GREEN SYNTHESIS AND CHARACTERIZATION OF TIN(IV) OXIDE NANOPARTICLES AND STUDY ON ITS ANTIMICROBIAL ACTIVITY

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Abstract

The tin oxide nanoparticles (SnO_2 NPs) were fabricated via eco-friendly process using lime (*Citrus aurantifolia*) peels extract as reducing agent. The main phytochemicals present in aqueous peels of lime are α–amino acid, carbohydrates, organic acids, glycosides, phenolic compound, reducing sugar, saponins, steroids and starch are responsible for bio-reduction of nanoparticles. The crystalline nature and lattice parameter was studied by x-ray diffraction (XRD) which confirmed the formation of tetragonal rutile SnO_2 NPs with average crystallite size of 40.8 nm. The functional groups present in prepared SnO_2 NPs were identified by using fourier transform infrared (FTIR) spectroscopy. Surface morphology of prepared nanoparticles was studied with the help of scanning electron microscopy (SEM). The percentage composition and purity of the SnO_2 NPs was determined by energy dispersive x-ray (EDS). SnO_2 NPs was screened against the selected microorganisms and the order of antimicrobial activities given as; C*andida albicans* > *Bacillus subtilis* > *Escherichia coli.*

Keywords: tin oxide nanoparticles, lime, *Citrus aurantifolia*, bioreduction, antimicrobial activities

Introduction

Numerous efforts have been made to development of semiconductor nanoparticles (NPs) in the last two decades due to their novel optical, chemical, photo-electrochemical and electronic properties which are different from that of bulk (Fu *et al*., 2015). Tin(IV) oxide (SnO_2) is a well know n-type wide band gap (E_g = 3.6 eV) semiconductor (Celina *et al*., 2017). Nano-sized SnO_2 is regarded as a highly preferred multitasking metal oxide, such as gas sensors and lithium rechargeable batteries (Fu *et al*., 2015). SnO_2 nanoparticles are commonly synthesized by wet chemical route, vapour phase process, hydrothermal method, precipitation, electrode position and sonochemical methods. However, chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in applications and environment. Thus, to design a simple and green route to synthesize SnO_2 nanoparticles is of considerable necessary. Recently, development of an eco-friendly method for the synthesis of nanoparticles via biological methods has been attracted lots of attentions. Using bacterial, fungi and plant extract are three main routes for biosynthesis of nanomaterials (Sudhaparimals *et al*., 2014) Among them, synthesis of nanomaterials using plant extract attracted lots attention due to its lower cost and simplicity. The biosynthesis of SnO_2 was only conducted by using aqueous peels extract of lime (*Citrus aurantifolia*). The *Citrus aurantifolia* (green source) used in the present work for the environment-friendly preparation of SnO_2 nanoparticles, belongs to the family Rutaceae and its peels are of medicinal importance. The lime is small, densely and irregular branched tree with short, sharp spains (Kose *et al*., 2014). For the synthesis of metal/metal oxide nanoparticles, plant biodiversity has been broadly considered due to the availability of effective phytochemicals in various plant extracts, such as α – amino acid, carbohydrates, organic acids, glycosides, phenolic compound, reducing sugar, saponins, steroids and starch. These components are found in peels of lime and capable of reducing metal salts into metal nanoparticles. Synthesized green SnO_2 nanoparticles was carried out using aqueous peels extracts of lime (*Citrus aurantifolia*) as reducing agent. The surface and structural properties of the prepared SnO_2 nanoparticles were studied by XRD, TG-DTA, FTIR, SEM-EDS and TEM. The SnO_2 nanoparticles were also screened for the antimicrobial

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activity against *Bacillus subtilis* as Gram Positive Bacteria, *Escherichia coli* as Gram Negative Bacteria, *Candida albicans* as fungi. The biological synthesis especially from bacteria, provides advancement over chemical and physical method as it is cost effective, environmentally friendly, easily scaled up for large scale synthesis and there is no need to use high pressure, energy, temperature and toxic chemicals.

Materials and Methods

Phytochemical Analysis of *Citrus aurantifolia* Peels Extract

The preliminary phytochemical screening of *C. aurantifolia* aqueous peel extracts were detected by qualitative assay. The more well-prepared aqueous peels essence was qualitatively proven for the existence of tannin, saponin, flavonoids, terpenoids, alkaloids, cardiac glycosides and anthraquinones using the standard protocol reported formerly.

Preparation of Tin Oxide Nanoparticles

A 30 g of lime (*C. aurantifolia*) peels were washed with distilled water and cut into small pieces. Then, it was blended with 100 mL of double distilled water, and filtered. A 40 mL of this extract was mixed with 0.02 M tin(II) chloride dihydrate solution with constant stirring. During mixing, the colour of mixture solution was found to be changed greenish yellow to pale yellow. Then the precipitate was washed with distilled water and dried in oven at 80 ºC. The obtained dried power was tin oxide nanoparticles and carefully stored in air-tight container (Junjie, 2015).

Characterization of Synthesized SnO_2 Nanoparticles

The SnO_2 nanoparticles were synthesized by using aqueous peels extracts of lime (*C. aurantifolia*). The crystal structure and crystallite size were determined by X- ray Diffraction technique. The FT IR spectrum was used to identify the functional groups presents in the sample. Surface morphology was analyzed by SEM and TEM and the nanoparticles purity was checked by energy - dispersive X- ray analysis.

Determination of Antimicrobial Activity

The crude extracts were screened for antimicrobial activity by determination the zone of inhibition against the test organisms using agar-well diffusion method. Sterile Mueller- Hinton agar plates were inoculated with prepared inoculum with sterile cotton swab. Then with the help of sterile cork borer, wells were made in the inoculated media plate. Next, 50 µL each of the working solutions were transferred into the wells with the help of micropipette. The control was also placed in the separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well (Haq *et al*., 2020).

Results and Discussion

Preliminary phytochemical screening

The green synthesis of SnO_2 nanoparticles from *Citrus aurantifolia* aqueous peels extract was carried out successfully. Lime peels extract contained the phytochemicals present in the extract are presented in Table 1.

Table 1 Phytochemical Constituents of Lime (*C. aurantifolia*) Aqueous Peels Extract

No.	Test	Extract	Test Reagents	Observation	Results
1.	Alkaloids	1% HCl	Dragendorff's reagent	No orange ppt	-
			Sodium picrate	No yellow ppt	-
			Wagner's reagent	No reddish brown ppt	-
			Mayer's reagent	No white ppt	-
2.	α-amino acids	H_2O	Ninhydrin reagent	Purple spot	+
3.	Carbohydrates	H_2O	10 % α-naphthol and conc.H_2SO_4	Red ring	+
4.	Cyanogenic glycosides	H_2O	Sodium picrate solution and conc. H_2SO_4	No brick red ppt	-
5.	Flavonoids	EtOH	Mg turning and onc.HCl	No pink colour	-
6.	Glycosides	H_2O	10% Lead acetate	White ppt	+
7.	Phenolic compounds	EtOH	5% $FeCl_3$	Deep blue colour	+
8.	Organic acids	H_2O	Bromocresol green indicator	Blue colour	+
9.	Reducing sugars	H_2O	Benedict's solution	Brick red ppt	+
10.	Saponins	H_2O	Distilled water	Frothing	+
11.	Starch	H_2O	1 % Iodine solution	No deep blue colour	-
12.	Steroids	PE	Acetic anhydride and conc. H_2SO_4	Blue or green colour	+
13.	Terpenoids	$CHCl_3$	Acetic anhydride and conc. H_2SO_4	No pink colour	-
14.	Tannins	H_2O	5% $FeCl_3$	No green ppt	-

Biosynthesis of Tin(IV) Oxide nanoparticles

The SnO_2 nanoparticles obtained through *Citrus aurantifolia* were white in colour, powdery, crystalline and insoluble in water Figure 1.

Figure 1 Green synthesized tin(IV) oxide nanoparticles

Characterization of Prepared SnO_2 Nanoparticles

X-ray diffraction analysis

The phase purity and levels of crystallinity of the prepared SnO_2 nanoparticles were analyzed by the X-ray diffractometer. Figure 2(a) shows the XRD pattern of SnO_2 nanoparticles obtained at 80 ºC. It was observed that noise peaks and some impurities were present in the SnO_2 sample.

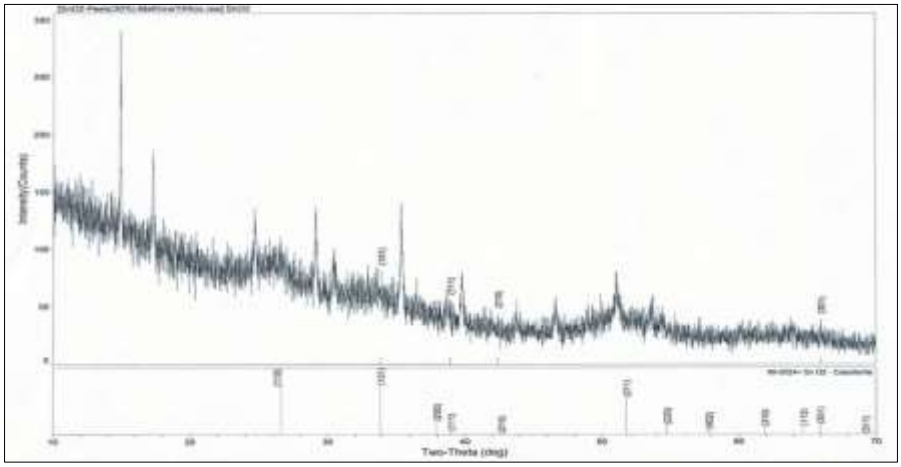

Figure 2(a) XRD pattern of prepared SnO_2 nanoparticles using aqueous peels extract of lime before calcination at 80 ºC

Figure 2(b) shows the XRD pattern of prepared SnO_2 nanoparticles using aqueous peels extracts of lime after calcination at 600 ºC. The peaks at 2θ values of 47.9º, 45.8º, 44.6º, 47.8º, 39.9º, 32.2º, 43.1º, 34.8º, 32.6º, 15.1º, 38.7º and 41.6º associated with (110), (100), (200), (111), (210), (211), (220), (002), (010), (221), (112) and (001), respectively, indicated the formation with tetragonal structure according to JCPDS File No. 99-0024. By using Scherrer equation, the average crystallite sizes of SnO_2 nanoparticles were found to be 40.8 nm by using aqueous peels extracts of lime at 600 ˚C. According to XRD, pure and crystallite SnO_2 nanoparticles with smaller size was obtained after calcination at 600 ˚C. On annealing the sample at higher temperature, the peak broadening decreased and the sharpness of the peak increased which clearly denoted the reduction of lattice strain and increased in crystallinity of the sample.

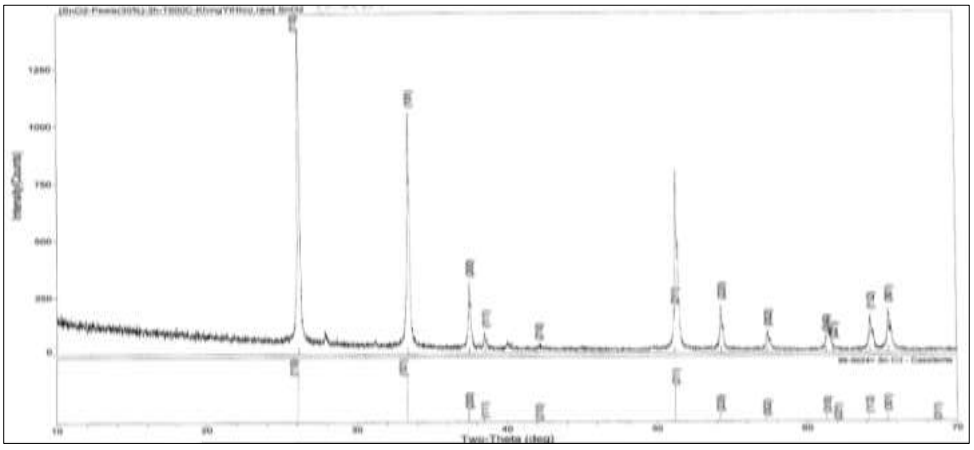

Figure 2(b) XRD pattern of prepared SnO_2 nanoparticles using aqueous peels extract of lime after calcined at 600 ºC

TG-DTA analysis

The TG-DTA thermogram of prepared powder (Figure 3) indicated an endothermic peak at 101.54 ºC which was due to the loss of water from the sample surface. The peak at 494.58 ºC was due to decomposition of hydroxide groups and phase transition from $Sn(OH)_2$ to SnO_2 (Jejenija and Damian, 2017). SnO_2 nanoparticles were found to be thermally stable beyond this temperature in this study.

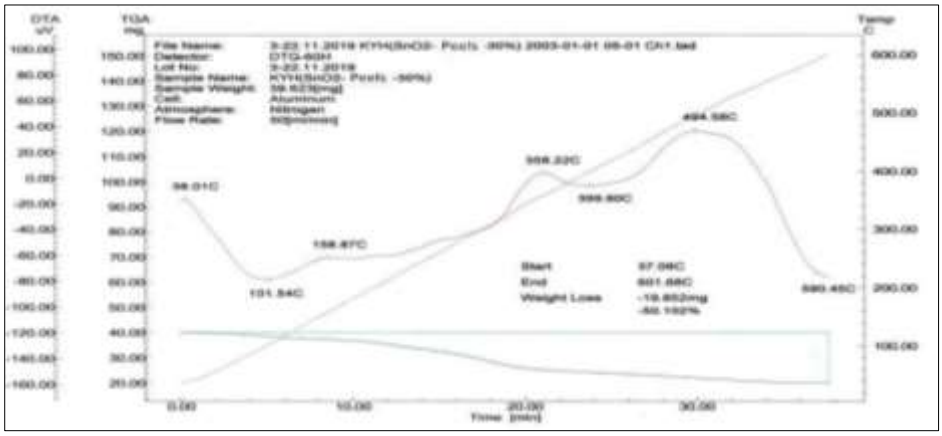

Figure 3 TG-DTA thermogram of green synthesized SnO_2 nanoparticles using aqueous peels extract of lime prepared at 80 ºC

FT IR analysis

Figure 4 shows FT IR spectrum of the prepared SnO_2 nanoparticles using aqueous peels extract of lime after calcination at 600 ºC. From this spectrum, it can be observed a strong band at 607 cm^{-1} associated with the anti- symmetric O-Sn-O stretching mode of the surface-bridging oxide formed by condensation of adjacent surface hydroxyl groups. This band confirms the presence of SnO_2 as crystalline phase formed at 600 ºC for 3 h.

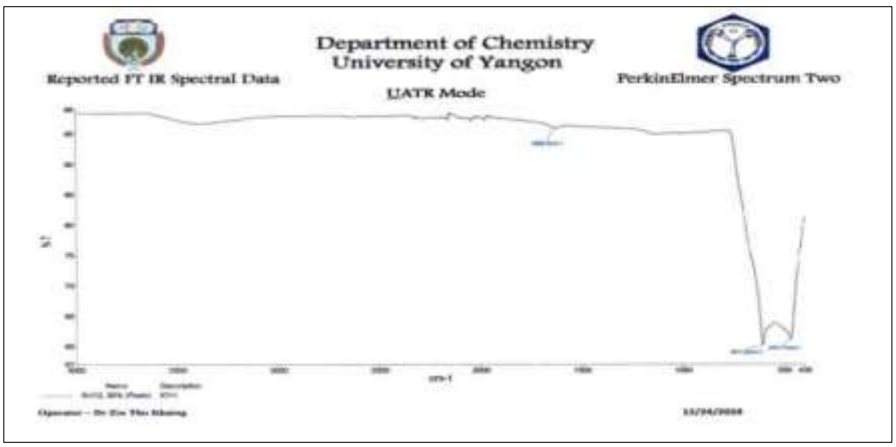

Figure 4 FT IR spectrum of the green synthesized SnO_2 nanoparticles using aqueous peels extract of lime after calcined at 600 ºC

SEM-EDS analysis

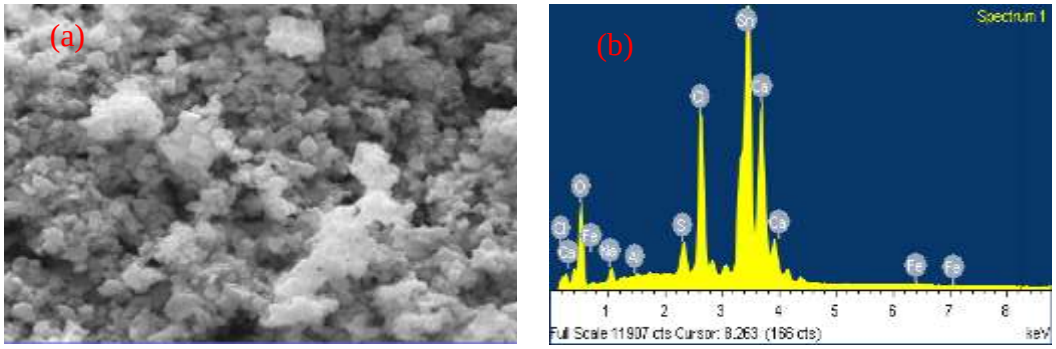

Figure 5 (a) SEM image, (b) EDS spectrum of green synthesized SnO_2 nanoparticles using aqueous peels extract of lime after calcined at 600 ºC

Figure 5(a) shows the SEM image of green synthesized SnO_2 nanoparticles. Small crystals of SnO_2 nanoparticles crystallized in tetragonal shape with slight agglomeration. This photograph indicated the porous nature of the surface. The elemental composition of fabricated SnO_2 nanoparticles was evaluated using EDS analysis as shown in Figure 5(b). The EDS spectrum showed the intense peaks of Sn which confirmed the formation of SnO_2 nanoparticles. The spectrum also showed the peaks of Na, Al, S, Cl, Ca and Fe in addition to Sn. The oxygen peaks were mainly due to the polyphenol groups present in *C. Aurantifolia* aqueous peels extract that could reduce and stabilize SnO_2 nanoparticles, while the Na, Al, S, Cl, Ca and Fe peaks were attributed to the impurities involved in synthesis process. The elemental weight percentage of the sample showed that 57.83% of samples consisted of Sn, 27.65% of O and small amount of impurities such as 2.76% of Na, 0.24% of Al, 0.82% of S, 8.27% of Cl 2.13% of Ca and 0.30% of Fe.

TEM Analysis

The TEM image of prepared SnO_2 nanoparticles after calcination at 600 ˚C is shown in Figure 6. It can be seen that SnO_2 nanoparticles consist of tetragonal structure with the particles size 37.5 nm. The small aggregations arise in synthesized nanoparticles are due to the hydrogen bonding between biomolecules used as reducing agents. The aggregations in the particles are dependent on the nature of the extracts and the biomolecules present in the extracts.

Figure 6 TEM image of SnO_2 NPs using aqueous peels extract of lime

Antimicrobial Behaviour of SnO_2 nanoparticles

The antimicrobial effects of SnO_2 nanoparticles was investigated using Gram positive, Gram negative and fungus strains such as, *Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Sacchromyces cerevisiae and Salmonella typhi*, respectively, by agar well diffusion method. Due to the large surface area the activity of the nanoparticles increases, therefore SnO_2 nanoparticles react efficiently with the cell membrane and inactivate the bacteria. The bactericidal efficacy for tin oxide nanoparticles against *B. subtilis, E. coli* and *C. albicans* were 17.93 mm, 17.73 mm and 19.12 mm, respectively. In Figure 7, it was observed that *C albicans* showed more significant activity than *B subtilis* and *E coli*. Other three microorganisms was not detected activity. Moreover, electrostatic interactions are directly responsible for the attachment of nanoparticles to bacteria. These interactions changes the integrity of cell membrane of bacteria and toxic free radicals are released which induce oxidative stress on bacteria (Khin Cho Khat, 2014).

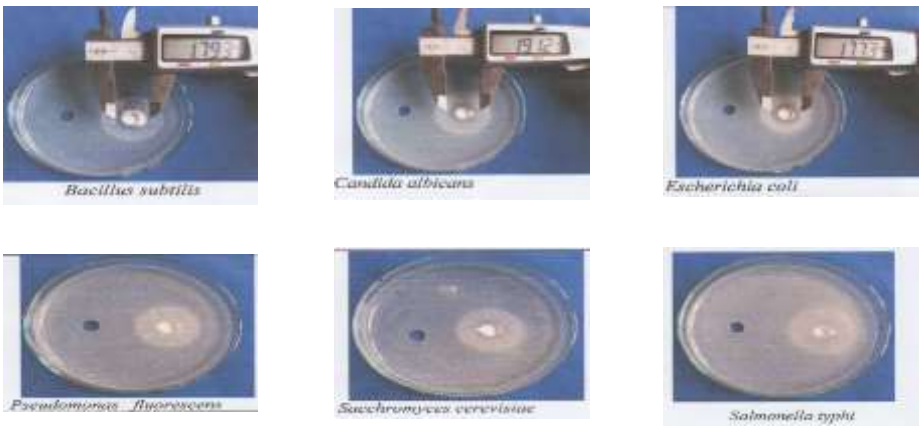

Figure 7 Antimicrobial activity of SnO2 nanoparticles

Conclusion

The green synthesis method is an economically beneficial and easy process which could eliminate several problems associated with the use of toxic reagents in the chemical synthesis procedure for SnO_2 nanoparticles. The phytochemicals act as good reducing agents in the formation of nanoparticles from aqueous peels extract. The XRD pattern of the SnO_2 nanoparticle indexed as tetragonal rutile structure. The peak at 494.58 ºC from TG-DTA curve indicated the formation of crystalline phase SnO_2. FT IR spectral data of SnO_2 nanoparticles related to the stretching vibration of O-Sn-O group. Based on SEM-EDS analysis, the small crystal of SnO_2 nanoparticles with porous and cluster structure with intense peak of Sn and O. By TEM analysis, SnO_2 nanoparticles obtained at 600 ºC showed tetragonal structure and the particles size distribution of SnO_2 nanoparticles was found to be 37.5 nm. The higher antimicrobial activity of SnO_2 nanoparticles was observed against *C. albican*, a fungus as compared to *B subtilis*, Gram positive bacteria and *E coli*, Gram negative bacteria. Therefore, the prepared SnO_2 nanoparticles can be used for the wastewater treatment. The eco-friendly green chemistry approach by the use of these peels extracts for the synthesis of nanoparticles will increase their economic viability and sustainable management. The potential nanofactories has heightened interest in the biological synthesis of nanoparticles.

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