Research Article

Synthesis Of Silver Nanoparticles By The Green Reagents (Polyphenolic Compounds) Prepared From *Ganoderma Lucidum,Phellinus Igniarius And Parmelia Perlata*

Sneha Paul[1], Changam Sheela Sasikumar [1] and Anita RJ singh[2]

[1]Department of Cellular & Molecular Biochemistry, Frontier Lifeline & Dr. K.M. Cherian Heart Foundation, R-30-C, Ambattur Industrial Estate Road, Mogappair, Chennai - 600 101, Tamil Nadu, India.
[2]Department of Biotechnology, Women's Christian college, College road, Chetpet, Chennai-600 006, Tamil Nadu, India.

ABSTRACT
Metal and metal oxide nanoparticles can be synthesized biologically by using different biological agents. In this study, we have come up with new and unique idea of synthesizing silver nanoparticles (SNPs) by polyphenolic compounds like tannin and flavonoid isolated from *Ganoderma Lucidum,Phellinus Igniarius and Parmelia Perlata*. Tannin and flavonoid are been used as green reagent in reducing and stabilizing SNPs. The size and formation of SNPs are been balanced and controlled by different parameters such as temperature, pH, tannin and flavonoid. Synthesized SNPs were confirmed by UV-Vis spectrophotometer and Scanning electron microscope, size of nanoparticles were obtained between 10-100nm.

Keywords: Tannin, flavonoid, green reagent, silver nanoparticles, *Ganoderma Lucidum*.

INTRODUCTION

Since long time silver colloids are present as metal nanoparticles that have been dispersed in the water and in other media.[1-6] These colloidal solution are been characterized by their colour but today it's known as due to surface Plasmon resonance whose origin is attributed to the collective oscillation of the free conduction electrons persuade by an interacting electromagnetic field form incident photon radiation[7] and another feature with regards the tyndall effect, scattering of light resulted by dispersed metal nanoparticles. In current era, the interest on nanomaterial's and their application has renewed the attention on metal nanoparticles colloidal dispersion[8-10].

There were different methods to synthesis metal nanoparticles but the preferred method for production was by chemical approach[6-14]. This method consists of addition of silver ions to a reducing agent containing solution, which helps in the reduction to nanoparticles. Readily available reducing agents are effective in the reduction of silver colloids. Researchers[8] have made complete statistics of various methods and about the chemical reduction the preferred source of reducing agent are sodium borohydride; it has been followed by various other agents such as citrate, amines, formaldehyde, aldehydes, ascorbic acid, sugars and polysaccharides. Chemical approach has advantages and disadvantages, as reducing agents used are being toxic to human system and environment. A typical use of citrate in reducing agent for the preparation of silver nanoparticles useful for surface enhanced Raman scattering application[15].

To overcome the disadvantages by chemical approach[16-19] in the medical field application, consequently biological method has been established. Polyphenolic compounds have been isolated from mushroom and lichen that has been used as reducing and stabilizing agent.

Tannin is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other macromolecules. Tannic acid, known also as gallotannic acid,it has been extracted from different plant sources or from plant galls or mushrooms or lichen thallus and there chemical structure forms a complex of pentadigalloyglucose and

pentagalloylglucose[20,21]. It acts an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannic acid is not toxic, as it form colloidal solution in water and also as reducing agent to convert silver ions to silver nanoparticles.

Flavonoids are known as Vitamin P and citrin, a class of plant secondary metabolites[22].Its one of important group of phytochemicals and have proven lot of pharmacological properties like anti-inflammatory, antiallergic, antibacterial, antiviral,antitumour properties and effective in neurodegenerative diseases[23-27].Scientist have found out in biological method,the flavonoids present in plant can be one of source acting as reducing agent of silver ions to silver nanoparticles[28].

This study attempts to synthesize silver nanoparticles using tannin and flavonoid isolated from *G.lucidum,P.Igniarius* and *P.perlata* directly rather than tannin and flavonoid rich plant or mushroom or lichen extracts.

MATERIALS AND METHODS

CHEMICALS

Silver nitrate, Acetone, ascorbic acid, Diethyl ether, Methanol, Folin Dennis reagent, HCl, Ethyl acetate , Amyl alcohol, Ethyl acetate, formic acid, boric acid, oxalic acid, Tannin, Querctin, chloroform, Liquid ammonia were purchased from HIMEDIA Laboratories Pvt. Ltd; Sigma laboratories Pvt.ltd and Fisher scientific, Thermo Electron Lls India Pvt. Ltd.

METHODOLOGY

Extraction and Purification of condensed Tannin

Condensed tannins were purified from the mushroom and lichen by the methods of Terrill et al. (1992) and Min et al. (2005) with the following procedures:-Samples were homogenized in 70% aqueous acetone (Hagerman, 1988) containing 0.1% ascorbic acid, and three rounds of diethyl ether extraction removed plant pigments.Remaining tannin fractions was freeze-dried, redissolved in 50%methanol (v/v) and purified by gel filtration using a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) washing with 50% methanol (v/v) and eluting with 70% acetone (v/v). The tannin extracts were freeze-dried and stored in the dark at 4^{o}C.Total condensed tannins was determined by using a Folin Dennis reagent and analyzed by thin layer chromatography. Freeze dried sample (condensed tannin) was used further for different purposes.[29-31]

Extraction and Purification of crude flavonoid

A small amount of mushroom and lichen is immersed in 2M HCl and heated in attest tube for 20-30min.The cooled extract is then filtered and re-extracted with ethyl acetate. Then the aqueous extract is further heated to remove the last trace of ethyl acetate and re-extracted with small amount of Amyl alcohol. The samples were evaluated by aluminum chloride and

analyzed by thin layer chromatography. Freeze dried sample (crude flavonoid) was used further for different purposes.[32-33]

Synthesis of silver nanoparticles by green reagent (condensed tannin and crude flavonoid)

For synthesis of silver nanoparticles, 1mM of 100ml silver nitrate was prepared and to that 1 gm of condensed tannin or crude flavonoid, both was added with constant stirring at 60-65^{o}C temperature and pH was brought to alkaline by 1N sodium hydroxide. Note for the colour change.

CHARACTERIZATION OF SNPs [34-36]

UV-VIS spectrophotometer

Ultraviolet- Visible spectrophotometry (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. It uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The silver nanoparticles solution was subjected to UV-Vis spectroscopy. Silver nitrate was used as blank solution.

Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) is a microscopy technique where the morphology and size of particles was determined. An image is formed by interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device. The SEM analysis was carried out at nanoscience and nanotechnology department, University of Madras, Chennai.

RESULTS AND DISCUSSION

Different method to synthesis silver nanoparticles, we are here coming with synthesis of nanoparticles by polyphenolic compounds especially tannin and flavonoid. Physical observation revealed the change in colour of the solution from colourless solution to dark brown within few minutes of adding tannin and flavonoid to aqueous silver nitrate solution. Silver nanoparticles exhibit a yellowish brown colour in aqueous solution

due to excitation of surface plasmon vibrations[37] and that indicates the formation of silver nanoparticles. Synthesis of nanoparticles explained in two steps:First step silver atoms are formed initially due to reduction of silver ions followed by formation of oligomeric clusters and these clusters eventually leads to the formation of colloidal silver particles.[38]

The purified compound of flavonoids are taken and confirmed by TLC plates and aluminum chloride, it is shown in fig 2,4 and table 2. The purified flavonoid compound isolated from different species is used to test for reduction property and it's compared with querctin. It confirms by change of pale colour to brownish, it's shown in fig 3 & 5 and table 3.Anish Stephen *et al*.,2013 relive almost similar result showing flavonoids can be responsible for the reduction of silver ions. From the study, we came to know the *ganoderma lucidum* gives the best source of flavonoids compared with other two species.[39]

The purified compound of tannin is confirmed by TLC plate and folin dennis reagent as shown in fig1,7 and table 1 .The purified compound was added to silver nitrate solution by altering pH it give brown colour shown in fig denoting the tannin also one of the compound that is responsible for the reduction and compared with standard tannin fig 5 & 8 and table 4. Venugopal Santhanam *et al.,*2009 shows the similar result using commercial available tannic acid compound**.** From the study, we come to know *Parmelia perlata* is the best source of tannin.[40]

UV-Vis spectroscopy was done in the wavelength range of 300nm and 700nm.the absorption peak was obtained can be seen in the table and absorption band for silver nanoparticles was observed in visible light region for silver nanoparticles,[41] as the particle size increase in size, the absorption peak usually shifts toward the red wavelengths which further reinforces the fact that there was no change in the particle size over a period of time. It was stable for almost 2 months. Scanning electron microscope was used to study the size and morphology of nano crystal domain and size of nanoparticles was in the range between 20-100nm, clumping of nanoparticles results in a larger particles was done as period of time. The size and morphology of the nanoparticles of the sample are shown in fig 9.

CONCLUSION

We have reported the synthesis of silver nanoparticles by green reagent such as condensed tannin and crude flavonoid, which provide simple and efficient ways for synthesis of silver nanomaterials. The method followed to synthesis silver nanoparticles was simple and cost effective. The characterization and confirmation of nanoparticles was done by UV-Vis spectroscopy and SEM. Polyphenolic compounds used to synthesis silver nanoparticles could be immense used in the medical field.

ACKNOWLEDGMENT

The authors are grateful to Centre of Nanoscience & Nanotechnology, University of Madras, Chennai to carry out the instrumentation works and Dr.K.M cherian for allowing to work in his laboratory, Frontier Mediville. I thank Mr. Joseph Varghese and Mr. Balakumaran for their valuable support throughout our work.

Table 1: The amount of total tannic acid present in the crude samples

Samples	Total Tannic Compound/ML
Ganoderma lucidum	42±2µg
Phellinus iginarius	16±0.6µg
Parmelia sulcata	60±0.2µg

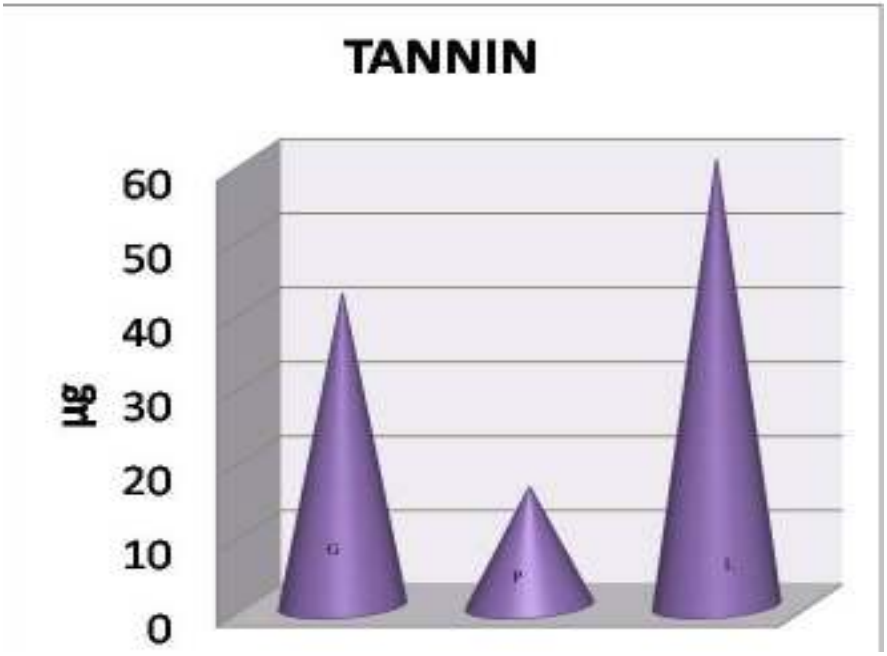

Fig. 1: Estimation of tannin

Table 2:The amount of total flavonoids present in the samples

Samples	Total Flavonoid /ML
Ganoderma lucidum	13.75±0.3µg
Phellinus iginarius	10±0.5µg
Parmelia sulcata	7.5±0.3µg

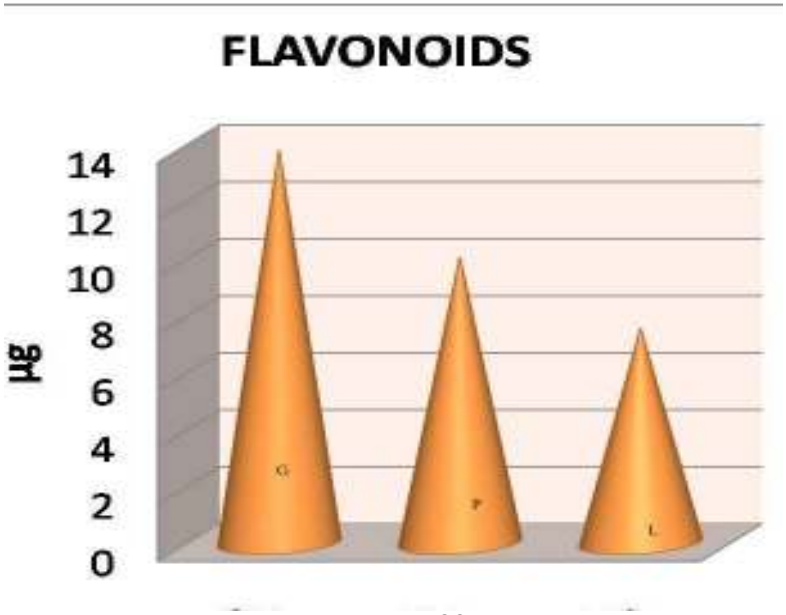

Fig. 2: Estimation of flavonoids

Table 3: The wavelength of flavonoids reduction compound

Samples	Wavelength
Querctin	**445nm**
Ganoderma lucidum	**410nm**
Phellinus igniarius	**426nm**
Parmelia sulcata	**450nm**

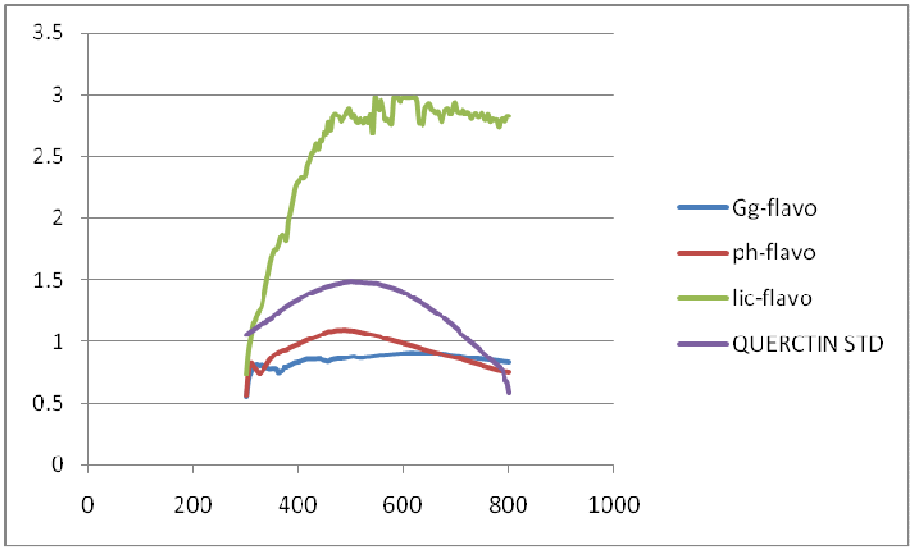

Fig. 3: UV-VIS absorbance spectra of silver nitrate solution with purified flavonoid compounds

Fig. 4: TLC plate showing the presence of flavonoids

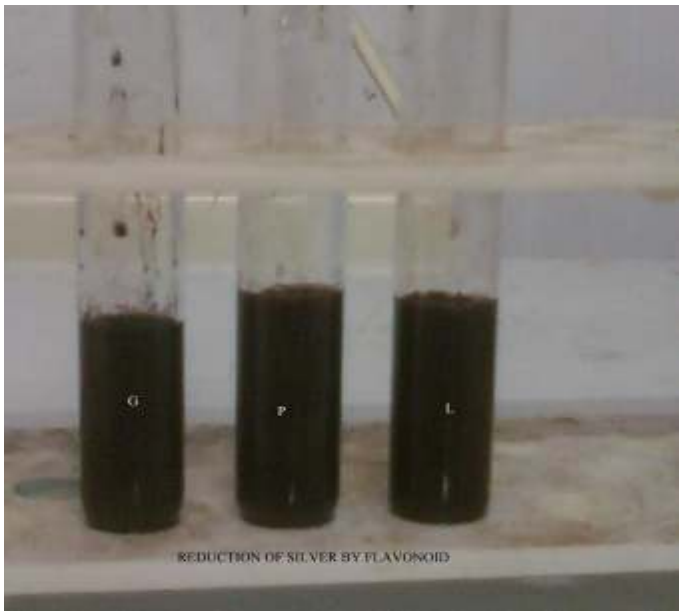

Fig. 5: The reduction of silver nitrate to silver nanoparticles using purified compound of flavonoids

Table 4: The wavelength by using tannin as reduction compound

Samples	Wavelength
Standard Tannic acid	**430nm**
Ganoderma lucidum	**415nm**
Phellinus igniarius	**420nm**
Parmelia sulcata	**425nm**

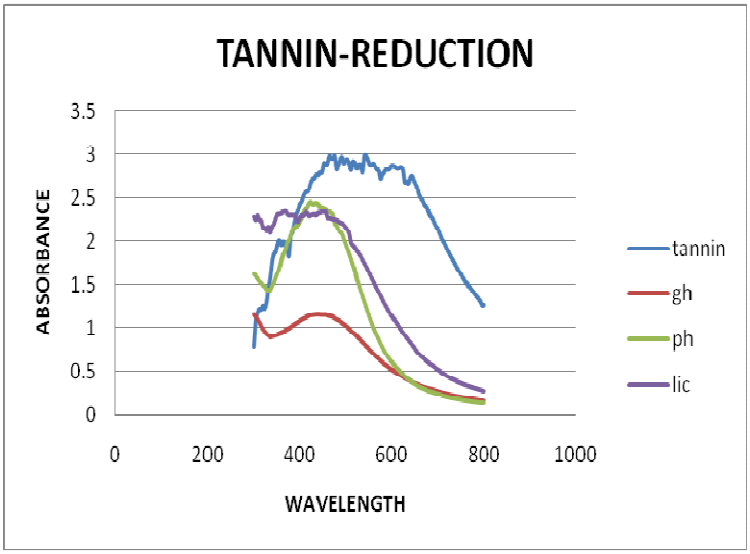

Fig. 6: UV-VIS absorbance spectra of silver nitrate solution with purified tannin compounds.

Fig. 7: TLC plate showing the confirmation of tannin

Fig. 8: The reduction of silver nitrate to silver nanoparticles using purified compound of tannic acid

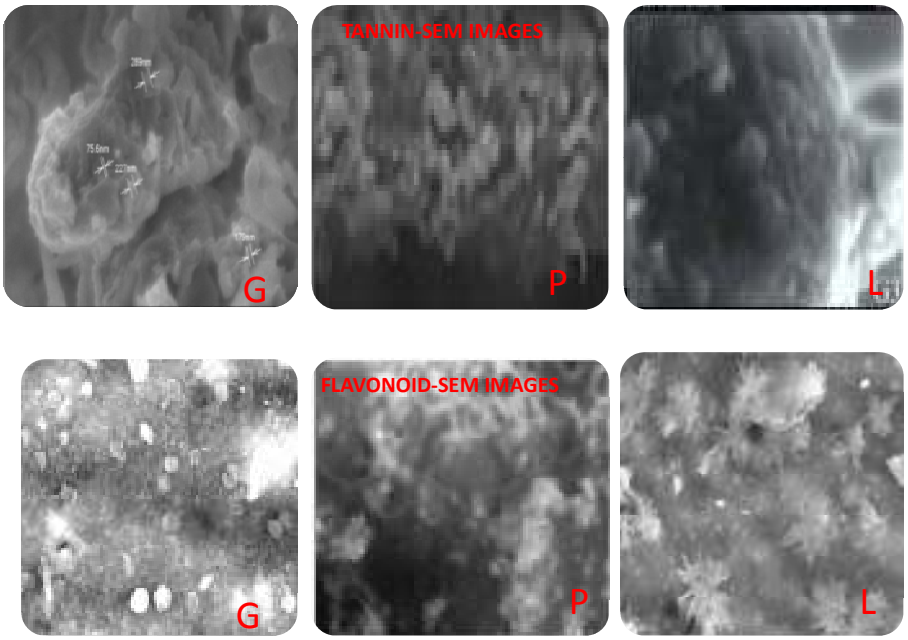

Fig. 9: SEM images of tannin and flavonois; G- *Ganoderma Lucidum, P-Phellinus Igniarius, L- Parmelia Perlata*

REFERENCES

1. Lettermoser A. J Prakt Chem.1903;68:357.
2. Schaum K, Lane H and Kolloid Z. 1921;28:243.
3. Guthier A, Huber J and Kuppinger O. Ber Deutsch Chem Ges. 1922;55:748.
4. Britzinger H and Kolloid Z. 1937;78:22.
5. Wiegel E. Z Phys. 1954;136:642.
6. Voyutsky S. Colloid Chemistry Mir Publishers, Moscow. 1978;Chapter 8.
7. Link S and El-Sayed MA. J Phys Chem B. 1999;103:4212.
8. Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP and Suidan M. Sci Total Environ. 2010;408:999.
9. Dallas P, Sharma VK and Zboril R. Adv Colloid Interface Sci. 2011;166:119.
10. Murphy CJ, Gole AM, Hunyadi SE and Orendorff CJ. Inorg Chem. 2006;45:7544.
11. Guzmán MG, Dille J and Godet S. Int J Chem Biomol Eng. 2009;2(3):04.
12. Solomon SD, Bahadory M, Jeyarajasingam AV, Rutkowsky SA, Boritz C, Mulfinger L. J Chem Educ. 2007;84:322.
13. Petica A, Gavriliu S, Lungu M, Buruntea N and Panzaru C. Mater Sci Eng B. 2008;152:22.
14. Rai M, Yadav A and Gade A. Biotechnol Adv. 2009;27:76.
15. Lucotti A, Casari CS, Tommasini M, Li Bassi A, Fazzi D, Russo V, Del Zoppo M, Castiglioni C, Cataldo F, Bottani CE and Zerbi G. Chem Phys Lett. 2009;478:45.
16. Yu DG. Formation of colloidal silver nanoparticles stabilized by Na+-poly (-yglutamic acid) silver nitrate complex via chemical reduction process. Colloids Surf B. 2007;59:171-178,
17. Tan Y, Wang Y and L Jiang. Tliiosalicylic acid-functionalized silver nanoparticles synthesized in one-phase system. J Colloid Interf Sci. 2002;249:336-345.
18. Petit C, Lixon P and Pileni MP. In situ synthesis of silver nanocluster in AOT reverse micelles. J Phys Chem. 1993;97:2974-12983.
19. Vorobyova SA, Lesnikovich AI and Sobal NS. Preparation of silver nanoparticles by interphase reduction. Colloids Surf A. 1999;152:375-379.
20. Steglich W, Fugmann B and Lang-Fugmann S. Rompp Encyclopedia of Natural Products. Georg Thieme Verlag, Stuttgart. 2000;630-631.
21. Budavari S. The Merck Index" 12th Ed. Merck Research Laboratories, Whitehouse Station. 1996;9221.

22. Foods Experimental Perspectives (7th edition) Hardcover – January 20, 2011, Margaret McWilliam..
23. Cushnie TPT and Lamb AJ. Antimicrobial activity of flavonoids," International Journal of Antimicrobial Agents. 2005;26(5):343–356.
24. Murray MT. Quercitin: nature's antihistamine," Better Nutrition. 1998;60(4):10.
25. Cook NC and Samman S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources," The Journal of Nutritional Biochemistry. 1996;7(2):66–76.
26. de Rijke E, Out P, Niessen WMA, Ariese F, Gooijer C and Brinkman UAT. Analytical separation and detection methods for flavonoids," Journal of Chromatography A. 2001;1112(1-2):31–63
27. Chebil L, Humeau C, Falcimaigne A,. Engasser JM and Ghoul M. Enzymatic acylation of flavonoids. Process Biochemistry. 2006;41(11):2237–2251,. ·
28. Pal RS, Ariharasivakumar G, Girhepunje K and. Upadhyay A. In-vitro antioxidative activity of phenolic and flavonoid compounds extracted from seeds of Abrus precatorius," International Journal of Pharmacy and Pharmaceutical Sciences. 2009;1(2):136–140.
29. Terrill TH, Rowan AM, Douglas GB and Barry TN. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. JSci Food Agr. 1992;58:321-329.
30. Min BR, Hart SP, Miller D, Tomita GT, Loetz E and Sahlu T. The effect of grazing forage containing condensed tannins on gastrointestinal parasite infection and milk composition in Angora does. Vet.Parasitol. 2005;130:105-113.
31. Hagerman AE. Extraction of tannin from fresh and preserved Leaves. J Chem Ecol. 1988; 14:453-461.
32. Tamanna Talreja. screening of crude extract of flavonoids of moringa oleifera against bacteria and fungal pathogen. Journal of Phytology. 2010;2(11):31-35.
33. Chang C, Yang M, Wen H and Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-182.
34. Instrumental Methods of Analysis. D Skoog Nieman and Holler.
35. A textbook of practical organic chemistry. Vogle.
36. Introduction to organic laboratory techniques: A small-scale approach., Donald l.pavia
37. Jae YS and Beom SK. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and Biosystems Engineering. 2009;32(1):79–84.
38. Kapoor S, Lawless D, Kennepohl P, Meisel D and Serpone N. Reduction and aggregation of silver ions in aqueous gelatin solutions. Langmuir.1994;10(9):3018–3022.
39. Anish Stephen and Sankar Seethalakshmi. Phytochemical Synthesis and Preliminary Characterization of Silver Nanoparticles Using Hesperidin. Journal of Nanoscience. 2013;Article ID 126564, 6 pages.
40. Sankar Kalidas Sivaraman, Iniyan Elango, Sanjeev Kumar and Venugopal Santhanam. A green protocol for room temperature synthesis of silver nanoparticles in seconds. Current Science. 2009;97(7):10.
41. Yamamoto S, Fujiwara K and Watarai H. Surface-enhanced Raman scattering from oleate-stabilized silver colloids at a liquid/liquid interface," Analytical Sciences. 2004;20(9):1347–1352.