Potential Therapeutic Activity of Bio-Synthesized Silver Nanoparticles as Anticancer and Antimicrobial Agent

Piyali Basak[1], RanabirMajumder[1], Amrita Jasu[1], Samrat Paul[2], Shreya Biswas[2]

School of Bioscience & Engineering, Jadavpur University, Kolkata- 700032, India

Abstract:

Aloevera is one of the oldest medicinal plants and it possesses different types of pharmacological properties including anti-microbial, anti-inflammatory, anti-cancer and antioxidant activity. More over Aloevera contains 75 bioactive compounds such as polyphenol, flavonoids, alkaloids, anthraquinone etc. Such bioactive compounds can be used to reduce silver ions to produce silver nanoparticles. In this study, we are trying to formulate bio-synthesised silver nanoparticle (**b- AgNps**) by green chemistry approach. It is a very simple, efficient and eco-friendly approach for silver nanoparticle synthesis that is formed by reduction of silver nitrate ($AgNO_3$) solution using Aloe vera leaf extract as a reducing agent.

Hydrothermal method was used to prepare b-AgNps using aloe vera leaf extract as both reducing and stabilizing agent. Then b-AgNps were characterized by FESEM, XRD, DLS and UV-VIS spectroscopy. Biological activity of b-AgNps were evaluated by performing cytotoxic test (MTT assay) against breast cancer cell lines and Fibroblast normal cell lines as well as screening antimicrobial activity using agar well diffusion method. The results revealed that there was significant amount of cell growth inhibition against breast cancer cell lines as well as microbes compare to normal healthy fibroblast cell lines.

Keywords: bio-synthesised silver nanoparticle (**b-AgNps**), Alovera, Reducing agent, Breast cancer cell lines.

Corresponding Email. piyalibasak@gmail.com

1. INTRODUCTION

According to the World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs [1]. The gel and dried leaf of Aloe species have been used as medicine since ancient civilizations of the Egyptians, Greeks, Mediterranean people and also for its cosmetic uses [2]. But the attention was drawn to the potential Alo gel after the Second World War when skin burns victims of the nuclear bombs in Japan were successfully treated with aloe gel [3].

The synthesis, delineation of design, and modification of nano-sized materials have found prodigious interest in recent times because of its broad application in medical and biological aspect. Among diverse nanomaterials silver (Ag) was focused with much interest due to its idiosyncratic properties, such as stability, catalytic and antibacterial property. Metallic nanoparticles are produced by various methods, the more common ones being chemical and physical methods. The aforesaid methods produce pure and well-defined nanoparticles, but the chemicals used in the synthesis are toxic, energy consuming, expensive, and unsuitable for various biological applications.
Silver and silver based products like Silver nanoparticles have received attention due to their physical, chemical, and biological properties that attributed to the catalytic activity and

antimicrobial activity against a wide range of microorganisms like bacteria, fungi, protozoa and recently virus. [4] Beside that, they also found applications in nanobiotechnological research [5,6].

Plant-mediated synthesis of nanoparticles [NPs] is a green chemistry approach that connects nanotechnology with plants. Such plant extracts have reportedly been used in the preparation of AgNPs[7]. Here Aloe extract is used as a reducing agent for the synthesis of biogenic-AgNPs [b-AgNPs]. Water-soluble organic compounds present in the plant materials are mainly responsible for the reduction of silver ions to AgNPs [8]. Besides that aloe vera leaves have been used as medicinal plants as they possess anti-inflammatory activity, anti-arthritic properties, and anti bacterial. They also provide protection against UV ray, promote wound and burn healing, and have antibacterial properties [9,10,11,12]. There are a number of biologically active constituents in aloevera leaves. These include lignin, hemicellulose, pectins which can be used in the reduction of silver ions [13]. As per reports the large enzymes and proteins in aloe vera extract are weakly bound to silver ions and function as a complexing agent. Due to their cost effectivity and environmentally friendly nature coupled with their reducing properties, we selected Aloe vera as the reducing and stabilizing agent to prepare AgNPs and test their anticancer activity.

Biosynthesis of silver nanoparticles (b-AgNPs using *Alo vera* leaf extract, which acts as reducing as well as stabilizing/ capping agent have been shown schematically:

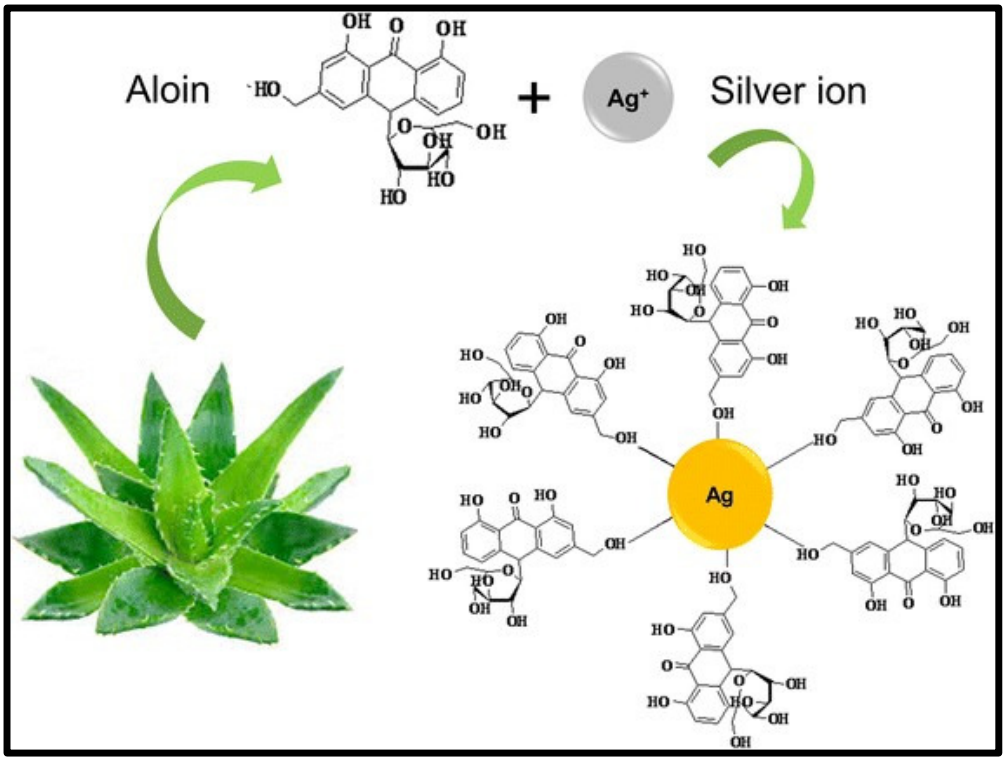


Figure 1 Biosynthesis of silver nanoparticles (b-AgNPs) using *Alo vera* leaf extract as reducing agent

2. MATERIALS AND METHODS

2.1. Chemicals

Silver nitrate ($AgNO_3$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), heat inactivated foetal bovine serum (FBS), minimum essential medium (MEM), glutamine, EDTA and trypsin were purchased from Sigma–Aldrich.

2.2. BioSynthesis of AgNPs

Alovera (AV) whole leaf was obtained from Ramkrishna Mission Ashrama, Narendrapur, Kolkata. Extract (10%) was prepared by mixing 50 g of whole leaf in 500 ml sterile distilled water, kept in boiling water bath for 10 min. The extract was filtered through Whattman filter paper No.1 and stored at 4◦C for further studies. Various concentrations of AV-AgNPs were prepared with 1 mM $AgNO_3$ and kept in ambient temperature. Generation of AgNPs was confirmed through periodical measurement using UV–visible spectroscopy in the range between 250-800 nm.

2.3. Characterization of AgNPs

The crystal phase analysis of the AgNPs was conducted using X-ray diffraction (XRD) pattern (Bruker D8 Adance) and was recorded by using Cu-Kα1 radiation (λ of 1.5406Å). The scanning was done in the region of 2θ (from 0º to 80º at 0. 02º/min). The particle size and morphology of the prepared AgNP samples were characterized using field emission electron microscope (FESEM) [Inspect F50, model Q150R with 1400 (VA) power and 50/60 Hz frequency. Size of particle distribution was studies by Zetasizer Nano ZS90. Careful UV–vis spectral analysis was performed by using Varian Cary 50 Bio UV-VIS spectrophotometer to determine the reduction of pure Ag+ ions and thereby the formation and stability of metal nanoparticles in aqueous solution.

2.4. In-vitro anticancer studies of synthesized AgNPs

2.4.1. Cell culture

Breast cancer cell lines (MCF-7) and (T47D) and Fibroblast cells (3T3) were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown as monolayer in MEM, supplemented with 10% FBS, 1% glutamine, and incubated at 37˚C in 5% CO_2 atmosphere. Stocks were maintained in 75 cm^2 tissue culture flask.

2.4.2. Cell viability

Cell viability was measured using MTT assay [16]. Cultured MCF-7 cells (1×10^5 cells/mL) were plated on 96 flat-bottom well plates, then cells were exposed to different concentration of AgNPs (1–100 µg/mL) and incubated for 24h at 37˚C in 5% CO_2 atmosphere. After incubation, MTT
0.5mg/ml was added to the incubated-cells, then further incubated for another 3 h at 37˚C, and 5% CO_2 atmosphere. Thereafter, the formazan crystals were dissolved in 200µl of DMSO and the
absorbance was measured at 570nm in iMark Microplate Absorbance Reader.

2.5 Antibacterial Activity Tests: Well diffusion Method

2.5.1. Bacterial Strains and Culture Media.

The bacterial strains of Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were used in this study. Bacterial suspensions were always prepared fresh by growing a single colony overnight at 37ºC in a Luria Bertani nutrient broth. The sample turbidity was adjusted to an

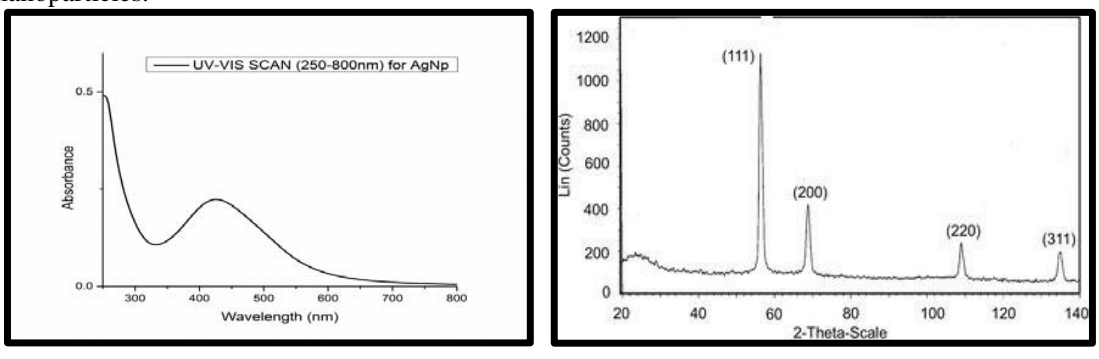
optical density of 0.1 at 600 (OD 600) before performing the antibacterial experiments. To obtain fresh culture growth, all agar plates were freshly prepared before the antibacterial tests. Cells were added to the luke-warm agar media before plating. These plates were immediately used for the antibacterial activity tests. Cup-Plate method was performed and wells were aseptically dug with a borer.

2.5.2. Determining Zone of Inhibition.
We determined the antibacterial activity of four AV-AgNP solutions (1, 2.5, 5 and 10mM) and each containing the equivalent of 1mM of $AgNO_3$. Simultaneously antibacterial activity of crude Alovera whole leaf extract, dH_2O (kept as control), and standard taken with antibiotic Streptomycin (50µg/ml) were observed. The samples with inoculated agar plates were then incubated for 24h at 37ºC. The zone of inhibition (ZOI) was determined as the total diameter (mm) of well with sample diameter plus the halo zone where bacterial growth was inhibited. All measurements were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Physico chemical Characterization and stability study of AV-AgNP

In metal nano particles such as in silver, the conduction band and valence band lie in very close proximity where the electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band. The absorption peak (SPR) is obtained in the visible range at 410 nm. UV-VIS spectral analysis (Figure 2a) shows the reduction of pure Ag+ ions and thereby the formation and stability of metal nanoparticles in aqueous solution. The crystalline nature of Ag NPs was confirmed by the XRD analysis. The diffraction peaks corresponding to the {111}, {200},
{220} and {311} facets of the fcc crystal structure [14]. The peak corresponding to the {111} plane is more intense than the other planes, suggesting that the {111} plane is the predominant orientation [15]. X-ray diffraction (XRD) pattern (Figure 2b) of AV-AgNPs demonstrates the crystalline nature of silver nanoparticles obtained with 500µL of extract. DLS study (Figure 3) of particle size distribution shows AgNPs with the size range between 85 and 465 nm with more than 75% of AgNPs between 150nm to 300nm and some below 100nm. FESEM micrographs (Figure 4) of different magnifications shows individual and clustered spherical particles as low as 25nm and above. The physicochemical characterization studies carried out using XRD, DLS, SEM, UV- Visible Spectrophotometer, confirmed the presence, distribution and pattern of silver nanoparticles.



(a) (b)

Figure 2 (a) UV-Vis spectroscopy, (b) XRD pattern of AV-AgNPs hybrids.

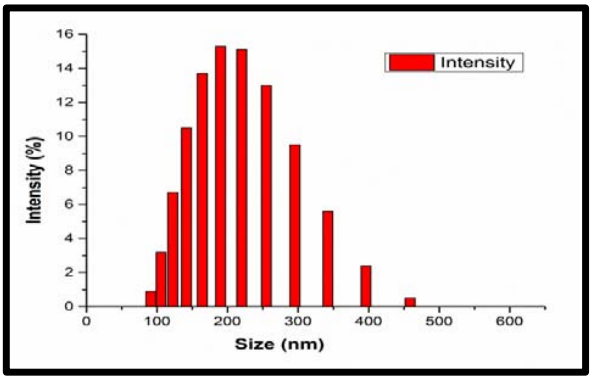


Figure 3 DLS study shows the particle size distribution

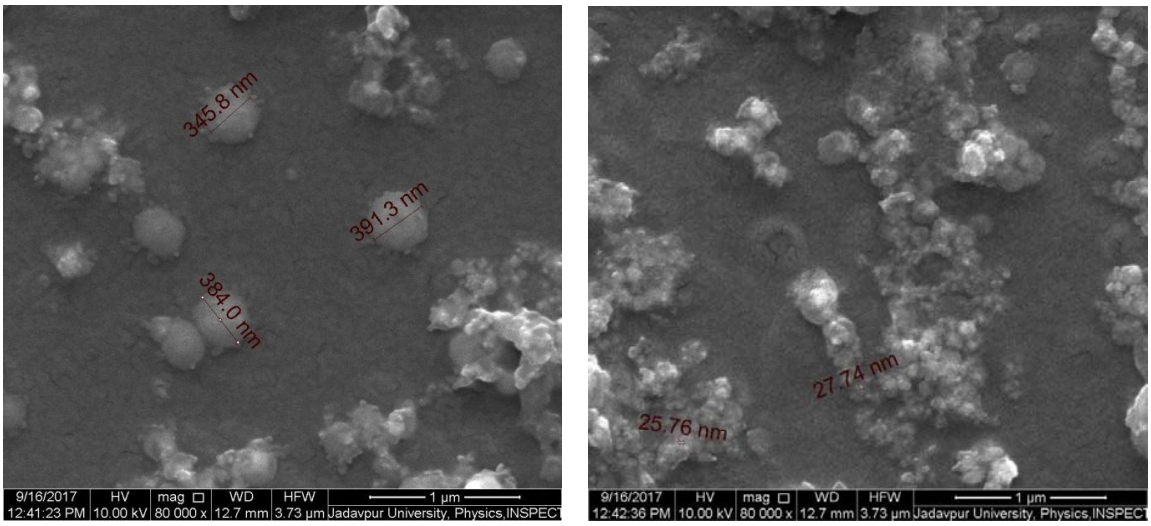
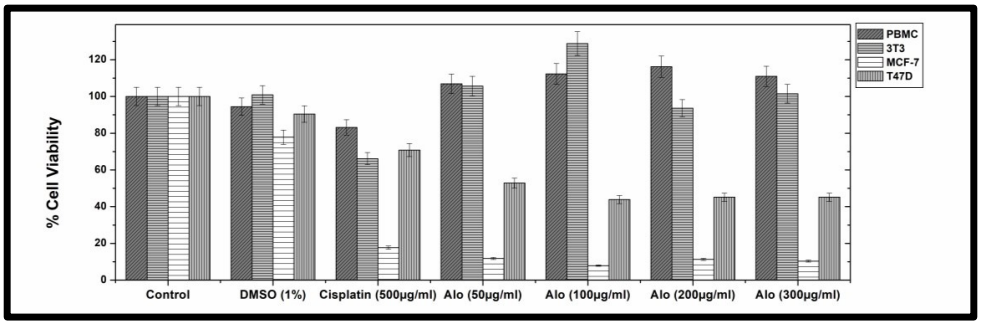


Figure 4 FESEM micrograph of AgNPs at different magnification

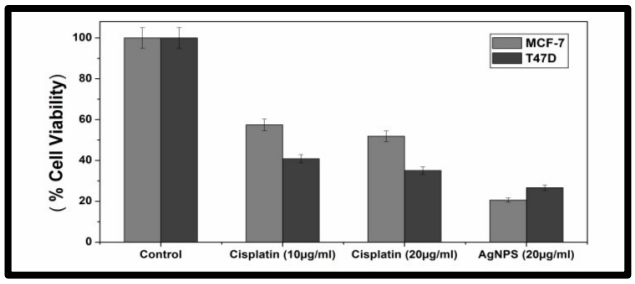
3.2. In vitro anticancer studies of synthesized AgNPs

3.2.1. Cell viability

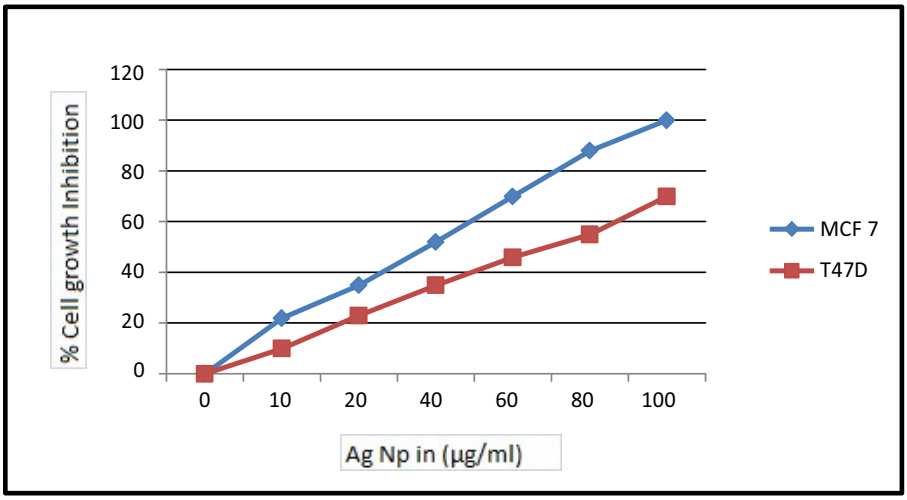
Alo vera crude leaf extract on Fibroblast cell line (3T3) and Breast cancer cell lines MCF-7 and T47D had promoted growth without any cytotxic effect. Whereas Cytotoxicity induced by biogenic AgNPs on MCF-7 and T47D cell lines were found to be higher with increase in concentrations of AgNPs. There was a change in the percentage of cell viability in control, standard with anti-cancer drug Cisplatin(10µg/ml and 20µg/ml) and AgNPs (0, 10, 20, 40, 60, 80 and 100 µg/ml) treated MCF-7 cells. IC_{50} of biogenic AgNPs was found to be 40µg/ml for MCF-7 and 80µg/ml for T47D.



(a)



(b)



(c)

Figure 5 a. Comparative effect of Aloe vera whole leaf extract on Normal and the Cancer cells, **b.** Cytotoxic effect of biogenic (AV)- AgNPs against MCF-7 & T47D cell line, **c.** Cytotoxicity of AgNPs on MCF-7 and T47D cells: With increased concentration of AgNPs.

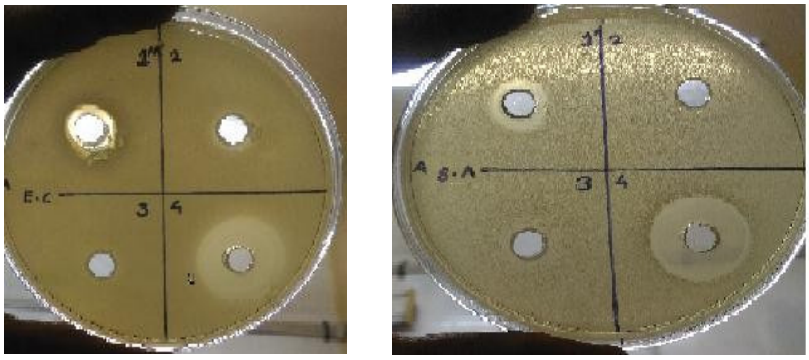
3.3 Antimicrobial Study

TheAV- AgNPs synthesized, showed inhibition zone against all test organisms. The ZOI of AgNP for both *Escherichia coli* and *Staphylococcus aureus* were greater than that of the standard

antibiotic streptomycin. The ZOI of Gram-positive *S. aureus* increased from 1.3 to 2.1 cm with increasing concentration of AV-AgNPs and Gram Negative *E.coli* showed much prominent effect than *S.aureus* with increasing ZOI from 1.2 to 2.5cm (Table 1).

Table: 1 Comparative study of ZOI of the two strains of Gram-negative *Escherichia coli* and
Gram-positive *Staphylococcus aureus* against the Standard of Streptomycin.

Sample Dose	Zone if Inhibition (cm) of Bacterial strain	
	Escherichia coli	*Staphylococcus aureus*
Standard drug (50µg/ml)	1.1	1.3
1µM	1.2	1.3
2.5 µM	1.5	1.6
5 µM	2.1	1.8
10 µM	2.5	2.1



(a) (b)

Figure 6 Plates showing zone of inhibition: (a) *Escherichia coli with 5mM AgNP in 4th quadrant* (b) *Staphylococcus aureus with 5mM AgNPin 4th quadrant.* 1st quadrant with the standard antibiotic drug and 2nd and 3rd quadrant with distilled water and crude Alovera extracts respectively.

4. CONCLUSIONS

Biogenic silver nanoparticles were synthesized using the extract of Alovera as a potent bio reducer. Spherical shaped nanoparticles were observed. Basic experimental evidence shows anticancer property against human breast cancer (MCF-7 and T47D) cell lines. It caused adverse effects of the various cellular components in the cancerous cells. Further in vivo studies and clinical level trails is necessary, to address the formulation of biogenic silver nanoparticles as an eco-friendly and biocompatible alternate to conventional anticancer drugs. Biosynthesized AgNP with Alovera showed clear antibacterial activity toward Gram-negative *E. coli* and Gram-positive *S. aureus*. As all the bacterial species showed similar susceptibility to AV-AgNP, thus can be concluded they show bactericidal action over a wide range of potentially pathogenic bacteria.

5. ACKNOWLEDGEMENTS

All the authors are thankful to School of Bioscience and Engineering Jadavpur University, Department of Physics, Jadavpur University, Department of Metallurgical and Material Engineering, Jadavpur University.

6. REFERENCES

1. Rates SM. 2001. Plants as source of drugs. Toxicon Vol. 39, pp. 603.
2. Evans WC. 2009. Trease and Evans' Pharmacognosy. Elsevier Health Sciences: London.
3. Reynolds T. 2004. Aloes: The Genus Aloe. CRC press: London.
4. M.I. Sriram, S. Barath, M. Kanth, K. Kalishwaralal, S. Gurunathan, 2010, International Journal Nanomed. Vol. 5, pp. 753.
5.V.K. Sharma, R.A. Yngard, and Y. Lin, 2009, Advances in Colloid and Interface Science, Vol. 145, pp. 83.
6.A.M. Fayaz, K. Balaji, M. Girilal, R. Yadav, P.T. Kalaichelvan, and R. Venketesan, 2010, Nanomedicine: Nanotechnology, Biology, and Medicine, Vol. 6, pp. e103.
7.Sun Q, Cai X, Li J, Zheng M, Chen Z, Yu C-P. 2014. Colloids and Surfaces A: Physicochemical and Engineering Aspects, Vol. 444, pp. 226.
8.Q. Feng, J. Wu, G. Chen, F. Cui, T. Kim, and J. Kim, 2000, Journal of Biomedical Materials Research, Vol. 52, pp. 662.
9. T. Reynolds and A. Dweck, 1999. Journal of Ethnopharmacology, Vol. 68, pp. 3.
10. B. Vazquez, G. Avila, D. Segura, and B. Escalante, 1996, Journal of Ethnopharmacology, Vol. 55, pp. 69.
11. T.H. Emaga, C. Robert, S.N. Ronkart, B. Wathelet and B,M. Paquot, 2008, Bioresource Technology, Vol. 99, pp. 4346.
12. Y. Zhang, X. Cheng, Y. Zhang, X. Xue, and Y. Fu, 2013, Colloids and Surfaces A: Physicochemical and Engineering Aspects, Vol. 423, pp. 63.
13. S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, 2006, Biotechnology Progress, Vol. 22, pp. 577.
14. P. Kannan and S. John, 2008, Nanotechnology, Vol. 19, pp. 085602.
15. L. Hart, A.Van der Berg, L. Kuis, H. Van Dijk, R. Labadie, 1989, Planta Medicine, Vol. 55, pp. 509512.