Research Article

Synthesis and Characterization of Ag Doped Tin Oxide Nanoparticles using Cleistanthus Collinus Plant and their Biological Activities

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Abstract

The Ag and SnO_2 nanoparticles are prepared by using Cleistanthus Collinus plant methanolic extract. Then Ag is mixed with SnO_2 nanoparticles to get Ag doped SnO_2 by chemical method. The formation of Ag doped SnO_2 nanoparticles are confirmed by XRD, SEM and EDAX analysis. The Average grain size of Ag doped SnO_2 is 27.84 nm. The Antibacterial Activity of Ag doped SnO_2 is studied by using Gram - negative bacteria *E.Coli* and Gram - positive bacteria *S.aureus*. Furthermore the antioxidant properties of Ag doped SnO_2 have been studied by DPPH Scavenging method.

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Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. New applications of nanoparticles and nano materials are emerging rapidly [1]. Nano sized tin oxide 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 [2]. The synthesis of nano materials with well controlled size, morphology and chemical composition may open opportunities in exploring new properties [3]. The crystalline structure, the size and shape of the particles are highly dependent on the method of synthesis [4, 5]. Nano crystalline silver particles have found tremendous applications in the field of high sensitivity bio molecular detection [5, 6] and diagnostics [7], antimicrobials [8] and therapeutics Catalysis[9] and micro-electronics[10-12]. These nanoparticles are synthesized by precipitation [13,14], Sol-gel method [15-17], pyrolysis and hydrothermal method [18]. These methods are toxic and give fewer yields. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver and other nanoparticles.

Biological methods of synthesis have paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nano technological applications [19]. The use of environmentally benign materials like plant extract [20], bacteria [21], fungi and enzymes [22,23] for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.

Cleistanthus Collinus contains lignan lactone glucosides like Cleistanthin A and Cleistanthin B. Genin [Diphyllin] is the major metabolite of these lignan lactone glycosides. The other lignin glycosidein in this plant is Collinusin. These are toxic aryl naphthalene lignin lactones. Cleistanthin A is used in China for medical purpose as sheng bai xin [24]. So this extract was chosen for the synthesis of the silver and tin oxide nanoparticles. In the present

study silver and tin oxide nano particles have been synthesized by using Cleistanthus Collinus methanolic extract. The prepared Ag nanoparticles have been doped in tin oxide by using chemical method [25]. Finally its Anti oxidant activities and antibacterial activities have been checked.

Experimental

All analytical reagents used in this experiment were of highest purity and obtained from Sigma Aldrich [Bangalore, India] and fresh, green leaves of Cleistanthus Collinus plant were collected from Mallur village [Tamil Nadu, India] in Salem district.

Synthesis of Ag, SnO_2 and Ag doped SnO_2 nanoparticles

Ag and SnO_2 nanoparticles were synthesized by using Cleistanthus Collinus plant methanolic extract and Ag doped SnO_2 nanoparticles were synthesized at room temperature by chemical methods [22].

X-Ray Diffraction Analyses

X-ray diffraction patterns were obtained by using a Philips X'pert Diffractometer with Cu-Kα radiation source [λ=1.54Å]. The powder samples were prepared by dispersion of a thin layer of the powder on a double-sided tape attached to a glass slide. The diffractometer was operated at 30KV and 25mA and a scanning step of 0.05^o in two theta and a dwell time of 1 second was used. Data was collected on a Bruker D8 Discover X-ray diffraction system. The diffraction patterns were collected in the 2θ range 20°–80°, with a step of 0.02° and 34 s measuring time per step. The width of the peaks, however, is relatively broad compared to the XRD pattern of Ag, SnO_2 nanoparticles and Ag doped SnO_2nanoparticles, indicating a small crystal size. The average grain sizes [D] of the nanoparticles were computed using Scherer's formula (1),

$$D = 0.94\,\lambda / \beta\, Cos\,\theta \qquad (1)$$

where, β and λ are the X ray wavelength [0.18 nm] and the full width half maximum of the diffraction peak [FWHM], respectively. The micro strain and dislocation density are calculated by using the following formula:

$$\text{Micro strain} \quad B(2\theta) = \frac{K\lambda}{L\cos\theta} \qquad (2)$$

$$\text{Dislocation Density } \rho = [\rho_D \,.\, \rho_\varepsilon]^{1/2} \qquad (3)$$

Scanning Electron Microscopy [SEM]

The morphology of the Ag doped SnO_2 nanoparticles was observed by using a Hitachi S-4000 Field Emission SEM and a Hitachi S-900 SEM. Powder samples were glued onto a copper stub using carbon conductive tape. They were subsequently sputtered with gold to improve conductivity of the samples.

Energy Dispersive X-ray Spectroscopy [EDAX]

The presence of the elemental Ag, Tin oxide and Ag doped Tin oxide can be seen in the graph presented by the EDAX analysis, which indicated the reduction of Ag and formation of Ag doped Bismuth oxide nanoparticles.

Antioxidant Activity

DPPH assay was carried out in two different ways using two different methods of mixing. The other parameters were same as described by Serpen et al[x, x]. In a typical process, 1.70 mL [100 μM] of DPPH was taken

in a small glass bottle, and 10 mg of eight different Ag doped Tin oxide samples were dispersed in to it. DPPH was used as the radical source and Ag doped Bismuth oxide was used as radical scavenger. DPPH radical has a deep violet color in solution, and gradually it becomes colorless or pale yellow in the presence of Ag doped Bismuth oxide nanoparticles. This property allows visual monitoring of the reaction, and the concentration of radicals is monitored from the change in percentage of absorption at 517 nm. The rate of the reaction was enhanced by vortexing the reaction mixture for 2 minutes at room temperature. The supernatant containing DPPH was collected for different time intervals. The time dependent DPPH scavenging was studied at an interval of 0, 5, 10 min. DPPH scavenging activity is calculated using the following equation:

$$\text{DPPH scavenging activity [\%]} = 1 - \frac{As}{Ac} X100 \qquad (4)$$

Where Ac and As are the intensity of peak at 517 nm for control [DPPH] and supernatant DPPH solvent respectively.

Antibacterial Activity

Antimicrobial activities of the synthesized Ag doped Bismuth oxide nanoparticles were performed against both Gram-negative [*E.coli]* and Gram-positive [*S.aureus*] bacteria. The antibacterial activity was done by modified Kirby-Bauer disk diffusion method. In brief, the pure cultures of organisms were subcultured in Muller-Hinton broth at 35°C ± 2°C on a rotary shaker at 160 rpm. For bacterial growth, a lawn of culture was prepared by spreading the 100 µL fresh culture having 10^6 colony-forming units [CFU]/mL of each test organism on nutrient agar plates with the help of a sterile glass-rod spreader. Plates were left standing for 10 minutes to let the culture get absorbed. Then disks were placed into the nutrient agar plates for testing nanomaterial antimicrobial activity. Using a micropipette, 50 and 75 µg of the sample of nano particle suspension was placed onto each of two disks on all plates. After overnight incubation at 35°C ± 2°C, the different levels of zone of inhibition were measured. Solvent blank was used as negative control. Antibiotic streptomycin was used as a positive control.

Result and Discussion

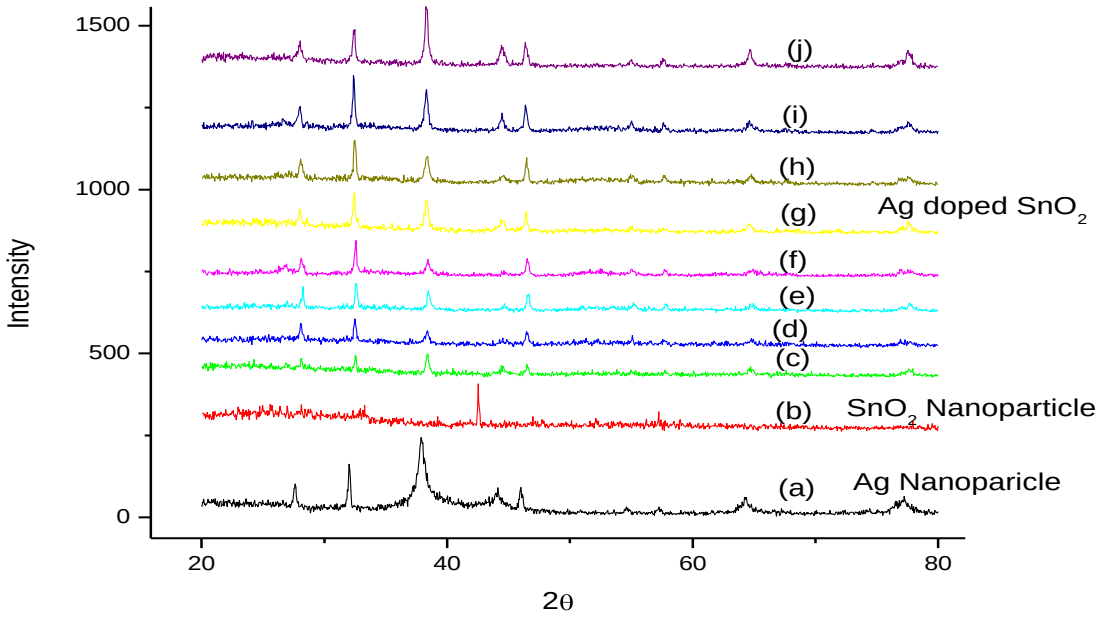
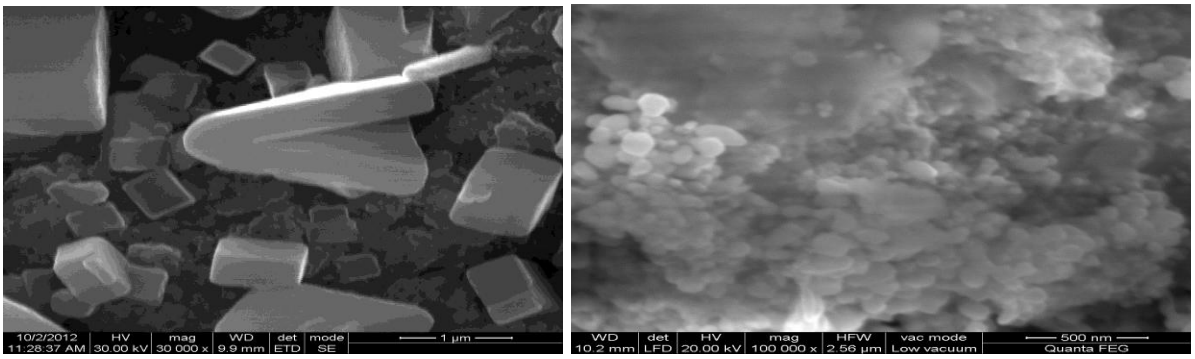


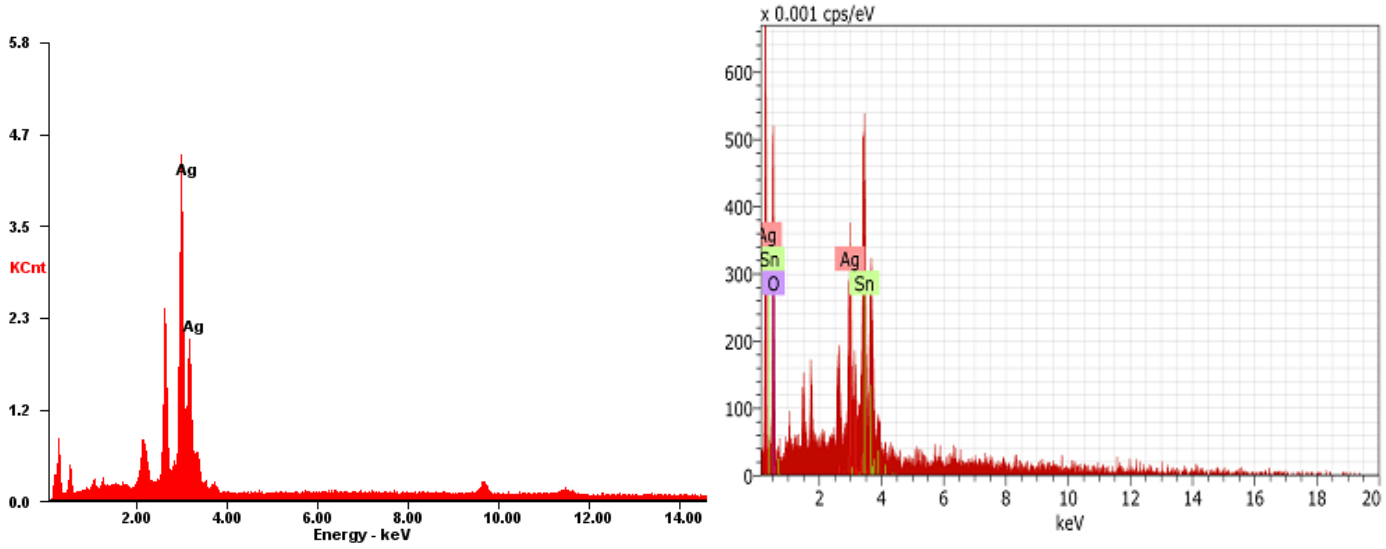
Figure 1. XRD results of [a]Ag,[b] SnO_2 Nanoparticles and [c-j] Ag doped SnO_2 nanoparticles 33%, 50%, 60%, 66.6%, 71.4%, 75%, 77.8%, and 80% of nanoparticles of SnO_2 in Ag doped SnO_2.

Figure 1 shows XRD of Ag, SnO_2 and Ag doped SnO_2.These peaks are attributed to crystal planes [110], [200], [220], [311] for Ag. This result clearly indicates the formation of predominantly cubic shaped Ag [JCPDS Pdf No. 65-2871] and orthorhombic shaped Tin oxide nanoparticles [JCPDS Pdf No.78 - 1063] .Also the results show the uniform distribution of Ag and SnO_2 nanoparticles. The average grain size of Ag nano particle was 158.11 nm and that of SnO_2 was 49.26 nm. After doping, the 2θ values of planes [004], [111], [106], [206] increased because crystal size got decreased [Figure.1]. FWHM of the peaks increased with increasing SnO_2 concentration. The reason is that the crystalinity increased with increasing SnO_2 concentration from 33% to 80% in the Ag doped Tinoxide nanoparticles. Due to this, the micro strain [ε_L] and dislocation density [ρ] are increase with decrease in the particle size. These results confirms the formation of hexagonal primitive structure of Ag doped Tinoxide Nanoparticles [JCPDS Pdf No. 86 – 2052].



(2a) (2b)

Figure 2. SEM Results For Ag (2a) and Ag doped SnO_2 nano particles (2b).



(3a) (3b)

Figure 3. Results For Ag (3a) and Ag doped SnO_2 nanoparticles (3b).

Figure 2 & 3 show the formation of predominantly cubic shaped Ag nanoparticles .Also the results show the uniform distribution of Ag and SnO_2nanoparticles. The average size of Ag nano particle was 131 nm. The average size of Ag doped SnO_2 is 27.84 nm. In EDAX results, the silver nanoparticles are represented by an optical absorption band peak at 3 keV and SnO_2 nanoparticles at an optical absorption band peak at 3.5 keV. This represents the typical absorption of Ag and SnO_2 nanoparticles .The SEM and EDAX results confirm the presence of noble Ag nanoparticles and Ag doped SnO_2 nanoparticles (Figures.2-3).

Table 1 Antioxidant Activity of Ag doped SnO_2 nanoparticles

Stock Value: 0.9253 Scan Range - 517 nm

Composition of Ag doped SnO_2 [SnO_2 : Ag]	Initial	Anti oxidant Activity	After 5 minutes	Anti oxidant Activity	After 10 minutes	Anti oxidant Activity
0.5 : 1 [33.3%]	0.8437	8.81870	0.8234	11.0126	0.8192	11.4665
1 : 1 [50%]	0.7840	15.2707	0.7774	15.9840	0.7723	16.5351
1.5 : 1 [60%]	0.5437	41.2406	0.5371	41.9539	0.5308	42.6348
2 : 1 [66.6%]	0.4589	50.4052	0.4581	50.4917	0.4563	50.6862
2.5 : 1 [71.4%]	0.4427	52.1560	0.4361	52.8693	0.4338	53.1179
3 : 1 [75%]	0.4199	54.6201	0.4195	54.6633	0.4189	54.7281
3.5 : 1 [77.8%]	0.3987	56.9112	0.4098	55.7116	0.4187	54.7498
4 : 1 [80%]	0.3918	57.6569	0.3891	56.8680	0.4094	55.8089

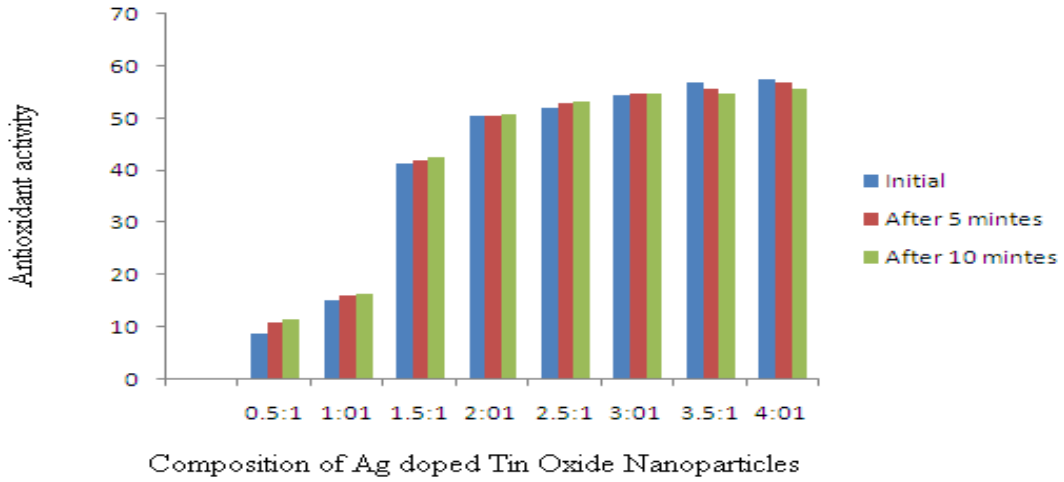


Figure 4 Antioxidant Activity of Ag doped SnO_2 nanoparticles

DPPH scavenging activity of Ag doped Tin oxide nanoparticles increases with increase in the ratio from 33.5% to 80%. The 80% ratio of Ag doped Tin oxide nanoparticles show maximum activity .Thus the vortexed samples show increase in antioxidant potency of Ag doped Tin oxide nanoparticles. This could be attributed to the increased chance of mixing of DPPH in methanol with nanoparticles. No DPPH scavenging was observed in the vortexed control DPPH solution. At 77.8% and 80% compositions initially gives better antioxidant activity and after vortex that activity slowly decreases. This result clearly indicates the optimum level of antioxidant activity of Ag doped Tinoxide could be observed at 75%. Composition.

Nano particles tend to adsorb on the bacterial surface and dehydrogenation due to respiration process occurs at the cell membrane in bacteria. Increasing the concentration of metal oxide in composition 33.3 % to 80 % the active oxide species concentration increases and antibacterial activity also increases. This result clearly indicates the antibacterial activity of *Escherichia coli* which was higher than that of *S.aureus*. The Gram - negative bacteria E.Coli does not have cell wall and gram - positive bacteria *S.aureus* have cell wall. Hence the sample easily reacts with E.Coli than that of *S.aureus*.

Table 2 Antibacterial Activity of Ag doped SnO_2 nanoparticles

S. No	Composition of Ag doped SnO_2 nanoparticles	Name of the Bacteria and Anti bacterial activity			
		Escherichia coli		*S.aureus*	
		50 µg	75 µg	50 µg	75 µg
1	0.5 : 1 [33.3%]	2	3	-	2
2	1: 1 [50%]	3	4	1	2
3	1.5 : 1 [60%]	3	5	2	4
4	2 : 1 [66.6%]	3	5	2	5
5	2.5 : 1 [71.4%]	5	7	3	5
6	3 : 1 [75%]	5	7	4	5
7	3.5 : 1 [77.8%]	6	7	4	7
8	4 : 1 [80%]	6	8	5	7

Conclusion

The Ag and SnO_2 nanoparticles successfully were green synthesized by using Cleistanthus Collinus plant methanolic extract. The antibacterial and antioxidant activity of Ag doped SnO_2 nanoparticles was evaluated. The formation of Ag dopedSnO_2 nanoparticles confirmed by XRD, SEM and EDAX methods. Ag doped SnO_2 show DPPH free radical scavenging activity up to 75% and after that activity slowly decreases. This result confirms that the optimum concentration of Antioxidant activity was 75% Ag doped SnO_2. Antibacterial studies, increase in the concentration of SnO_2 from 33.3% to 80% of Ag doped SnO_2 nanoparticles, increases the antibacterial activity.

References

[1] H.S. Naiwa, Ed. HandBook of Nanostructural Materials and Nanotechnology Academic Press, New York, 2000, p1.
[2] Green synthesis of nanostructured materials for antibacterial and antifungal activities, Ayeshamariam A, Tajun Meera Begam M, Jayachandran M, Praveen Kumar G and M Bououdina, *Int. J. Bioassays*, **2013**, 2, p304.
[3] T Pradeep, Nano: The Essentials, Understanding Nanoscience and Nanotechnology, Tata Mc Graw- Hill Publishing Company Limited, New Delhi, **2007**, p19.
[4] H.I.Chen, H.Y. Chang, Ceramic International, **2005**, 31, 795.
[5] Burda, C, Chen, X.; Narayanan, R.; El-Sayed, M. A, *Chemistry Rev*., **2005**, 105, 1025.
[6] Haes, A. J.; Van Duyne, R. P. *Anal. Bioanal. Chem,*. **2004**, *379*, 920.
[7] N.L.Rani, C.A.Mirkin, Chem.Rev., **2005**,105, 1547.
[8] Thiruppathi S, Ramasubramanian V, Sivakumar T, ThirumalaiArasu V. et al., J. Bio sci. Res., **2010**, 1[4], 251.
[9] Roucoux AI, Schulz J, Henri P, Chem. Rev., **2002**, 102, 3757.
[10] Anderson, N. A, Lian, T. Q. Annu, Rev. Phys. Chem. **2005**, 56, 491.
[11] S.A. Maier, M.D. Friedman, P.E. Barclay, and O. Painter, Appl. Phys. Lett, **2005**, 86[7], 071103.
[12] M. Fichtner *Adv. Eng. Mater.*, **2005**, 7, 443.
[13] Jha, R. K.; Pasricha, R.; Ravi, V, *J. Ceram Int.* **2005**, 31,495.

[14] Liu A T, Kleinschmidt P and Davidge R W, [Eds.], Novel Ceramic Fabrication. Process and Applications, Institute of Ceramics, Staffs, UK, 1986, 38, p1-10.
[15] Anilkumar, M.; Pasricha, R.; Ravi. V, *J Ceram Int.*, **2005**, *31*, 889.
[16] M.D. Wildberger, J.-D. Grunwaldt, M. Maciejewski, T. Mallat, A. Baiker, Appl. Catal. A, **1998**, 175, 1.
[17] S. Gnanam ,V. Rajendran, J Sol-Gel Sci Technol., **2010**, 53, 555.
[18] A. Cabot, J. Arbiol, R. Ferré, J. R. Morante, Fanglin Chen, J. Appl. Phys., **2004**, 95, 2178.
[19] G. S. Metraux, Y. C. Cao, R. Jin, and C. A. Mirkin, *Nano Letters*, **2003**, vol. 3, 519.
[20] R. Sathyavathi, M. B. Krishna, S. V. Rao, R. Saritha, and D. N. Rao, J. Adv. Sci. Lett, **2010**, 3, 138.
[21] J. K. Fu, W. D. Zhang, Y. Y. Liu, Z. Y. Lin, B. X. Yao, S. Z. Weng, Chem. J. Chinese Universities,1999, 20[9], 1452.
[22] A. Ahmad, P. Mukaherjee, S. Senapati etal, **2002**, j.of *Chem Bio Chem*,3, 461.
[23] K.C.Bhainsa, S.F.D'souza, colloids and surface B: Biointerfaces, **2006**, 47, 160.
[24] Donald G. Barceloux.; *Medical toxicology of natural substances, Cleistanthin, diterphene esters and the spurge Family*, John wiley & sons, 2008, 1st vol, 1st ed.; *Chap. 123*.
[25] Susha, V. S, Chinnamuthu, C. R, *Research J. of Nanoscience and Nanotechnology*. **2012**, *2*, 79.

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