemical method and its expensive production (Figure 7).

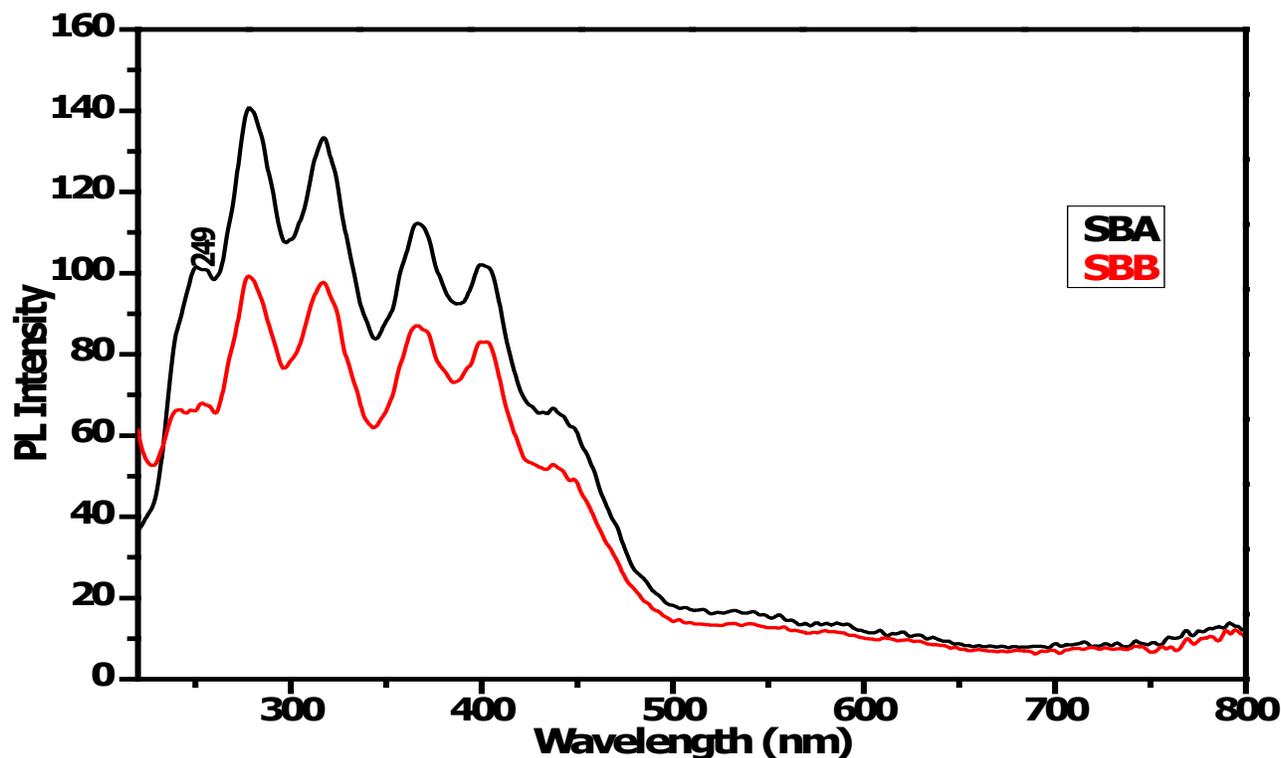
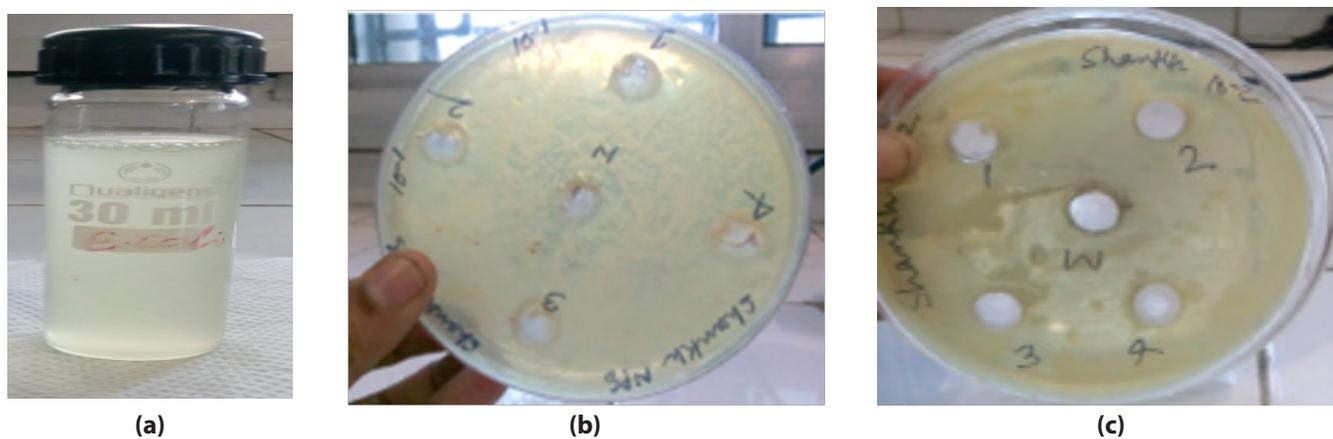


Figure 7. Photoluminescence spectra of SBA and SBB.

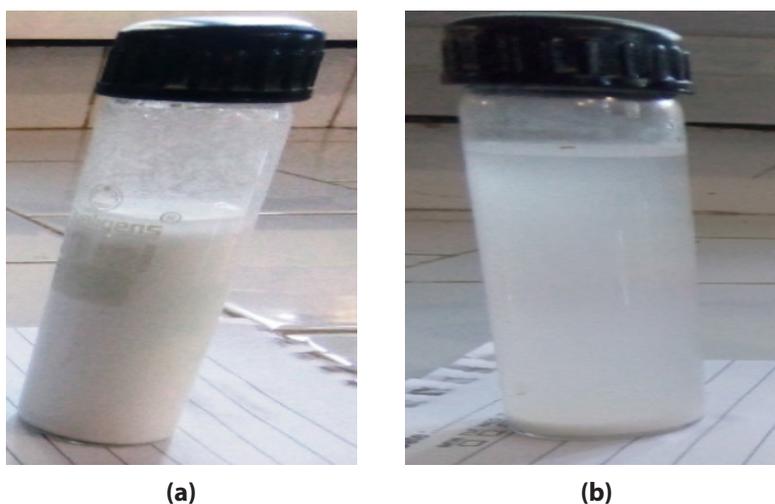
#### 4. Antimicrobial Property

The effect of Shankh Bhasma nanoparticles (SSA) and SSB having average particle size 73.71 nm and 86.78 nm were checked on *Escherichia coli*, a gram negative bacteria. The zone of inhibition was not observed after incubation of 36 hrs as shown in Figure 8 (a), (b) and (c). Similar result was also reported by research group<sup>21</sup>. Same sample were treated as a hand wash and the result is shown in Figures 9 (a) and (b) respectively. There were microbial colonies observed in both distilled water treated sample and Shankh Bhasma as calcium based nanoparticles. This finding suggest that natural calcium based powder is not a potential agent for hand wash. While other CaO based shows in hand wash functional property (which were prepared using egg shell<sup>22</sup>. Thus present study shows calcium based materials prepared using marine

based conch shells are not used in hand wash behaviour (Figures 10 and 11). Further study is required for difference in behaviour of calcium based nanomaterials and their applications from different sources with different crystalline size. In this research Shankh Bhasma as nonmaterial shows negative result treated with bacteria *E.coli* and also less antimicrobial effects in hand wash. However, size dependent behavior may result in some different property. Generally, the antimicrobial efficacy depends on materials employed for the preparation of the materials, crystalline size and surface morphology. Some Literature reviews also revealed that calcium based Bhasma shows very less antibacterial effect against *S. aureus*, *P. aeruginosa* and *E. coli*<sup>22</sup>. Thus, we can say that shankh bhasma containing enriched calcium has less antibacterial effects but may be effective in case of other microbes. This can be verified in future research work.



**Figure 8.** (a) *Escherichia Coli* subculture (b) Antimicrobial activity for SBA (c) Antimicrobial activity of SBB NPs.



**Figure 9.** (a) SBA stock solution (b) SBA Hand – Wash water.



**Figure 10.** Distilled water as a Hand wash.



**Figure 11.** Shankh Bhasma Nps as a Hand wash.

In nutshell, we can highlight the exploration of physical property measurement of shankh bhasma as nanomaterials support a foundation for the development of natural marine based ayurvedic bhasma, as evidence based nanomedicine not for various diseases but in other area of science and technology.

## 5. Conclusions

Marine origin Indian subcontinent based *Shankh Bhasma as nanomaterials* has been prepared by conch shell using aloe vera and buttermilk as ingredient. This shows that natural marine resources can be transformed into technological grade material at nanoscale using green approach. The preparation method was ecofriendly with hazardous chemicals free used or emitted during preparation. The bhasma are characterized by the modern scientific techniques. TGA-DTA measurement shows that major weight loss found in the temperature range 680–750 degree celcius and shift in the exothermic peak in DTA curve at 743 degree celcius and this may be due to formation of CaO and elimination of other elements like S,N,C and P. Thermal analysis measurement highlights the ancient method of preparation at particular temperature. X-ray diffractometer and Scanning Electron Microscopy measurement shows the particles sizes are less than 100nm and may be treated as Indian origin ayurvedic natural nanomedicine. An FTIR measurement reveals the bond stretching during the incineration process and found peaks of carboxylic group, calcium carbonate and calcium hydroxide. The photoluminescence measurement shows that broad spectrum in UV region and one prominent emission peak in visible region at 405nm. Efficacy was not observed in Shankh bhasma treated on *E. coli*. The present research opens a new window that low cost ayurvedic nanomedicine can be prepared using ecofriendly approach for benefit of huge population. The characteristics of this research also shows that such calcium based materials prepared using natural based techniques can be used in other area of science and technology due to its significant physical properties.

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