International Journal of Pharmaceutical Research and Development

ISSN Print: 2664-6862
ISSN Online: 2664-6870
Impact Factor: RJIF 8
IJPRD 2024; 6(1): 105-109
www.pharmaceuticaljournal.net
Received: 04-01-2024
Accepted: 11-02-2024

Amandeep Swami
Associate Professor, Department of Pharmacology, Sciences, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Dr. Rajesh Asija
Principal, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Sakshi Yadav
Research Interns, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Synthesis, characterization and anti-cancer activity of *Tinospora cordifolia* extract medicated Silver nanoparticles

Amandeep Swami, Dr. Rajesh Asija and Sakshi Yadav

DOI: https://doi.org/10.33545/26646862.2024.v6.i1b.47

Abstract
With great authority various processed metals like silver, gold, etc were used by the traditional Indian systems to treat the various disorders; the usage of green synthesized metal nanoparticle preparations can be termed as a unique feature in healthcare system. Green synthesized silver nanoparticles (Ag NPs) come under the group of costly metals having high therapeutic value. The silver nanoparticles (Ag NPs) are one of the most indispensable and fascinating nanomaterials among various metallic nanoparticles that are involved in biomedical applications. In the present research, we discuss the green synthesis of Silver nanoparticles (AgNPs) and characterization is performed using a variety of analytical techniques, including photoluminescence spectroscopy, X-ray diffractometry (XRD), TEM and UV vis, spectroscopy. The cell viability assay results showed that *Tinospora cordifolia*- Ag nanoparticles inhibited proliferation of human cancer cell line A549.

Keywords: Silver nanoparticles, XRD, UV-vis, PL and anti-cancer activity

Introduction
The silver-based products were used by peoples from thousands of years as utensils and ornamental jewelry, however, the development of bioactive nanocrystals from silver has only recently been documented in scientific literature. Recently, too many applications of bioactive Ag have been reported in the food industry as a preservative, antibacterial activity against multiple drug-resistant bacteria, as an antifungal agent, agriculture improvement, diagnostics, cosmetics, electronics and catalysis. Recently, *Tinospora cordifolia* extract medicated silver nanoparticles were used in several biomedical activities, medicines, textiles, and so on [1-4].

Because of their applications, several methods have been developed, including chemical and biological. Generally, chemical and physical method are very expensive and hazardous while biological methods are non-toxic, reliable, simple, high yield, rapid and solubility dependable that could produce uniform Ag NPs underoptimized condition. Numbers of protocols have been well established and optimized to prepare the silver nanoparticles from the biological materials like plant parts (flowers, stem, leaves, roots and fruits, barks, seeds), microbes including fungi. Previously, Ag NPs were successfully developed by using different plant extract of *Aloe vera*, *Mangifera indica*, *Ocimum tenuiflorum*, and *Syzygium cumini*. The metabolites (Alkaloids, tannins, ascorbic acid etc.) present in plant extract act as size reducing and stabilizing agents. The characterization of nanoparticles is an essential step to understand the surface area, size and shape, solubility, size distribution etc.; it needs to be evaluated before assessing biocompatibility or toxicity (Shin *et al*., 2015). Several analytical techniques were used for synthesized nanomaterials, including UV-Vis spectroscopy, XRD, PL and TEM. The Ag NPs with control structures that are uniform in size and shape and morphology, successfully used in many biomedical applications [5-7].

Medicinally *Tinospora cordifolia* extract medicated silver nanoparticle is very important and it is used as drug against various diseases like antistress, anti-anxiety, neuroprotective activity, etc. This nano drug is also used in eye diseases, cough, jaundice, anemia, psychological diseases, abdominal diseases, liver and kidney problems [8, 9].

Corresponding Author:
Amandeep Swami
Associate Professor, Department of Pharmacology, Sciences, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Materials and methods

Material synthesis: Recently, *in vitro* technique has provided new approaches for the biological preparation of nanoparticles. This technique is simple and faster than the synthesis of nanoparticles from the whole plant, as it cuts off the time required for the diffusion and uptake of metal ions via the plant. This technique provides flexible control on the morphology of green nanoparticles by changing the various physical conditions. Also the morphology of nanoparticles depends on plant explant, which is used for the extract preparation. Recently, nanoparticles were developed successfully by using different metals (silver, gold and copper) and plant extract prepared from different explants such as *Tinospora cordifolia*.

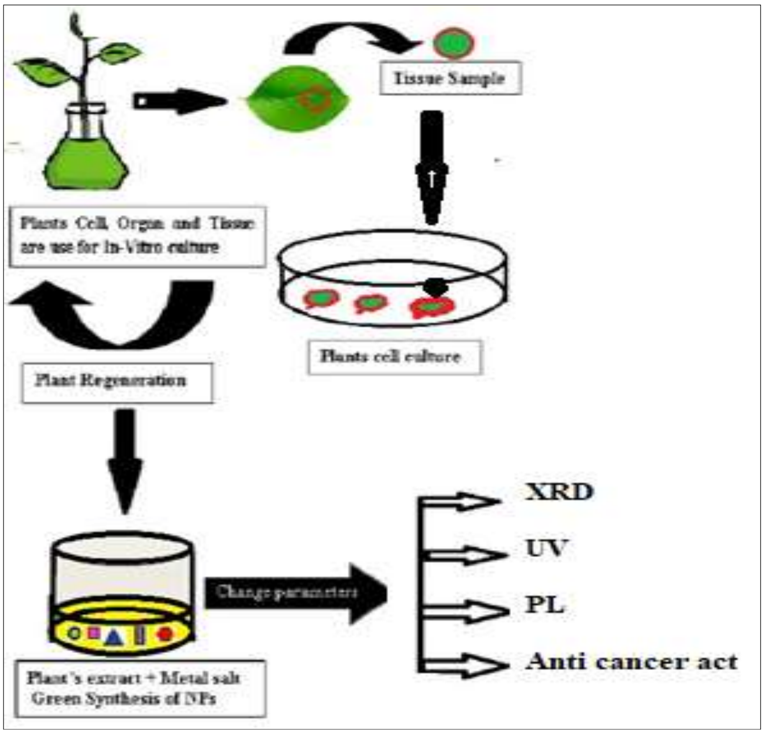

Fig 1: Synthesis technique of plant extract capped Ag NPs

Characterization Techniques

Material characterization is an important role in identifying the nanoparticles size, shape and also their porosity, pore size, particles size distribution etc. Following techniques are used for characterization: Transmission Electron Microscopy (TEM), UV-Visible Spectroscopy, PL and X-ray-diffraction technique (XRD).

UV-Visible Spectroscopy

It is a commonly used characterization technique that identifies and analyzes various nanoparticles by measuring their plasmon resonance. In general, the wavelength range of between 300 - 600 nm is confirmed for the preparation of nanoparticles.

X-ray-diffraction (XRD)

This technique is useful for the study of structure and crystalline size of the synthesized nanoparticles. The XRD pattern confirmed the nanocrystal form and crystalline nature properties of the nanoparticles. The high peaks produced by XRD technique indicated that the synthesized nanoparticles are face-centred cubic, and crystalline.

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

Transmission Electron Microscopy (TEM)

TEM techniques are used to confirm the surface the surface morphology and morphological characterizations at the nanometer and micrometer scale. This is very costly and time-consuming techniques.

Cell viability Assay of Green Synthesized Silver Nanoparticles

The cell line A549 was purchased from National Centre for Cell Science (NCCS), Pune, India and was grown in Dulbecco's Modified Eagle's Medium (DMEM). Cells were seeded into 96 well plates approximately as 5*104 cells at 37ºC in a 5% carbon dioxide atmosphere.

Results

Optical absorption and photoluminescence spectrum

Photoluminescence (B) and UV-Visible absorption spectra (A) of bio-inorganic nanoparticles with the corresponding nano-composite thin film excited with 300 nm was monitored (Figure 2 A & B). The excitation & emission peak positions of the bio-inorganic Ag NPs nanoparticles samples were determined by fitting these bands to Gaussian curves. The appearance of surface plasma resonance (SPR) peak at 414 nm confirms the formation of *Tinospora cordifolia*-Ag nanoparticles. The observed symmetric sharper SPR band with a single peak is due to spherical particles. The excitation & emission peak positions of the *Tinospora cordifolia*- Ag nanoparticles samples were determined by fitting these bands to Gaussian curves. The observed photoluminescence could be deconvoluted into five bands designated as E1 (418 nm), E2 (455 nm), E3 (479 nm), E4 (554 nm) and E5 (619 nm). The observed red shift of the photoluminescence emission peak with respect to the fundamental absorption edge at 414 nm may be attributed to electron-phonon or Frohlich interaction.

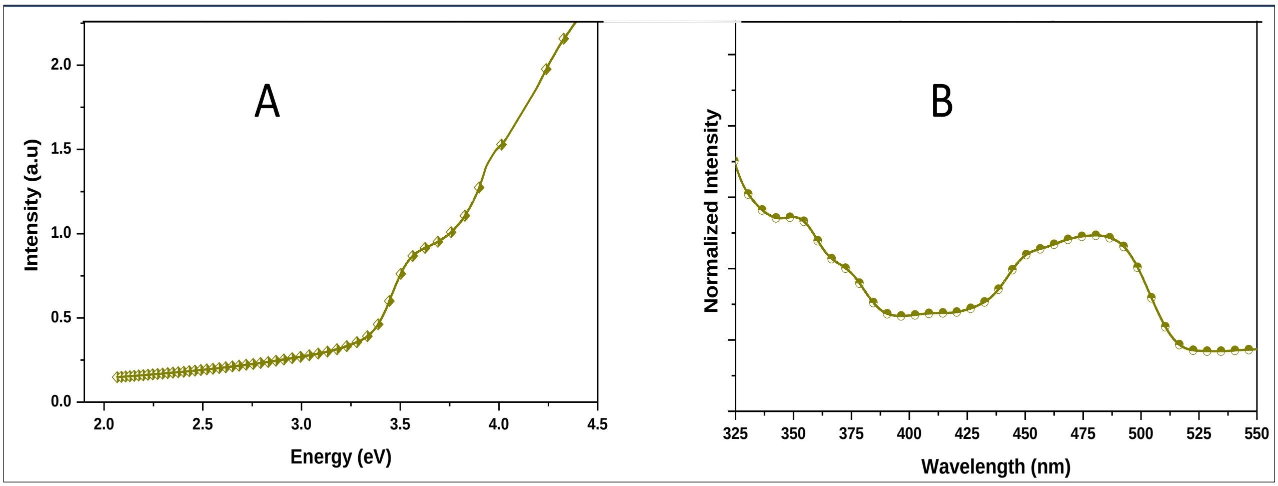

Fig 2: Optical absorption (A) and photoluminescence (B) spectrum of Ag nanoparticles

Transmission Electron Microscopy

The green synthesized bio-inorganic Ag nanoparticles were further confirmed by TEM images. The two dimensional image of the synthesized bio-inorganic Ag nanoparticles is presented in Figure 3. It has been reported that the bio-inorganic Ag nanoparticles nanoparticle size was measured using line profile determination of individual particles in the range of 32.85 x 32.85 nm. The Ag nanoparticles were imaged by TEM to understand the accurate configuration of clusters and also to verify that the Ag NPs nanoparticles were more or less homogenous in shape & size. The average cluster size of Ag nanoparticles was measured using TESCAN line profile determination of individual cubic shaped clusters in the range of 108±20 nm.

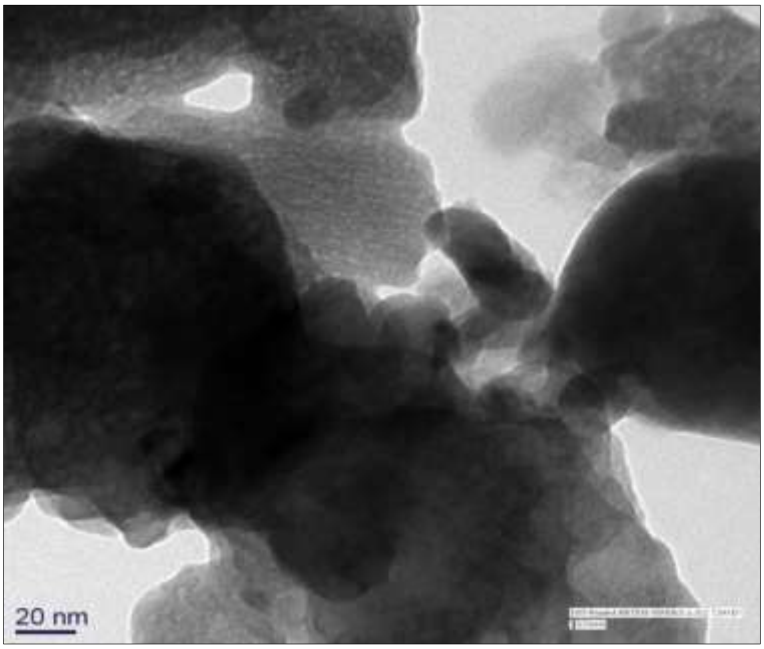

Fig 3: Transmission Electron Microscopy of AgNPs

X-ray diffraction analysis

The crystallographic structure of biologically synthesized silver nanoparticles (Table 1; Figure 3) depicted four distinct peaks at 37.47°, 46.05°& 64.76° which were the corresponding values at (1 1 1), (2 0 0) and (2 2 0) hkl planes of face centered cubic (FCC) Ag crystals, closely matching with the reported crystal values. All diffraction peaks could be well indexed with the cubic structure of Ag [JCPDS No. 087-0720]. The absence of any other chemical phase indicated purity and crystallinity of as traditionally synthesized AgNPs. The systematic increase in the broadening (FWHM) of the diffraction peaks with increasing 2θ indicated a significant reduction in particle size of AgNPs. The obtained diffraction spectrum of the sample strongly suggested the presence of Agnanoparticles in accordance of SEM and optical analysis. The angle positions ofhkl planes were shifted towards the higher angle because of tensile strain generation in the surface of AgNP snanocrystals due slightly to the presence of polymer extracts as cappant. Additionally, small hump like peaks were also observed at 34.15°, 41.57°, 52.08° and 60.1° which corresponds to the different polymers (Bhavnadrvya) coated on the AgNPs nanoparticles surface. These XRD results confirm the formation of highlynano-crystalline inorganic core and organic shell with some amount of strain in lattice. This is in good agreement with TEM & Optical absorption data.

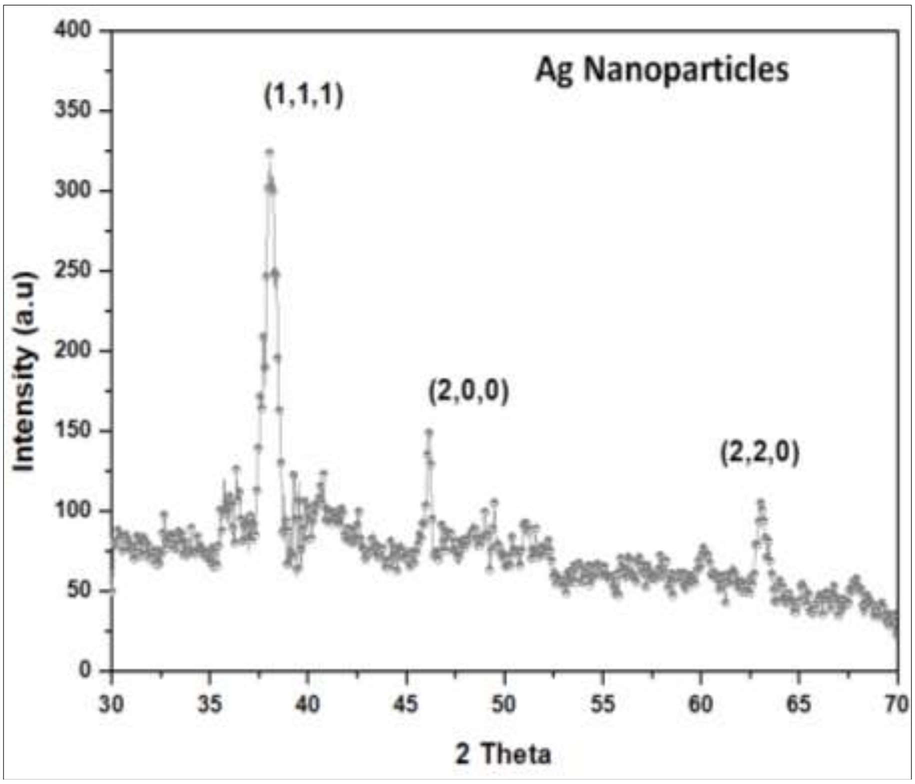

Fig 4: X-Ray diffraction spectra of Ag Nanoparticles

Table 1: Summary of the XRD peak positions (2 theta), corresponding (hkl) value, different compounds,and crystalline phase for AgNPs

Sample Name	2θ (Degree)	FWHM (Degree)	Lattice constant (A)	Miller (hkl)	Standard d-value	Observed d-value
Ag NPs	37.4°	0.64	4.106	(111)	2.36	2.36
	46.5°	1.15		(200)	2.04	2.03
	64.7°	0.74		(220)	1.44	1.41

Cell Line Studies

Anti-cancer Activity of *Tinospora cordifolia* Synthesized Ag nanoparticles

The observations of experiments showed that green synthesized Ag nanoparticles inhibited proliferation of human breast cancer cell line A549 (Figure 5). These research results suggest that Ag nanoparticles may exert its anti-proliferative effect on cell line by suppressing its growth. Hence, the inhibitory concentration at 50% (IC50) was observed at 55 µg/mL of Ag nanoparticles s for A549 cells. (12) The anti-proliferative effect of Ag nanoparticles synthesized from leaves extract upon staining with PI showed apoptotic changes and nuclear condensation. In case of control cells, a very negligible number of PI positive cells were noticed. By contrast a progressive increase in the number of PI positive cells was noted in Ag nanoparticles treated cells. This data suggest that Ag nanoparticles can induce cell death in cancer cells through the reactive oxygen species mediated apoptotic process. The increased reactive oxygen species (ROS) levels and subsequent loss of mitochondria membrane potential might be the reason for increased apoptotic morphological changes in Ag nanoparticles treated cells.

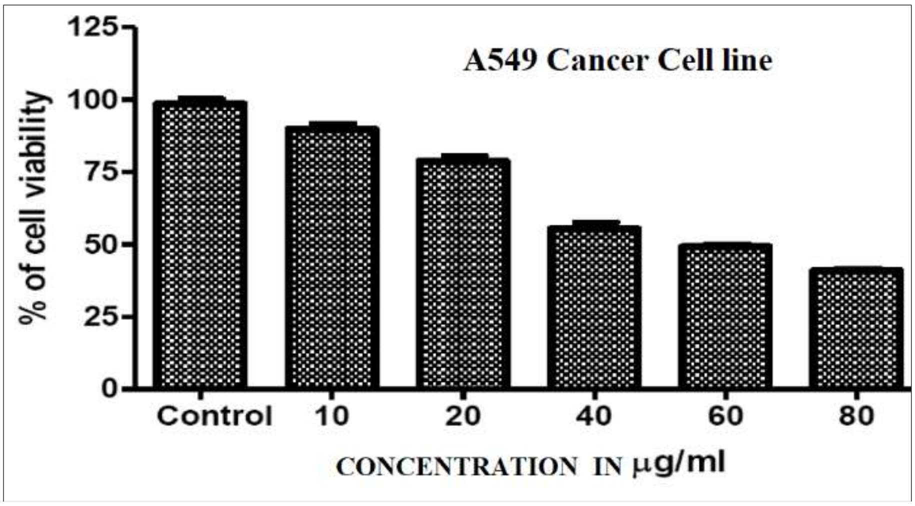

Fig 5: Anti-Cancer activity of

Discussion

The present findings give directions for economically feasible and eco-friendly method for green synthesis of silver nanoparticles. Since this method does not involve the use of any hazardous chemicals, the strategy for synthesis is called 'green synthesis of silver nanoparticles' also. In this study, to understand the acting mechanism of plant phytochemicals on silver nitrate deeply, we identified the biomolecules of stem extracts and studied the interaction between plant phytochemicals and silver nitrate through UV. Studies have revealed that water-soluble phytochemicals such as flavonoids and phenols are responsible for the immediate reduction and stabilization of silver nanoparticles.

In this study, we investigated the anti-cancer activity of silver nanoparticles gainst A549 cell line. It was conclusively proved that silver nanoparticles synthesized through extract of *Tinospora cordifolia* used in combination with antibiotics to show no any synergistic effect, except linezolid. On the other hand, bactericidal assessment of nano-silver showed a good bactericidal action compare to antibiotics (Ofloxin). The binding affinity of nano-silver towards bacterial membrane induced loss of catalytic activity for respiratory chain dehydrogenases.

Our results showed that *Tinospora cordifolia*- Ag nanoparticles inhibited proliferation of human breast cancer cell line A549 (Figure 5). These results suggest that *Tinospora cordifolia*- Ag nanoparticles may exert its antiproliferative effect on cell line by suppressing its growth. Hence, the inhibitory concentration at 50% (IC50) was observed at 57 µg/mL of *Tinospora cordifolia*- Ag nanoparticles for A549 cells. The anti-proliferative effect of *Tinospora cordifolia*- Ag nanoparticles synthesized from leaves extract upon staining with PI showed apoptotic changes and nuclear condensation. In case of control cells, a very negligible number of PI positive cells were noticed. By contrast a progressive increase in the number of PI positive cells was noted in *Tinospora cordifolia*- Ag nanoparticles treated cells. This data suggest that *Tinospora cordifolia*- Ag nanoparticles can induce cell death in cancer cells through the reactive oxygen species mediated apoptotic process. The increased reactive oxygen species (ROS) levels and subsequent loss of mitochondria membrane potential might be the reason for increased apoptotic morphological changes in *Tinospora cordifolia*- Ag nanoparticles treated cancer cells.

Conclusion

We have synthesized Silver nanoparticles using the green synthesis method and studied the crystallographic and morphologicl characterization using X- ray diffraction, Transmission electron microscope, UV, and Photoluninescene techniques. All the analysis confirmed the formation of plant extract capped silver particles in nanometer regime. The optical absorption showed intense peak of silver nanocrystals at 461.20 nm with signs of surface plasmon states near. As prepared Tinosfora capped silver nanoparticles thin film gave rise to another interesting feature with higher intra-band transition were predicted around 490 nm. The XRD & TEM analysis further confirmed the size of nanoparticles is around ~48nm. Silver nanoparticles are showing emission spectra near to 548.00 nm. The nanostructure medicine or nanomedicine is newly used word but in traditional Indian medicine the form of rasayana the nanostructures of herbal and minerals are being used as a bioavailable and effective medicines. Similarly, the diffraction pattern of green synthesized silver revealed existence of peaks (111), (200) and (220) which matched with the standard JCPDS.

Acknowledgement: I wish to express my deep gratitude and respects Principal Sir Dr Rajesh Asija Maharishi Arvind Institute of Pharmaceutical Science, Jaipur Rajasthan, India for his keen interest and valuable guidance, strong motivation and constant encouragement during the course of the work. I thank Dr Nayan Mishra Director of Innovation Incubation center JVWU Jaipur for his experimental help and extensive discussions around my work.

References

1. Pileni MP. Fabrication and physical properties of self-organized silver nanocrystals. Pure Appl. Chem. 2000;72:53-65.
2. Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, *et al*. Heavy metal content of Ayurvedic herbal medicine products. JAMA. 2004;292:2868-2873.
3. Dhanalakshmi D, Dhivya R, Manimegalai K. Antibacterial activity of selected medicinal plants from South India. Hygeia J Drugs Med. 2013;7:63-68.
4. Adhvaryu MR, Reddy N, Parabia MH. Anti-tumor activity of four Ayurvedic herbs in Dalton lymphoma ascites bearing mice and their short-term *in vitro* cytotoxicity on DLA-cell-line. Afr. J Tradit. Complement Altern. Med. 2008;5(4):409-418.
5. Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR, *et al*. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B Biointerfaces. 2010;81:81-86.
6. Ahmed S, Ahmad SM, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat. Res. Appl. Sci. 2016;9:1-7.
7. Aranha I, Clement F, Venkatesh YP. Immunostimulatory properties of the major protein from the stem of the Ayurvedic medicinal herb, guduchi (*Tinospora cordifolia*). J Ethnopharmacol. 2012;139(2):366-372.
8. Vagbhata. Rasa Ratna Samuchchya, 5/14, commented by Kulkarni DA. 3rd ed. Delhi: Mehar Chand Lachman Das; c1982.
9. Pal S, Tak YK, Song JM. The antibacterial activity of silver nanoparticles depends on the shape of the nanoparticle - A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ Microbiol. 2007;73(6):1712-1720.
10. Sanpui P, Murugadoss A, Prasad PV, Ghosh SS, Chattopadhyay A. The antibacterial properties of a novel chitosan–Ag-nanoparticles composite. Int. J Food Microbiol. 2008;124(2):142-146.
11. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 2007;18(22):1.
12. Kuppusamy P, Solachuddin J. Ichwan A. *In vitro* anticancer activity of Au, Ag nanoparticles synthesized using *Commelina nudiflora* L. aqueous extract against HCT-116, colon cancer cells. Biol. Trace Elem Res. 2016;120:1-6.