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GREEN SYNTHESIS OF NANOSTRUCTURED MATERIALS FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract: New materials hold the key to fundamental advances in antibacterial and antifungal activities, both of which are vital in order to meet the challenge of global warning of microorganism's advantages and limitations and the finite nature of medicinal plants. The use of additive to augment the effect of a synthetic or natural drug candidate is well known. Here we report the use of nanoparticles of tin oxide (SnO_2) to enhance the antibacterial and anti fungal potency of *Alovera* extract when compared to bulk tinoxide (SnO_2). The possible advantage and limitations of this result will be discussed. It is hoped that this study would lead to the establishment of nanomaterial compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Antibacterial activity of *Alovera extracts* was checked against these gram positive isolates of *Staphylococcus aureus, Escherichia Coli E, Salmonella Typhi, Streptococcus pyogenes* and gram negative isolates of *Pseudomonas Aeruginosa.* We observed that effective anti-bacterial and anti-fungal activities for SnO_2 nanoparticles, particularly for *Streptococcus pyogenes* microorganisms and antifungal microorganisms of *Aspergillus niger, Mucor indicus* microorganism than bulk SnO_2.

Keywords: Antibacterial Activity; Antifungal Activity, Alovera Extract, Nanoparticles, SnO_2

INTRODUCTION

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance [1]. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a battery of detoxifying proteins the generation of reactive oxygen free radicals [2].

Figure.1: *Alovera* plant and its extracts

Aloe vera L. (syn.: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the *Aloeaceae* family (sub-family of the *Asphodelaceae*) [3]. Out of 400 *Aloe* species, *Alovera* is most widely used for various medical and cosmetic purposes. The plant is made of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of two parts: an outer green rind (skin) and an inner clear pulp (gel). The plant contains a large amount of phenolic compounds. With

a high content of 1, 8-dihydroxyanthraquinone derivatives (*aloe emodin*) and glycosides (aloins), which are used as cathartic. Various studies have revealed that *Aloe vera* leaf *skin (AVLS)* possesses many pharmaceutical activities, including purgative, antibacterial, anticancer, antifungal and antioxidant [4]. The alovera plant and its extract is shown in Figure 1. Mannose and glucose are the most significant sugars found in the *Alovera* gel, and they can be used to assay the activity of *Alovera*. Alovera is a member of liliaceae family. Alovera (L.) Burm. Fil (Synonym *A. broabadensis* Miller) is a cactus like plant with green, dagger shaped leaves that are fleshy tapering, spiny marginated and filled with clear viscous gel [5]. The *Aloe* Vera cell, visible at a magnification of about 40 000, is surrounded by a cell wall, has large nucleus and two cell membranes the cytoplasm of which manufacturers *mucopolysaccharide*. The mucopolysaccharide is stored within the lumen of the cell.

Alovera is used in cosmetics, drinks, detergents, as well as in stockings, diet foods, toothpaste and clothing. It is marked as a remedy for atherosclerosis, allergies, AIDS, prevention of radiation-induced dermatitis, wound healing, psoriasis, insomnia, cancer, pulpits and several other diseases. Scientific studies investigating these claims are few in number and the majority of them have been unable to diminish the intuitive skepticism against miracle cures. The conformational change of original form could mean a loss in its biological activities. The increasing use of biocompatible SnO_2 in pharmaceutical applications leads us to investigate the possible effects on SnO_2. However, the changes in activities with *Alovera* extract after conjugation of nanoparticles are not very clear and this needs some basic understanding before such complexes are used in biomedical applications.

Ibrahim *et al* [6] investigated the phyto-constituents and antimicrobial activity of aqueous, ethanol and acetone extracts of the *A. vera* gel against some human and plant pathogens by disc diffusion method. Among the three extracts, ethanol and acetone extracts recorded significant antimicrobial activity against all test pathogens. Antibacterial and antifungal activity of the acetone extract was found to be quite impressive as compared to ethanol and aqueous extracts.

Cock [7] studied the antimicrobial activity of *A. barbadensis* leaf gel components. Methanolic extracts of *A. barbadensis* inner leaf gel were fractionated by RP-HPLC and the resultant fractions were tested for inhibitory activity against a panel of bacteria and fungi. Five fractions were identified as having antimicrobial activity. Of which fraction 1 had the broadest antibacterial activity.

Agarry et al [8] compared the antimicrobial activities of ethanolic extracts of *A. vera* gel and leaf against *S. aureus*, *P. aeruginosa*, *Trichophyton mentagraphytes*, *T. schoeleinii*, *M. canis* and *C.albicans*. Antimicrobial susceptibility test showed that both the gel and the leaf inhibited the growth of *S. aureus*. Only the gel inhibited the growth of *T. mentagrophytes*, while the leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans*.

Thiruppathi *et al* [9] conducted a study to determine the antimicrobial activity of *A. vera* juice with different solvents viz., hexane, ethyl acetate, petroleum ether and ethanol against Gram positive bacteria (*B. subtilis*, *S. aureus*), Gram negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*). The result showed that more antimicrobial activity in ethyl acetate (1-9 mm) and ethanol extract (7-12mm). The least inhibitory effect on petroleum ether extract was 2 mm.

In addition, Arunkumar and Muthuselvam [10] used three different solvents aqueous, ethanol and acetone to extract the bioactive compounds from the leaves of *A. vera* to screen the antimicrobial activity against selected human clinical pathogens by agar diffusion method. They observed the maximum antibacterial activities in acetone extracts (12±0.45 nm, 20±0.35 nm, 20±0.57 nm and 15±0.38 nm) other then aqueous and ethanol extracts. The maximum antifungal activity was observed in acetone extracts (15±0.73 nm and 8±0.37 nm) when weighed against other extracts.

Nano-sized SnO_2 materials have been reported to possess specific properties and advantages of high sensitivity, including conductivity, transparency in the visible region, in addition to mechanical and chemical stabilities. The synthesis of nanometerials with well-controlled size, morphology and chemical composition may open opportunities in exploring new properties. The crystalline structure, the size and shape of the particles are highly dependent on the method of synthesis. Thus, it is necessary to adjust the synthetic method of the nanoparticles to assure high quality materials.

In general, gaseous electron donors or acceptors adsorb on the metal oxides and form surface states, which can exchange electrons with the semiconductor metal oxide. An acceptor molecule will extract electrons from the metal-oxide semiconductor and thus decrease the conductivity. The opposite holds true for an electron-donating molecule. A space charge layer will thus be formed. By changing the surface concentration of donors/acceptors, the conductivity of the space charge region is modulated so that the conductivity of metal-oxide semiconductor materials changes in response with respect to *alovera* extract

concentration which increases the reaction of microbial activities [11].

Here we reported that the antimicrobial activity of *Aloe vera* extract and *Alovera* extract with tin oxide (SnO_2) bulk and nnao particles in distilled water against *Staphylococcus Aureus* (MTCC-737), *Streptococcus pyogenes* (MTCC-1923), *Pseudomonas Aeruginosa* (MTCC-3542), *Escherichia Coli* (1576) and *Salmonella Typhii were discussed.* The number of bacterial colonies was significantly reduced by the application of extracts ($P < 0.05$) and the antimicrobial activity was especially strong against *E. coli* and *Vibrio* ($P < 0.001$).

In this article we report how SnO_2 + *Alovera* extract induces some modifications in the antibacterial activity also in antifungal activity leading to the growth of the extended assemblies and how it undergoes changes at the surface boundary of the nanoparticles.

MATERIALS AND METHODS

Synthesis of SnO_2 Nanopartides: In a typical synthesis, an aqueous solution containing ions of SnO_2 was prepared by dissolving typical amount of high purity the analytical grade chemicals i.e., tin metal ingots (99%, Aldrich, India supplied from Merck Chemical Company), distilled water (~ 100 ml) and nitric acid (99% Merck, India) in a glass beaker. Urea (99%, Merck, India) and 25% NH_3 (0.91 g cm^{-3}) was then added to the solution containing Sn ions. Amount of urea was calculated based on total valence of the oxidizing and reducing agents for maximum release of energy during combustion [12].

According to the principle of propellant chemistry [13], for a stoichiometric combustion reaction between a fuel and an oxidizer, the ratio of the net oxidizing valency of the metal nitrate to the net reducing valency of the fuel should be unity. The total oxidizing valency of Sn $(NO_3)_2.3H_2O$ works out to be -15. Urea (N_2H_4CO) was taken as a fuel, which contains one carboxylic and four hydroxyl group for coordinating the metal ions, which facilitate the formation of a viscous gel [14]. The total reducing valency of urea works out to be +6. Hence, in order to have the stoichiometric combustion reaction, one mole of tin nitrate needs 15/6 (or 2.5) mol of urea. This ratio of oxidant: fuel (1:2.5) gives a satisfactory viscous gel to initiate combustion. Since oxidant-fuel composition was optimum, sluggish decomposition was not possible and therefore the reaction not leftover carbonaceous material in the as-prepared powder [15].

Characterizations methods: The X-ray diffraction (XRD) pattern of the samples were measured by using using X-ray diffractometer (PANalytical X'Pert) with CuK_α radiation ($\lambda = 0.15406$ nm). The crystallite size D of the prepared powders was estimated using the Scherrer equation (1),

$$D = \frac{0.94\lambda}{\beta Cos\theta} \qquad (1)$$

where λ, β and θ are the X ray wavelength (0.15406 nm) and the full width half maximum of the diffraction peak (FWHM), respectively. Transmission electron micrograph (TEM) images were taken with a JEM-100CX transmission electron microscope.

Extraction of antimicrobial ingredients from *Aloe vera* leaves: Powder was made from *Alovera leaf extract* solution using a freeze dryer (Bondiro, Korea) by first rapidly freezing the peel and then eliminating the water by sublimation. The phosphate buffered saline (PBS) used in this study had a final concentration of 137 mM of sodium chloride, 2.7 mM of potassium chloride, 4.3 mM of sodium phosphate (dibasic) and 1.4 mM of potassium phosphate (monobasic), distilled water (DW) was used for the extraction taking into consideration the economic use for large scale industrial applications [16]. 50 g of material was *added* to 500 ml of DW and was shaken (250 rpm) for 8 hrs at room temperature. The solution was then centrifuged at 3000 rpm for 50 min. The supernatant was filtered through No. 5A filter paper (Advantec, Tokyo, Japan) and the filtered solution was freeze-dried again. 1 g of the dried powder was dissolved in 1.5 ml of DW to form a saturated solution.

To get the (*Alovera* leaf extract + SnO_2), 1 g of the dried powder was dissolved in 1.5 ml of DW. 1 g of the above said nano powdered particle was dissolved in 1.5 ml of DW equal ratio of these two solution were stirred well and dried in hot plate at 40 ° C (within the in nature temperature of *alovera* leaf extract) we get the dried powder. Like that 1 g of bulk powder of SnO_2 was dissolved in 1.5 ml of DW and the same ratio of *alovera* extracts was taken by repeating the same procedure we get the (*Alovera* extracts + Bulk SnO_2)

Evaluation of Antibacterial and Antifungal Activities: The supernatant was collected and stored in refrigerator at 4°C. Different concentration of *Alover* extracts was subjected to antimicrobial studies. Pure bacterial and fungal culture was obtained from Vivek's Laboratory, Nagercoil. Five bacterial cultures were maintained in nutrient agar medium at room temperature and were subcultured into newly prepared nutrient agar slants, every two-week.

The extracts of the *Alovera* plant and *Alovera* + SnO_2 were used throughout the study. The extracts were dissolved in sterile distilled water to form dilution such as 5, 10 and 25µg. Each concentration of the drug was tested against different bacterial pathogens for antibacterial activity. It was demonstrated by well diffusion assay. One species each of Gram positive

bacteria (*Staphylococcus Aureus* MTCC-737) and Gram negative bacteria (*Pseudomonas Aeruginosa* MTCC-3542) were used for the antibacterial assays.

The methanolic and aqueous extract of 100, 200 & 500 mg were tested against different fungal pathogens such as *Alovera* extract, *Alovera* extract with Bulk SnO_2 and *Alovera* extract with nano SnO_2 for the antifungal activities. This study revealed that the tested *Alovera* plant extract possesses potential antibacterial activity against *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*. The extracts with SnO_2 showed highest inhibitory activity against the tested bacteria are shown in Figures 7 and 11. The extracts with Bulk SnO_2 showed inhibitory activity lesser than the effect of nano SnO_2.

(i) Bacterial media (Muller Hindon Media): 36 g of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15 liquid bowl pound (lbp) pressure for 15 min. The sterilized media was poured into petridishes. The solidified plates were pored with 5 mm diameter cork borer. The plates with wells were used for the antibacterial studies.

(ii) Fungal Media (*Sabouraud Agar* Media): 10 g of peptone were taken and mixed with distilled water. 40 g of dextrose was mixed with peptone infusion. 20 g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were pored with 6 mm diameter cork borer.

(iii) Bacterial strains: The bacterial and fungal pathogenic strains were obtained from the Microbial Type Culture Collection (MTCC), the Institute of Microbial Technology, Sector 39-4 Chandigarh, India. Bacterial strains were *Staphylococcus Aureus* (MTCC-737), *Streptococcus pyogenes* (MTCC-1923), *Pseudomonas Aeruginosa* (MTCC-3542), *Escherichia Coli* (1576) and *Salmonella Typhii*.

(iv) Fungal strains: Fungal strains were *Aspergillus Niger* (MTCC-1344), *Aspergillus Flavus* (MTCC-1973), *Aspergillus Fumigatous* (MTCC-2132), *Rhizopus Indicus*, and *Mucor Indicus* (MTCC-918).

RESULTS AND DISCUSSION

Structure and microstructure SnO_2 nanopartides:

The crystallinity of the sample is clearly evident by the sharper diffraction peaks at respective diffraction angles which can be readily indexed for its rutile tetragonal structure of SnO_2. Obtained rutile phase is comparable with the standard JCPDS data (Card No. 88-0287).

In the 2θ = 34.3° region, the nonsymmetrical line shape originated from 101 peak situated. When it is mixed with *alovera* plant extract this peak was shifted and slightly amorphous in nature. Similarly in the 2θ=60° region, non-symmetrical peak shape is due to the indexed peak (310) and (301) situated at 2θ=62.1° and 65.8° respectively. But SnO_2 with *alovera* extract it was absent.

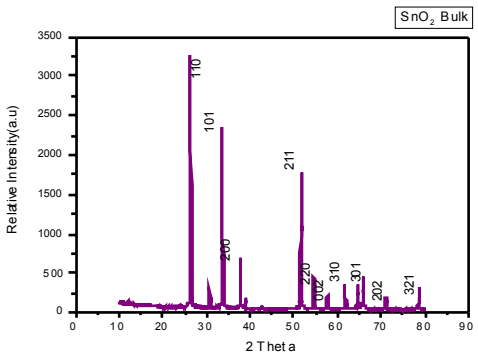

Figure.2a: XRD of Bulk SnO_2

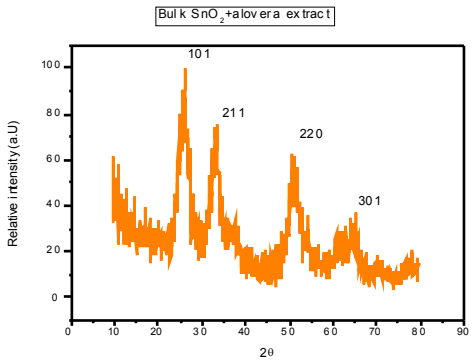

Figure.2b: XRD of Bulk SnO_2 + Alovera extracts

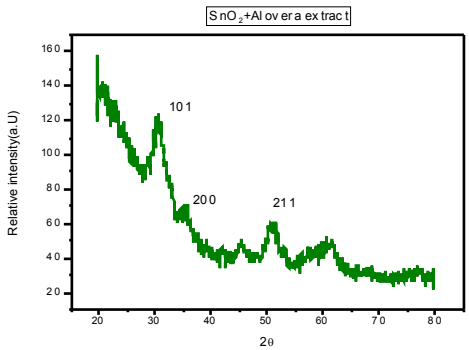

Figure.2c: XRD of SnO_2 nanoparticles

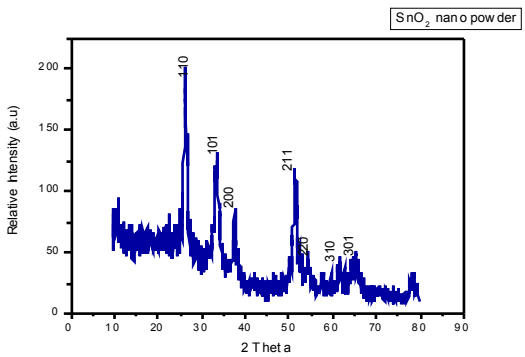

Figure.2 (d): XRD of SnO_2 nanoparticles + Alovera extracts

Figure 3 (a) shows a TEM micrograph of the as-

prepared SnO_2 powder, which reflects the highly agglomerated nanometer powder having nearly spherical nanocrystals with facets of size around 17 nm. The crystallite size observed by TEM is in good agreement with that estimated by X-ray line broadening (10 nm for the as prepared powder particles) of the X-ray diffraction pattern shown in Figure 2 (a). Figure 2(c) and Figure 2 (d) indicates that structural planes are disturbed when *alovera* extract was added with the mixture of SnO_2 (nano) and SnO_2 (bulk). This study again favors for the evolution of tetragonal phase of the product for the present preparative conditions probably due to the high degree of agglomeration which can reduce the surface energy. Figure 3 (b) shows TEM micrographs of SnO_2 powder with *alovera* plant extracts, the particle size increases nearly to 100 nm. Figure 3(c) shows the TEM micrographs of bulk SnO_2 with alovera plant extracts, the particle size increases above 100 nm, Figure 3(b) and 3(c), showed the disturbances in the diffraction planes.

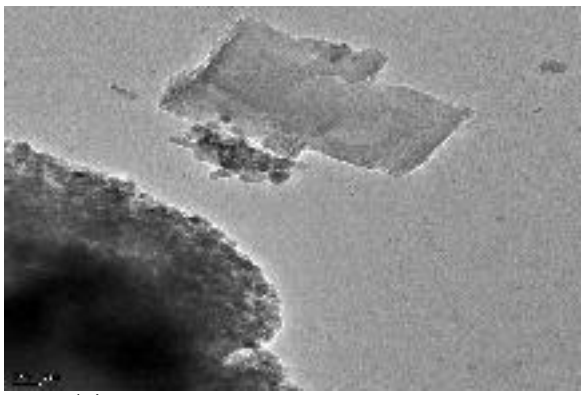

Figure.3 (a): TEM micrograph of SnO_2

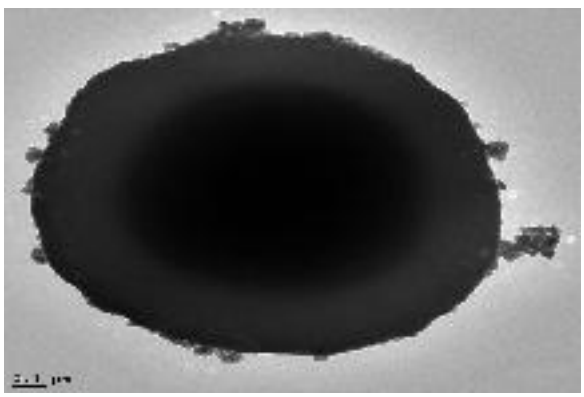

Figure.3 (b): TEM micrograph of SnO_2 nanoparticles + Alovera extracts

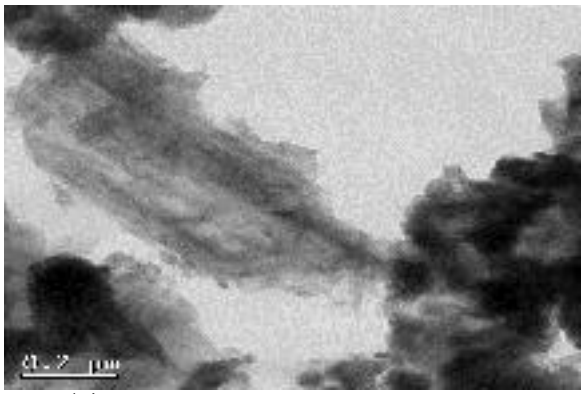

Figure.3(c): TEM micrograph of SnO_2 bulk powder + Alovera extracts

Anti-bacterial and Anti-fungal activity:

Anti-microbial activity was checked by agar gel diffusion method. The cultures were grown in nutrient broth and incubated at 37°C for 24 hrs. After incubation period was over, 0.1 ml of culture was seeded in 25 ml molten nutrient agar butts, mixed and poured into sterile petri plates and allowed to solidify. The well was bored with 6 mm borer in seeded agar. 0.1 g of each *Alovera* extract or *Alovera* extract with SnO_2 sample was added in each well. Plates were kept at 10°C as a period of pre-diffusion for 30 min. When the plates reached the room temperature; the plates were incubated at 37°C for 24 hrs in case of bacteria and at 27°C for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with Hi-antibiotic zone scale. Antibacterial activity of the plant extract was tested using well diffusion method [17]. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 5 mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 24 hours for bacterial activity. The plates were observed for the zone formation around the wells. The extracts were dissolved in sterile distilled water to form dilution such as 5, 10 and 25 µg. Each

Figure.4: *Staphylococcus aureus microorganism in alovera extract*

Figure.5: *Staphylococcus aureus microorganism in alovera extract with SnO_2 nanoparticles*

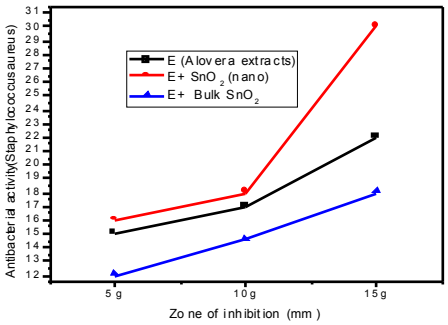

Figure.6: *Staphylococcus aureus microorganism in alovera extract with bulk SnO_2* Figure 7. Comparison studies of antibacterial activity by using micro organism of (*Staphylococcus aureus*) with nano SnO_2 and bulk SnO_2

concentration of the drug were tested against different bacterial pathogens *Staphylococcus Aureus*, *Pseudomonas aeruginosa* are shown in Figures 4, 8, and 12 for pure extract (E) and Figures 5, 9, 13 and 15 extract (E) + SnO_2 (E+SnO_2). The antifungal activities are shown in Figures 16 and 17 only for Alovera extract + nano SnO_2. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were plotted Figure 18. The comparison studies of 3 bacterial activities are shown in Figures 7, 11 and 15. From this study we observed that there is no effective bacterial and fungal activities for bulk SnO_2, particularly for *Streptococcus pyogenes* microorganisms and antifungal microorganisms of *Aspergillus niger*, *Mucor indicus microorganism.*

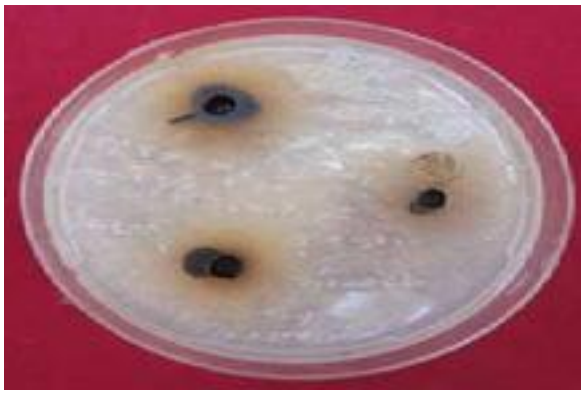

Figure.8: *Pseudomonas aeruginosa microorganisms with alovera extract*

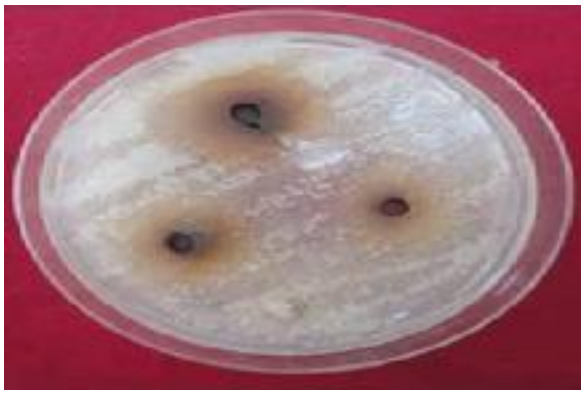

Figure.9: *Pseudomonas aeruginosa microorganism with extract + SnO_2 (nano)*

Figure.10: *Pseudomonas aeruginosa microorganism with extract + bulk SnO_2*

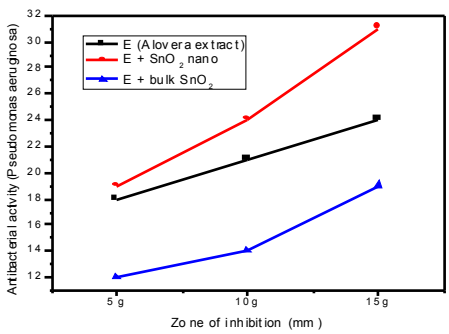

Figure.11: Comparison studies of antibacterial activity by using micro organism of (*Pseudomonas aeruginosa*) *with E, E + nano SnO_2 and E + bulk SnO_2*

Figure.12: *Streptococcus pyogenes microorganisms with alovera extract*

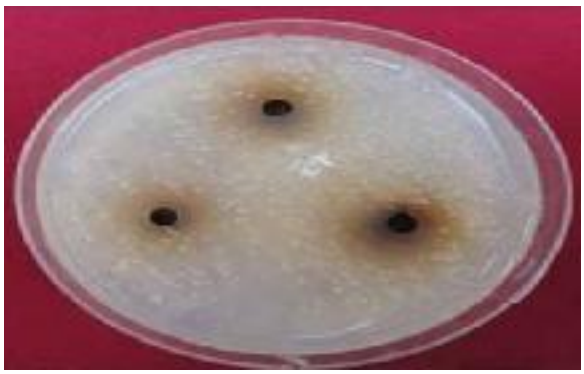

Figure.13: *Streptococcus pyogenes* microorganisms with alovera extract + nano SnO_2

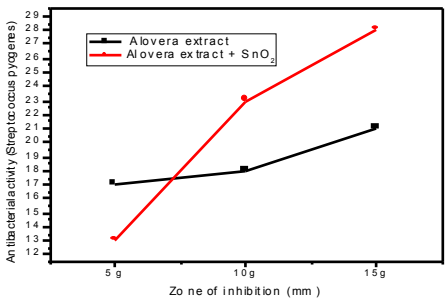

Figure.14: Comparison studies of antibacterial activity of by using microorganism (*Streptococcus pyogenes*) with E and E+ nano SnO_2

Table1. Percentage of gram positive and gram negative clinical skin infection isolates

Isolate	Total number of organism	Total percentage (aloe vera extract)	Total percentage (aloe vera extract + SnO_2)
Gram Positive	100	--	--
Staphylococcus aureus	55	48.1	49.2
Escherichia coli	35	30.8	31.1
Salmonella typhi	20	19.0	19.2
Streptococcus pyogenes	10	8.3	8.9
Gram negative	15	--	--
Pseudomonas aeruginosa	15	13	13.5

Determination of Minimum Inhibitory Concentration (MIC):

Numerous studies report on the effectiveness of this plant when used topically for the healing of burns, sunburns, inflammatory skin disorders and wounds [18]. The present study was designed to identify the antibacterial activity of *Alovera extract* and *Alovea* extract + Bulk SnO_2 and Alovera extract + SnO_2 nao powders which were also compared with five standards anti bacterial against clinical isolates from community acquired skin infections. The antibacterial activity was monitored using agar-well diffusion and agar disc diffusion method; the activity was determined by noting the zones of inhibition around the wells or discs [19]. Antibacterial activity of *Alovera extracts* was checked against these isolates shown in Table.1. It shows the percentage of gram – positive isolates was as follows: *Staphylococcus Aureus* for E (48.1%), E+Bulk SnO_2 (27.3%) and E+SnO_2 nano powder (49.2%) , *Escherichia Coli* E (30.8%), E + Bulk SnO_2 (25.3%) and E+SnO_2 nano powder (31.1), *Salmonella Typhi* for E (19%), E+Bulk SnO_2 (17%) and for E+SnO_2 nano powder (19.2) and *Streptococcus pyogenes* for E (8.3%), E+ Bulk SnO_2 (0%) but E+ SnO_2 nano powder (8.9%) while the percentage of gram negative isolates includes: *Pseudomonas Aeruginosa* for E (13%), E + Bulk SnO_2 (-0-%) and for E+SnO_2 nano powder (13.5%). *Alovera* has been used as a cosmetic and medical remedy since ancient times and has gained increasing antimicrobial activity and when SnO_2 is added with extract it enriches the activities. *Alovera extracts* showed 100% activity against gram negative isolates and 75.9% against all tested gram positive isolates. This result could be responsible for the popular use of *Alovera extracts* to relieve many types of gastrointestinal irritations, since *Saureus* form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract [20]. Also the leaf extract is said to promote wound healing due to the presence of some components like *Anthraquinones* and hormones [21], which possess antibacterial antifungal and antiviral activities. However, most of the constituents are found in the leaf extract, hence the leaf gel extract is likely to be more active [22].

Figure.15: Antifungal activity *Aspergillus niger microorganism alovera extract with nano SnO2.*

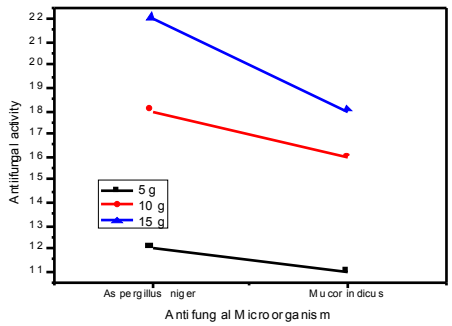

Figure.16: Comparison studies of antifungal activity of alovera extract with nano SnO_2

CONCLUSION

Here we report the findings of the use of nanoparticles of SnO_2 with Aloe vera for anti-bacterial and anti-fungal properties. These studies highlight the size modified inorganic salt that modulates the biological property of a natural compound. Further studies to optimize the ratio and concentration of SnO_2 nanoparticles for maximum potency will be conducted. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to identify the bioactive compound and to evaluate the mechanisms of action of *Alovera* extracts on some organisms associated with human diseases.

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