CHAPTER – III

3. Experimental Details

3.1. Materials and Chemicals

Titanium tetra(IV)isopropoxide [Ti(OC₃H₇)₄] was purchased from Acros Organics Limited, India. Tin tetrachloride pentahydrate [SnCl₄.5H₂O], Zirconium oxychloride octahydrate [ZrOCl₂.8H₂O] and citric acid were availed from Merck Private Limited, India. All the chemicals were used without further purification. The leaves of Aloe vera and flowers of Nyctanthes arbor-tristis plants were collected from Karaikudi, Tamil Nadu, India. Woven cotton fabric was purchased from Ayyappa Textiles, Karaikudi. Double distilled water was used throughout the reaction process.

3.2. Preparation of Aloe vera gel extract

Freshly collected leaves of Aloe vera plant were first washed thoroughly with pure water before extraction. Inner gel of the leaf was extracted by peeling the outer skin manually by cleaned knife. About 50 g of the collected inner gel were finely cut into small portions and extracted by boiling with 100mL of double distilled water. The resulting extract was cooled to room temperature and filtered using a pure cotton cloth followed by filtration with Whatman No.1 filter paper. The filtered extract was collected in a closed container and stored in refrigerator at 4ºC for further use. The colour of the collected extract is light green. This Aloe
vera gel extract was used as the solvent and hydrolyzing agent instead of external chemical agents for the synthesis of TiO$_2$, SnO$_2$ and ZrO$_2$ nanoparticles.

3.3. Preparation of Nyctanthes arbor-tristis flower extract

Fresh taxonomically authenticated healthy flowers of Nyctanthes arbor-tristis collected from our local garden were washed with pure water. The corolla tubes of flowers were separated and dried at room temperature for about 10 days under dust free condition. Then the dried tubes were cut into fine pieces and grinded to get the very fine powder. About 10g of the grinded corolla tubes of flowers were extracted by boiling with 100mL of double distilled water. The resulting extract was cooled to room temperature and filtered using a pure cotton cloth followed by filtration with Whatman No.1 filter paper. The filtered aqueous flower extract was collected in closed container and stored in refrigerator at 4ºC for further use. Orange colour was observed for the Nyctanthes flower extract. This Nyctanthes arbor-tristis flower extract was used to perform multifunctions on the synthesis of TiO$_2$, SnO$_2$ and ZrO$_2$ nanoparticles.

3.4. Biosynthesis of TiO$_2$ nanoparticles using Aloe vera gel extract

TiO$_2$ nanoparticles were prepared by a biological route using aqueous extract of Aloe vera gel. A starting solution of about 0.4M titanium tetra(IV)isopropoxide (TTIP) was prepared using 50mL of aqueous extract of Aloe vera gel. The prepared TTIP solution with the Aloe vera gel extract was subjected to vigorous stirring for about four hours. The precipitation of the titanium hydroxide [Ti(OH)$_4$] precursor was predicted by the light greenish white colloidal
particles, which can be believed that due to the progress of the starting material, titanium tetra(IV)isopropoxide to form hydrated Ti(OH)$_4$. The formed (Ti(OH)$_4$ particles with the Aloe vera gel extract was allowed to stand for one day. The particles were then separated from the reaction solution by centrifugation at 10000 rpm (KUBOTA-6800 Centrifuge Machine) for 10 minutes at room temperature. Separated Ti(OH)$_4$ particles was then washed with water repeatedly to remove the impurities and dried in an hot air oven at 80°C. The dried precursor was powdered by grinding and crushing using mortar and pestle. The powdered Ti(OH)$_4$ was then subjected to calcination in muffle furnace at 500°C for 3 hours to receive the pure and white coloured TiO$_2$ nanoparticles by the evaporation of adsorbed natural extract and dehydration of the Ti(OH)$_4$ precursor.

3.5. Biosynthesis of TiO$_2$ nanoparticles using Nyctanthes arbor-tristis flower extract

TiO$_2$ nanoparticles were prepared using a biosynthesis method at room temperature. About 0.4M of TTIP, a starting material was dissolved in 50 mL of Nyctanthes extract followed by stirring for the complete dissolution of starting material. During stirring, the precipitation of the hydrated form of titanium oxide, Ti(OH)$_4$ product was appeared. After the completion of the reaction by 4 hours of stirring, the precipitate was allowed to settle for whole day. The settled precipitate was separated from the supernatant by centrifugation for 10 minutes at 10000 rpm followed by repeated washing with distilled water to remove the aggregated and entrapped impurities. The centrifuged particles were dried in hot air oven at 80°C. The dried yellow coloured Ti(OH)$_4$ was grinded to get the fine powder. The powdered material was calcinated at
500°C to produce the nanosized TiO₂ nanoparticles. Finally formation of pure white coloured TiO₂ particles were observed after evaporation or decomposition of biological matters of the natural extract by the calcination process.

3.6. Biosynthesis of SnO₂ nanoparticles using Aloe vera gel extract

Biological method of synthesis was followed to prepare SnO₂ nanoparticles using the natural matters of Aloe vera gel extract. In a typical procedure, 0.4M solution of hydrated tin tetrachloride (SnCl₄) was prepared with 50 mL of aqueous extract of Aloe vera gel extract. The starting reaction mixture was stirred for about four hours at room temperature. After the required time of stirring, precipitation of tin hydroxide [Sn(OH)₄] was recognized by the dirty greenish white coloured colloidal particles at the bottom of the flask. It is believed that the colour change indicates the progress of the hydrolysis of tin chloride to Sn(OH)₄. The precipitated precursor Sn(OH)₄ was allowed to stand for one day before centrifugation. The Sn(OH)₄ particles were separated from the reaction solution by using centrifuge machine at 10,000 rpm for 10 minutes at room temperature. The precursor was then washed with water repeatedly to remove the organic and aggregated impurities and dried in hot air oven at 80°C. The dried material of Sn(OH)₄ precursor was calcinated in muffle furnace at 500°C for 3 hours. After the heat treatment, dirty greenish white coloured precursor was dehydrated and white coloured SnO₂ nanoparticles were formed by removing the adsorbed organic functional groups of Aloe vera gel extract.
3.7. Biosynthesis of SnO\(_2\) nanoparticles using *Nyctanthes arbor-tristis* flower extract

In a typical procedure, 0.4M of SnCl\(_4\).5H\(_2\)O was added with 50mL of water extract of *Nyctanthes arbor-tristis* flower hosted in a conical flask. The solution was refluxed at room temperature for a certain time with constant stirring under atmospheric pressure for 4 hours. During refluxing colour of the contents changes from dark orange yellow into slight yellow colloid when the time increases. It is believed that the colour change indicates the progress of the hydrolysis of tin chloride into Sn(OH)\(_4\). The particles were separated by centrifugation at 10000 rpm for 10 minutes from the supernatant solution after settled for one day. For removing the unwanted organic waste after they were centrifuged, washed with deionized water and dried in hot air oven at 80°C. A dry powder of Sn(OH)\(_4\) precursor was crushed into fine powder using mortar and pestle to subject heat treatment of Sn(OH)\(_4\) sample at 500°C in muffle furnace for 3 hours. After the required time of heat treatment, dehydrated white coloured tin oxide nanoparticles were obtained by the removal of colouring matter and organic moieties of *Nyctanthes arbor-tristis*.

3.8. Biosynthesis of ZrO\(_2\) nanoparticles using *Aloe vera* gel extract

Synthesis of ZrO\(_2\) nanoparticles were performed by biological approach using *Aloe vera* gel extract. About 0.4M of zirconium oxychloride octahydrate [ZrOCl\(_2\).8H\(_2\)O] was prepared using 50mL of aqueous extract of *Aloe vera* gel. The reaction mixture was subjected to vigorous stirring at room temperature for about 4 hours. The precipitation of the product was predicted by the turbid white colloidal particles at the bottom of the flask, which can be believed that due to
the progress of the starting material, ZrOCl$_2$.8H$_2$O to form zirconium hydroxide [Zr(OH)$_4$]. The precipitated sample was allowed to stand for one day before centrifugation. The particles were separated by centrifugation at 10000 rpm for 10 minutes at room temperature. To remove the impurities on the surface of the Zr(OH)$_4$ precursor, it was washed with deionized water and dried in hot air oven at 80°C. The dried particles were crushed into fine powder before calcination. The dried sample was subjected to calcination in muffle furnace at 500°C to form the ZrO$_2$ nanoparticles by dehydration of the Zr(OH)$_4$ with the simultaneous evaporation of other organic compounds of Aloe vera extract.

3.9. Biosynthesis of ZrO$_2$ nanoparticles using Nyctanthes arbor-tristis flower extract

In a typical experiment, about 0.4M ZrOCl$_2$.8H$_2$O was prepared using 50mL of aqueous extract of Nyctanthes arbor-tristis. Zr(OH)$_4$ precursor was precipitated by subjecting the starting solution under vigorous stirring for about four hours. After the completion of the reaction, the formed yellow coloured precipitate was allowed to settle for whole day. The precipitate was separated from the reaction solution by centrifugation at 10000rpm for 10 minutes, washed with water repeatedly to remove the impurities and dried in hot air oven at 80°C. The dried as-prepared sample i.e. Zr(OH)$_4$ was powdered by grinding and crushing using mortar and pestle. The powdered Zr(OH)$_4$ particles was then subjected to calcination in muffle furnace at 500°C for 3 hours. After the calcination process, dehydrated white coloured ZrO$_2$ nanoparticles were obtained by removing the yellow colour of as-prepared sample which is due to the adsorption of bioorganic matters of Nyctanthes extract.
3.10. Treatment of cotton fabric with biosynthetic metal oxide nanoparticles

The prepared metal oxide nanoparticles such as TiO$_2$, SnO$_2$ and ZrO$_2$ using two different plant extracts were applied separately on cotton fabric. Treatment of fine medium weight of woven cotton fabric with metal oxide nanoparticles were carried out by direct application system of pad dry cure method. The cotton fabric specimen of dimension 12 cm × 12 cm was immersed in the solution containing 6% of citric acid, a crosslinking agent and 3% of biosynthetic metal oxide nanoparticles for about 3 hours. Then the fabric was padded on the laboratory padder. The fabric was passed between the rollers at a padding pressure of 3psi for the uniform distribution of nanoparticles and 100% wet pickup on the fabric. Finally the padded fabric was subjected to air drying and curing at 80°C and 150°C for 5 and 3 minutes respectively.

3.11. Characterization of as-prepared metal oxides

3.11.1. Thermogravimetric analysis

Thermal analysis is a group of techniques in which changes of physical or chemical properties of the sample are monitored against time or temperature, while the temperature of the sample is programmed. Thermogravimetric analysis (TGA) is a technique in which the mass of the sample can be monitored during the temperature program. The schematic diagram of TGA is shown in Fig. 3.11.1.
TGA measurements were done on a SDT Q600 V8.3 101 thermogravimetry system with the temperature programming software for the furnace. TGA of the metal oxide samples before calcination i.e. as-prepared sample was carried out in an air atmosphere between room temperature to 800°C. The weight of the sample used was less than 10mg and the rate of elevating temperature was 10°C/min. The sample weight loss (TG signal) functions of time and temperature were recorded continuously under dynamics conditions in the range of 30 - 800°C for the temperature measurements. In this investigation, TGA data were used to determine the thermal decomposition, crystallization temperature effect of phase transitions of the as-prepared samples [1, 2].

Working of TGA

Measurements of changes in sample mass with temperature are made using a thermobalance. This is a combination of a suitable electronic microbalance with a furnace and associated temperature programmer. The balance should be in an enclosed system so that the atmosphere can be controlled. X axis is temp (or time, since usually a linear heating rate) and Y
axis is percentage of mass loss. As the specimen changes weight, its tendency to rise or fall is
detected by linear variable differential transformer (LVDT). A current through the coil on the
counterbalance side exerts a force on the magnetic core which acts to return the balance pan to a
null position. The current required to maintain this position is considered proportional to the
mass change of the specimen.

3.11.2. Differential thermal analysis

Differential thermal analysis (DTA) of as-prepared sample was carried out on SDT Q600
V8.3 101 thermogravimetry system between room temperature to 800°C. The default ramp rate
was 10°C/min. The weighed sample was placed into an aluminium sample pan for DTA using an
empty aluminium pan as a reference. The instrument measures the difference in heat flow
between the sample and the reference material. A graph of heat flow versus temperature was
plotted to show the exothermic and endothermic events associated with the material.

Working of DTA

The sample and reference chambers are heated equally into a temperature regime in
which a transformation takes place within the sample. As the sample temperature deviates from
the reference temperature, the device detects it and reduces the heat input to one cell while
adding heat to the other in order to maintain a zero temperature difference between the sample
and reference. The quantity of electrical energy per unit time which must be supplied to the
heating element in order to maintain null balance is assumed to be proportional to the heat
released per unit time by the sample.


3.12. Characterization of synthesized metal oxide nanoparticles

3.12.1. X-ray Diffraction Analysis

X-ray Diffraction (XRD) is a powerful, non-destructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations and other structural parameters such as average grain size, crystallinity and crystal defects. The XRD pattern is the fingerprint of periodic atomic arrangements in a given material. When electrons strike a metal anode with sufficient energy, X-rays are produced (Fig. 3.12.1). This process is typically accomplished using a sealed X-ray tube, which consists of a metal target (often copper metal) and a tungsten metal filament, which can be heated by passing a current through it, resulting in the emission of electrons from the tungsten filament. These electrons are accelerated from the tungsten filament to the metal target by an applied voltage. The collision between these energetic electrons and electrons in the target atoms results in electron from target atoms being excited out of their core-level orbitals, placing the atom in a short-lived excited state. The atom returns to its ground state by having electrons from lower binding energy levels making transitions to the empty core levels. The difference in energy between these lower and higher binding energy levels is radiated in the form of X-rays. This process results in the production of characteristic X-rays [3, 4]. X-ray powder diffractometers record all reflections using a detector. XRD analysis was performed for as-prepared and calcinated metal oxide nanoparticles of TiO₂, SnO₂ and ZrO₂ synthesized using the extract of Aloe vera gel and Nyctanthes arbor-tristis flower. XRD instrument used was PANalytical X-ray diffractometer with a Cu-Kα X-ray source operated at 40 kV with a current of 30 mA (λ=0.15406 nm).
From the XRD analysis, the size and the shape of the unit cell for any compound can be determined most easily using the diffraction of X-rays with the aid of JCPDS software. The crystallite size of prepared metal oxide nanoparticles [5] were calculated using Debye-Scherrer’s equation, \( D = \frac{0.89 \lambda}{\beta \cos \theta} \) where D is the crystallite size, \( \lambda \) is the wavelength of the radiation and \( \beta \) is the full width and half maximum and \( \theta \) is the diffraction angle.

![Figure 3.12.1: Schematic diagram of XRD spectrometer](image)

### 3.12.2. Scanning Electron Microscopy

Scanning Electron Microscope (SEM) is commonly used to study surfaces, structures, morphologies and forms of materials. The images viewed using SEM are created by detecting secondary electrons ejected from samples as they are bombarded by focused, high energy electron beams. Unlike optical microscopy, one does not look through lenses at the actual sample, but one observes images of the sample created by the instrument’s electronics. SEM can
achieve higher magnifications than optical microscopes. When samples are probed with focused electron beams, a variety of signals can be collected and displayed on the view screen. In addition to secondary electron signals (Fig. 3.12.2), X-rays characteristic of the elemental composition of the sample can be mapped to sample images, and back-scattered electrons can also be collected and displayed. When SEMs are fitted with appropriate detectors, one can not only see images of the samples (using secondary and back-scattered electron signals) but one can also see images which map the elemental compositions of the samples.

Figure 3.12.2: Schematic diagram of SEM instrument

SEM analysis was conducted in vacuum environments. Surface morphology and size distribution of particles were examined by scanning electron microscopic images [6, 7]. SEM images of prepared metal oxide nanoparticles and treated cotton with nanoparticles were
recorded on a JEOL JSM 6390 SEM instrument using an accelerating voltage of 15 kV. Scanning rate of 0.02°/s was employed in the 2θ range of 20° to 80° at the intervals 0.02°.

3.12.3. Energy Dispersive Spectroscopy

Interaction of an electron beam with a sample target produces a variety of emissions, including X-rays. An energy-dispersive (EDX) detector is used to separate the characteristic X-rays of different elements into an energy spectrum and EDX system software is used to analyze the energy spectrum in order to determine the abundance of specific elements. EDX can be used to find the chemical composition of materials. EDX capabilities provide fundamental compositional information for a wide variety of materials.

![Figure 3.12.3: Schematic diagram of EDX instrument](image-url)
EDX systems are typically integrated with either a scanning electron microscope or transmission electron microscope instruments. EDX systems include a sensitive x-ray detector, a liquid nitrogen chamber for cooling, and software to collect and analyze energy spectra. The detector is mounted in the sample chamber of the main instrument. An EDX detector contains a crystal that absorbs the energy of incoming X-rays by ionization (Fig. 3.12.3), yielding free electrons in the crystal that become conductive and produce an electrical charge bias. The x-ray absorption thus converts the energy of individual X-rays into electrical voltages of proportional size; the electrical pulses correspond to the characteristic X-rays of the element. The advantages of EDX are a user can acquire a full elemental spectrum in only a few seconds. Supporting software makes it possible to readily identify peaks, which makes EDX a great survey tool to quickly identify unknown phases prior to quantitative analysis [8, 9]. EDX images of synthesized metal oxide nanoparticles and treated cotton with prepared metal oxide nanoparticles were recorded on a JEOL JSM 6390 SEM instrument with thermo EDX attachment using an accelerating voltage of 15 kV to determine the exact elemental composition.

3.12.4. Atomic Force Microscopy

Atomic force microscopy (AFM) has a broader range of capabilities than scanning tunneling Microscopy (STM). AFM can be used to investigate any surface, even poorly or non-conducting ones, which broadens its potential applications significantly. The instrument measures forces on a surface by scanning the sample with the tip attached to a flexible cantilever. The resolution obtained by AFM is determined in large part by the size of the probe tip used for imaging. Pyramidal or needle shaped silicon (Si) or silicon nitride (Si$_3$N$_4$) are the two main tips
used in AFM. These probes have end radii of curvature as small as 10 nm but are often much larger. Advances are always being made to create a better tip, for example, SuperSharpSilicon™ tips are offering radii of typically 2 nm. This way of AFM operates is similar to the principle behind the record player in that the tip moves up and down in response to the surface features. An optical readout or a piezoelectric crystal translates the motion of the cantilever into an electronic signal. The outcome is a three-dimensional image of the surface structure displayed on a screen. Maximum resolution is typically on the atomic scale in the lateral and vertical directions [10].

Figure 3.12.4 shows the schematic diagram of AFM instrument. AFM technique is used in order to study the accurate height and width distribution of the synthesized nanoparticles and shape of the desired nanoparticles. In this work, prepared metal oxide nanoparticles such as TiO$_2$, SnO$_2$ and ZrO$_2$ were analyzed using SPM Solver P47H PRO model AFM instrument for their size distribution.

![Figure 3.12.4: Schematic diagram of AFM instrument](image_url)
3.12.5. Transmission Electron Microscopy

Transmission electron microscopy (TEM) is an imaging technique whereby a filament (Tungsten) is heated to produce a beam of electrons [6]. The voltages generally range from 100 kV to 200 kV. The sample that is being observed is held in a high-vacuum object chamber that can be reached from outside through an inside chamber. The electron beam is focused with the help of the first and second condenser lenses which are user selectable and allowed to focus on the sample. Most of the times the sample has to be thin enough so that the electrons can pass through them to form the image. The electrons interact with the sample and hit the phosphor screen at the end. The image is then imposed on a photographic film or detected by a sensor. (Fig. 3.12.5).

![Schematic diagram of TEM instrument](image)

Figure 3.12.5: Schematic diagram of TEM instrument
The darker areas of an image represent those areas of the sample that fewer electrons were transmitted through it (they are thicker or denser). The lighter areas of the image represent those areas of the sample that more electrons were transmitted through it (they are thinner or less dense). The advantages of TEM are small sample loading, less preparation time and moreover the ability to see nanometer range particles. Bright field images of TiO$_2$, SnO$_2$ and ZrO$_2$ nanoparticles was undertaken with a Philips/CM 200 TEM microscope instrument operating at an acceleration voltage of 200kV with a resolution of 2.4Å. Ultrasonically dispersed samples used for TEM measurements were coated on copper grids and dried at room temperature.

3.12.6. Ultraviolet-Visible Spectroscopy

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of light when it is passed through a sample. The principle of UV-Vis spectroscopy is based on the ability of molecule to absorb ultraviolet and visible light [11]. Absorption spectra of the prepared metal oxide nanoparticles were recorded by using Perkin Elmer LAMBDA 35 UV-VIS spectrophotometer. The light generated from a Xenon flash lamp and is passed through the monochromator which splits the beam into different wavelengths out of the continuous spectrum. The intensity ‘$I_0$’ is measured by the fraction of beam redirected using beam splitter. The transmitted intensity ‘$I$’ of the light beam is measured at photodetector and the absorbance is calculated by the formula, $A = \log I_0/I$. The absorption spectrum can be plotted as a function of the wavelength in an absorption spectrum. The schematic representation of UV-Vis spectrophotometer is shown in Fig. 3.12.6.
3.12.7. Fourier Transform Infrared Spectroscopy

The functional groups present in the prepared metal oxide nanoparticles were identified by Fourier transform infrared spectroscopic (FT-IR) technique. The vibrational frequency of bond stretching/bending can be approximated by relative contributions of bond strengths and atomic masses [12].
FT-IR instrument (Fig.3.12.7) was calibrated by adopting standard test procedures. Once the calibration process is over, the instrument is made ready for analysis of the unknown samples. The sample under investigation is taken in a mortar containing few milligrams of pure moisture free KBr followed by the grinding of the mixture using pestle. The well ground mixture is then cast in a die set. The die set is closed after loading the ground sample. This is now pressed in a hydraulic applying little pressure on to the die set. Once this press is over, the die set is removed from the hydraulic press and opened carefully to get thin transparent pellets of uniform size. The above made pellet is then placed in the sample holder provided in the instrument for running the spectrum followed by the closing of sample compartment doors ensuring no interference of moisture inside the instrument. The instrument software is opened and the required parameters like recording range (4000 – 400 cm$^{-1}$) are furnished before beginning the recording of spectra for the unknown sample. Now, the scanning of the instrument is started to record the characteristic absorbance/transmittance spectra for the sample under investigation. The plot of wavenumber Vs absorbance/transmittance is manipulated using the characteristic behaviour of various functionalities present in the unknown chemical substance [12]. FT-IR spectra of the Aloe vera gel extract, Nyctanthes arbor-tristis flower extract, as-prepared samples and calcinated TiO$_2$, SnO$_2$ and ZrO$_2$ nanoparticles were recorded in transmission mode on KBr pellets using a Perkin Elmer RX1 spectrometer with 4 cm$^{-1}$ resolution.

3.12.8. Antibacterial and antifungal study by agar diffusion method

Antimicrobial activity such as antibacterial and antifungal study of the synthesized metal oxide nanoparticles and metal oxide nanoparticles treated cotton were carried out by agar
Agar diffusion method. Agar diffusion method, also known as Kirby-Bauer method is a relatively quick and easily executed semiquantitative test for the testing of antimicrobial activity. Antibacterial activity of the samples was evaluated against *Staphylococcus aureus* ATCC-11229 (*S. aureus*), gram positive bacteria and *Escherichia coli* ATCC-25923 (*E. coli*), gram negative bacteria by agar diffusion method. Antifungal activity was determined against *Candida albicans* ATCC-10231 (*C. albicans*) and *Aspergillus niger* ATCC-16888 (*A. niger*) fungal strains. The antimicrobial activity of the prepared metal oxide nanoparticles and treated cotton was evaluated by measuring the zone of inhibition against the test organisms. Zone of inhibition is the area in which the bacterial or fungal growth is stopped due to bacteriostatic or fungistatic effect of the compound and it measures the inhibitory effect of compound towards a particular microorganism [13, 14]. Finally, diameters of zone of inhibition of the control strain and test can be measured with a ruler or calipers (Fig. 3.12.8).

![Figure 3.12.8: Antimicrobial study by agar diffusion method](image)

Figure 3.12.8: Antimicrobial study by agar diffusion method
The bacterial culture medium used was nutrient agar medium which consists of peptone -5g/l, meat extract -1.0g/l, yeast extract – 2.0g/l, sodium chloride – 5.0 g/l and agar - 15.0g/l of in double distilled water. The medium used for the growth of fungal strains are dextrose medium comprised of malt extract – 3.0g/l, peptone – 10.0g/l and dextrose – 10.0g/l of double distilled water. Respective media of bacterial and fungal cultures was autoclaved and cooled. The media was poured in the petri dishes and kept for 30 minutes for solidification. After 30 minutes, the cultured medium was inoculated with the test organisms.

Sterile made of Whatman filter paper, 5 mm diameter dipped in 25 µg/l of prepared metal oxide nanoparticles along with the standard antimicrobial agent containing disc were placed on each plate. Metal oxide nanoparticles treated cotton of size 1 cm × 1 cm was used to study the antimicrobial activity. The standard antibacterial agent and antifungal agent used were Amikacin and Ketoconazole respectively. The cultured agar plates were incubated at 37°C for 24 h. After 24 h of incubation the zone of inhibition was measured using the ruler for all the tested samples. The inhibition cleared zone in and around the sample decides the efficiency of the antimicrobial agent to inhibit the growth of microorganisms.
References


