

GREEN SYNTHESIS AND CHARACTERIZATION OF MARINE YEAST-MEDIATED SILVER AND ZINC OXIDE NANOPARTICLES AND ASSESSMENT OF THEIR ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: The present study focuses on the synthesis of silver and zinc oxide (ZnO) nanoparticles from marine yeasts, isolated from the sediments of the Bay of Bengal, Bakkhali coast, West Bengal, and India.

Methods: The marine sediment samples were diluted through serial dilution and cultured onto yeast malt agar medium by the spread plate method. The selected yeast isolates were screened for the biosynthesis of silver and ZnO nanoparticles. Characterization of both the nanoparticles was done by applying ultraviolet (UV)-visible spectroscopy, atomic force microscopy, scanning electron microscopy, and Fourier transform infrared spectroscopy.

Results: A total of five marine yeasts isolates were able to synthesize silver and ZnO nanoparticles as evidence of the color change. Optical density was measured in UV-spectrometer at different time interval for the conformation of production of nanoparticles. The size of silver nanoparticle was 31.78 nm and ZnO nanoparticle was 86.27 nm. The synthesized nanoparticles are then used for antioxidant assays.

Conclusions: We are concluding that marine yeast isolates SAG1 and SAG2 both are potential marine yeast isolates which can synthesize both the silver and ZnO nanoparticles. They also showed good antioxidant activity.

Keywords: Marine yeast, Silver nanoparticle, Zinc oxide nanoparticle, Scanning electron microscopy, Ultraviolet-spectrophotometry, Fourier transform infra-red, Atomic force microscopy.

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INTRODUCTION

Nanotechnology is an innovative field which acts on all aspects of human's life [1,2]. Nanoscience is applicable for the industrial and commercial objectives, which is referred as nanotechnology [3]. A nanoparticle is a very small particle with a size of approximately 100 nm. Inorganic nanoparticles contain magnetic nanoparticle, the noble metal nanoparticle such as silver and gold, also zinc oxide (ZnO) and titanium oxide which are semiconductor nanoparticles [4]. Recent studies have shown that nanoparticle from some materials such as metal oxides are capable of inducing eukaryotic cell death and in prokaryotic, cell growth is inhibited by its cytotoxic effect [5].

Nanoparticles can be synthesized by different methods involving physical methods, chemical methods, and biological methods [6]. In recent years, the demand to develop eco-friendly procedures with low cost and non-toxic methods for nanoparticle synthesis is increased [7,8]. There are reports of using different groups of microorganisms such as yeast, bacteria, fungi, and actinomycetes for the synthesis of nanoparticles. A few examples include, *Aspergillus fumigatus* (Ag nanoparticle), *Pseudomonas aeruginosa* (Au nanoparticle), *Candida glabrata* (cadmium nanoparticle), and *Fusarium oxysporum* (silver, gold, and zirconia nanoparticles) [9-12]. Nanoparticles synthesized biologically are multifunctional with diverse applications in biomedical field such as in therapeutics [13], tissue regeneration [14], drug delivery systems [15], separation techniques [16], and cancer therapy.

Nanoparticles, obtained from silver have unique electrical, thermal, and optical properties. In addition, silver nanoparticles are used for antimicrobial coatings, wound dressings, keyboards, and many textiles. Biomedical devices now are having silver nanoparticles in them, which

can release a very low level of silver ions in a continuous action to provide protection against bacteria. ZnO is having several applications in gas sensing, optical, magnetic, and piezoelectric methods. Moreover, ZnO nanoparticles have strong adsorption ability, high catalytic efficiency, and they are also used for sunscreens production [17], rubber processing, as a fungicide, and in waste water treatment [18,19]. ZnO usage on the environment can overtake nano-titanium dioxide in the near future because it absorbs both ultraviolet (UV)-A and UV-B radiation, whereas, TiO₂ can block UV-B only, and thereby giving better protection to the environment. ZnO has antimicrobial activities. It is capable of interacting with the cell surface of bacteria or with bacterial core and generates different bactericidal mechanisms [20]. It is recognized that among eukaryotes, yeast have the potential for synthesis nanoparticles.

Yeasts, single-celled eukaryotic organisms, are a type of fungi regarded as the "model organism" [20,21]. The major sources of yeast include fruits, grains, plants, and carbohydrate containing foods. Although some yeasts cause disease and spoilage in the foods, they are considered very important in the food industry [22].

There are reports on production of various bioactive compounds or secondary metabolites such as glucans, vitamins, killer toxins, and glutathione from yeasts. They are also having a variety of applications in food, pharmaceutical industries, cosmetic industries, and chemical industries and they also play a great role in environmental protection. They are used in brewing of alcohol, alcohol distillation, baking industries, and carbon dioxide production. Yeasts are having some other applications, namely, single-cell protein production, biofuel production, nanoparticles, fatty acids, pigment production, and also production of enzymes such as amylase, protease, lipases, and inulinases [23].

Marine yeasts can survive in sea water for a longer time than in fresh water. They have unique features in producing enzymes compared to terrestrial yeasts. Some examples of marine yeast genera are *Candida*, *Clavispora*, *Mrakia*, *Cryptococcus*, and *Saccharomyces* sp. [24]. There are reports of production of silver nanoparticles from marine yeast such as *Candida* sp. and *Yarrowia* sp. [25,26].

The purpose of the current study is to synthesize silver and ZnO nanoparticles from isolated marine yeasts from marine sediment samples.

METHODS

Sample collection

The marine sediment sample was collected from Bay of Bengal, Bakkhali Coast, West Bengal, India in January, 2016. It was then brought in sterile condition and stored in molecular biology and microbiology laboratory, VIT University, Vellore, at 4°C for further use.

Isolation of marine yeast

Isolation of marine yeast was done using serial dilution and spread plate technique. Yeast malt agar medium was prepared by the addition of 50% marine water and 50% distilled water for the cultivation of yeast isolates. The inoculated plates were incubated at room temperature (30°C) for 2-3 days. Pure cultures from the master plates were selected and streaked on to fresh plates under aseptic conditions.

Identification of potential yeast strain

Identification and characterization of the yeast isolates were done through colony morphology, Gram staining, scanning electron microscopy (SEM), and biochemical tests, namely, methyl red, Voges-Proskauer test, indole test, citrate utilization test, urease and carbohydrate utilization tests.

Fermentation process

Yeast cells were inoculated into production medium (containing glucose, yeast extract, maltose, and peptone) and incubated in shaking condition at 30°C for 3 days.

Biosynthesis of silver and ZnO nanoparticles

The cultured broth was collected after incubation and centrifuged at 8,000 rpm for 15 minutes. The supernatant was then used for the synthesis of silver and ZnO nanoparticles. In each 250 ml Erlenmeyer conical flasks, 100 ml of the supernatant from each culture was added and in that 1 mm of silver nitrate (AgNO_3) and ZnO was added separately. The inoculated conical flasks were incubated in a rotary incubator shaker at room temperature for 3-4 days [27,28].

Characterization of the ZnO and AgNO_3 nanoparticles

After the biosynthesis of silver and zinc nanoparticle, a portion of the broth was subjected to UV spectrophotometric analysis in different wavelengths and in specific time intervals. Atomic force microscopy (AFM) analysis was done to determine the shape and size of the silver and ZnO nanoparticles. Fourier transform infrared (FTIR) spectroscopy is carried for determining the functional groups which are present on the surface of the microbial cell that could be an important factor for the synthesis of silver and ZnO nanoparticles. SEM is done for getting the two-dimensional image.

Antioxidant test

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Standards of the samples were prepared with 20, 40, 60, 80, and 100 concentrations and to each of those standards, 1 ml DPPH was added. Absorbance of those given standards was measured at 570 nm in UV spectroscope. Ascorbic acid was prepared as the positive control, and on the other hand, negative control was then prepared by mixing properly

2 ml methanol and 1 ml DPPH. Total 3 ml of that mixed methanol with DPPH was used as blank [29].

Equation for calculating DPPH scavenging activity:

$$(A_0 - A_1 / A_0) \times 100$$

Where,

A_0 = Absorbance of control and A_1 = Absorbance of sample

RESULTS AND DISCUSSION

Isolation of marine yeast

A total of 20 yeast isolates were recovered from the marine sediment samples. Morphologically distinct colonies were selected for the further studies.

Screening for the synthesis of silver and ZnO nanoparticles

Among the 20 isolates screened for the synthesis of both the nanoparticles, only five isolates showed a color change (Figs. 1 and 2) after the addition of AgNO_3 and ZnO. These five isolates were named as SAG1, SAG2, SAG3, SAG4, and SAG5. SEM images of the potential two isolates are shown in Figs. 3 and 4.

Characterization of yeast isolates

Some biochemical tests such as methyl red, Voges-Proskauer, citrate utilization test, and indole tests were done. SAG1 and SAG4 showed positive results here. SAG2 and SAG3 isolates showed negative results for indole and MR, and the SAG5 strain showed negative results for VP. All the isolates were positive for urease production. Carbohydrate utilization tests were done using glucose and galactose. All the isolates showed positive results for this test (Table 1).



Fig. 1: Before (a) and after (b) the addition of silver nitrate

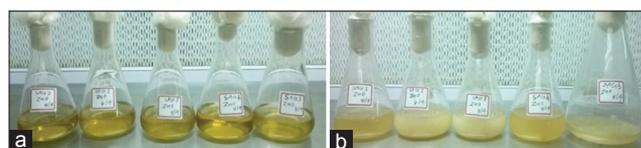


Fig. 2: Before (a) and after (b) the addition of zinc oxide

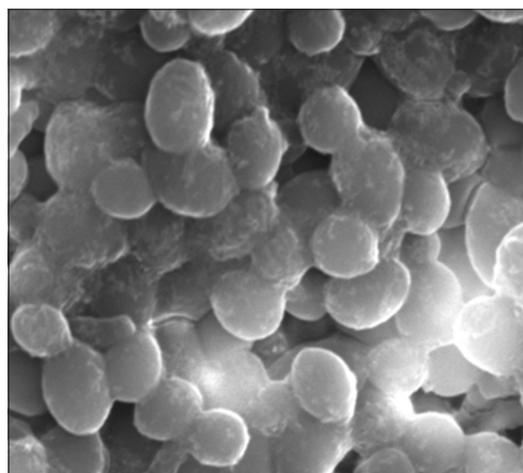


Fig. 3: Scanning electron microscopy image of SAG1

Characterization of silver and ZnO nanoparticles

UV-spectrometer analysis of silver and ZnO nanoparticles

Among the five isolates, only two isolates (SAG1 and SAG2) showed a maximum absorbance for both the silver and ZnO. Through two-way ANOVA analysis, both SAG 1 and SAG 2 showed a broad peak at 430-450 nm (Fig. 5) which confirmed the presence of silver nanoparticles, as surface plasmon resonance for silver nanoparticles generally occurs within that range only. The formation of the highest peak at approximately 330 nm (SAG1) and at 325 nm (SAG2) through the two-way ANOVA analysis is a confirmation of the presence of ZnO

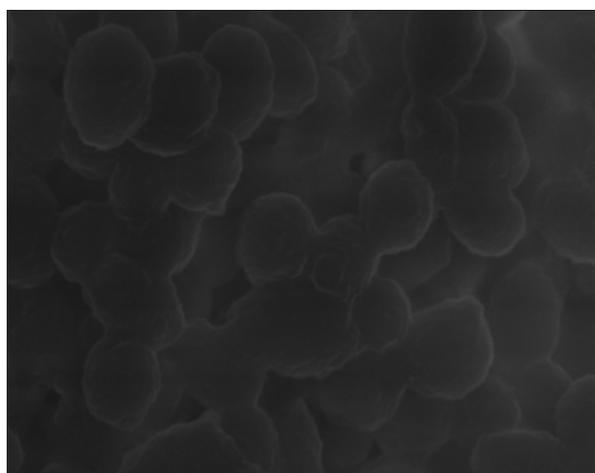


Fig. 4: Scanning electron microscopy image of SAG2

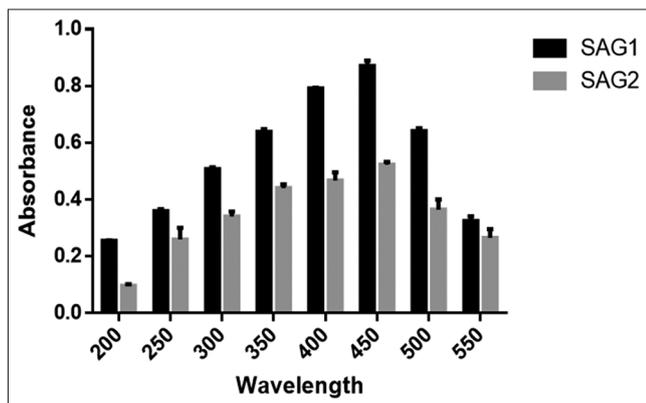


Fig. 5: Ultraviolet-visible spectrum of SAG1 and SAG2 for silver nanoparticles

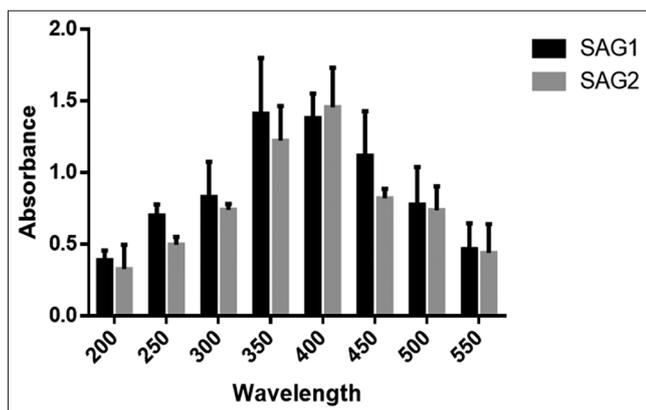


Fig. 6: Ultraviolet-visible spectrum of SAG1 and SAG2 for zinc oxide nanoparticles

nanoparticles (Fig. 6). Absorbance was taken in UV-spectrometer in different time intervals with both the isolates in the particular wavelengths where they have shown higher activity in silver nanoparticles and ZnO nanoparticles production. Both the graphs showed an increase in absorbance in various time intervals (0, 6, 12, 18, 24, 30, 36, 42, and 48 hrs) (Figs. 7 and 8). The color changed from yellow to dark brown with the increase of time period.

AFM analysis for the nanoparticles

To analyze the topographical structure of the synthesized AgNPs, AFM analysis was performed. Based on the results obtained in the UV-spectroscopy, AFM analysis was carried out for both the nanoparticles synthesized by the isolate SAG1 only. From the AFM images, it can be

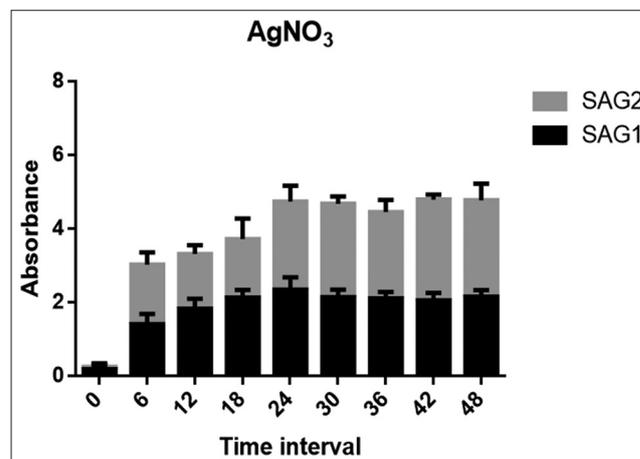


Fig. 7: Increase in absorbance in different time intervals of SAG1 and SAG2 for silver nanoparticles

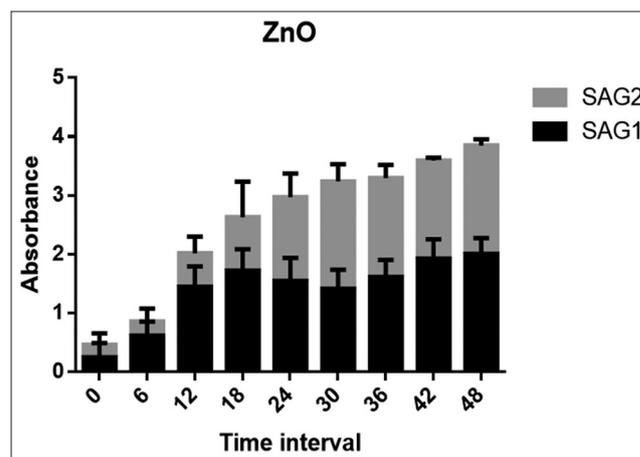


Fig. 8: Increase in absorbance in different time intervals of SAG1 and SAG2 for zinc oxide nanoparticles

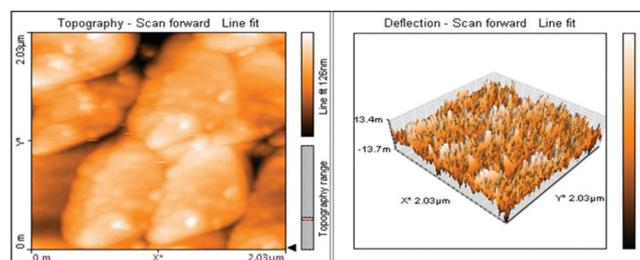


Fig. 9: Atomic force microscopy topography image of SAG1 silver nanoparticles

Table 1: Biochemical characteristics of isolates

Isolates	Indole	MR	VP	Citrate	Urease	Glucose fermentation	Galactose fermentation
SAG 1	+	+	+	+	+	+	+
SAG 2	-	-	+	+	+	+	+
SAG 3	-	-	+	+	+	+	+
SAG 4	+	+	+	+	+	+	+
SAG 5	+	+	-	+	+	+	+

Table 2: DPPH assay for analyzing antioxidant activity - AgNPs

Samples	Concentrations	% of DPPH activity
SAG1	100	63.63
SAG2	80	65.12

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

Table 3: DPPH assay for analyzing antioxidant activity - ZnONPs

Samples	Concentrations	% of DPPH activity
SAG1	100	54.10
SAG2	80	48.95

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

concluded that the shape of silver nanoparticle is round and size is approximately 31.78 nm (Fig. 9). On the other side, the shape of the ZnO nanoparticles is almost round and size is approximately 86.27 nm (Fig. 10).

FTIR analysis of silver and ZnO nanoparticles

The maximum peak for the synthesis of ZnO nanoparticles from the FTIR resulted in the range of 1300-1000 cm^{-1} . The functional group C-O is involved in the synthesis of this nanoparticle (Fig. 11). For AgNO_3 , maximum peak showed in the range of 3300-2500 cm^{-1} and the functional group involved here is O-H (Fig. 12).

SEM analysis of the nanoparticles

The SEM images of the silver nanoparticles have been given as Figs. 13 and 14. The images revealed the uniformity in the size of the nanoparticles.

DPPH assay for analyzing antioxidant activity

DPPH is a biological free radical which is violet in color. The antioxidant ability of the sample can be expressed by their capacity to scavenge DPPH radical. On the basis of this scavenging property, free radicals will turn to yellow color by the antioxidants present in the sample. This change of color from violet to yellow indicates a positive test. SAG1 and SAG2 silver nanoparticles showed 63.63% and 65.12% scavenging activity (Table 2). On the other hand, SAG1 and SAG2 ZnO nanoparticles showed 54.10% and 48.95% scavenging activity (Table 3).

DISCUSSION

In the previous study, UV spectrophotometer analysis showed highest peak on the range of 400-450 nm which confirmed the presence of silver nanoparticles, and they also observed a broad peak in the range of 300-350 nm confirming the synthesis of ZnO nanoparticles which was quite similar to our study [30]. Similarly, in a recent study, they have checked the absorbance in increasing time intervals and got color change in the broth from yellow to dark brown after 24 hrs only [31]. However, in another study, they did not observe any color change after 72 hrs also indicating there was no synthesis of silver nanoparticles [27]. We have got very similar AFM results for the characterization of the silver and ZnO nanoparticles observed in some previous studies, respectively [30,32]. We have got similar functional groups through FTIR analysis in some previous studies for the confirmation of AgNPs and ZnONPs,

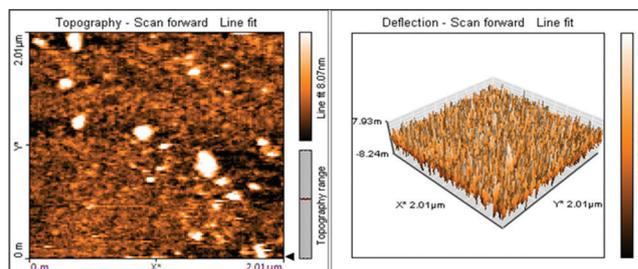


Fig. 10: Atomic force microscopy topography image of SAG1 zinc oxide nanoparticles

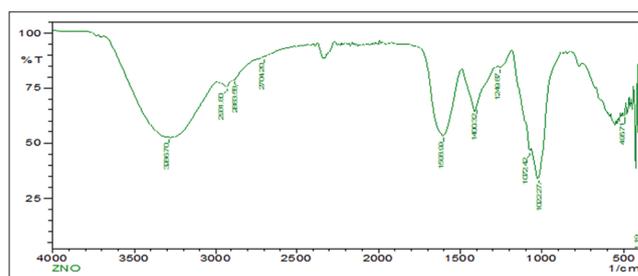


Fig. 11: Fourier transform infrared analysis of zinc oxide nanoparticles

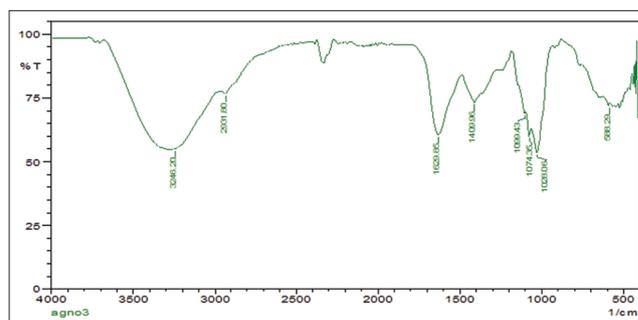


Fig. 12: Fourier transform infrared analysis of silver nanoparticles

respectively [25,33]. On the other hand, in a recent study, they have observed DPPH scavenging activity of synthesized silver nanoparticles extracted from leaf samples and they have found highest activity as 4.30 $\mu\text{g/ml}$ [34]. Similarly, in Lingaraju *et al.* [35], synthesized ZnO nanoparticles showed good scavenging activity with inhibitory concentration (IC_{50}) value of 9.34 mg/ml . In a recent study, they have also got the similar results [36]. In a similar study, methanolic extract of *Lantana camara* showed minimum IC_{50} value for DPPH assay ($48.75 \pm 2.34 \mu\text{g/ml}$) [37].

CONCLUSION

In this paper, green synthesis of silver nanoparticles and ZnO nanoparticles was done using marine yeast isolated from marine sediment sample of Bay of Bengal and West Bengal. Characterization and identification of both the nanoparticle were

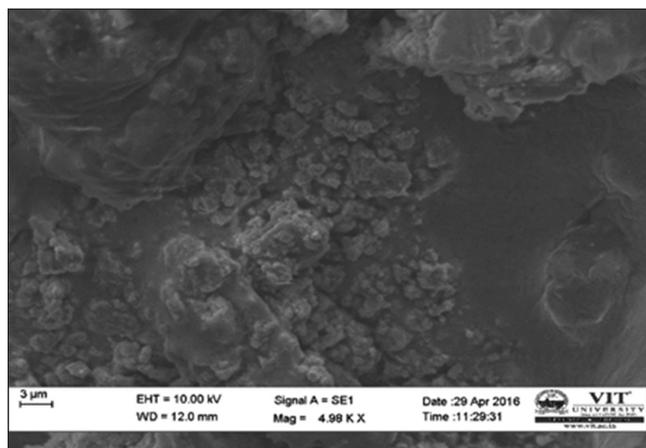


Fig. 13: Scanning electron microscopy image of silver nanoparticles

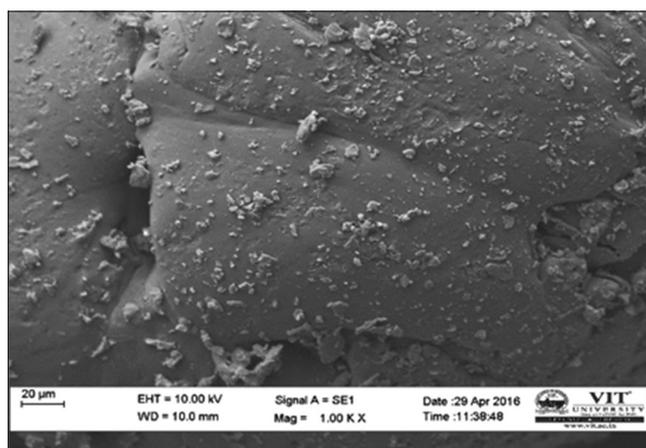


Fig. 14: Scanning electron microscopy image of zinc oxide nanoparticles

performed through UV-visible, SEM, and AFM. Antioxidant activity (DPPH) of the nanoparticles was also tested. We can conclude that marine yeast isolates SAG1 and SAG2 are very potential isolates and further work includes identification of these isolates using 18S rRNA sequencing.

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