Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.7202

PLANT MEDIATED SYNTHESIS, CHARACTERIZATION AND ANTI-NEOPLASTIC EVALUATION OF AGNPS USING AQUEOUS EXTRACT FROM LEAVES OF *CALLISTEMON VIMINALIS*

Farhat Qayyoum[1], Eiman Ismail[2], Uswa Siddique[3], Noor Talaat Nassar[4], Afifa Kousar[5], Iqbal Nisa[6], Qudsia Begum[7], Nehala Naz[8], Farrukh Hussain[1#], Farah Siren[9*]

[1]Institute of Biological Sciences, Sarhad University of Science & Information Technology, Peshawar, Pakistan
[2]Department of Biosciences, Comsats University Islamabad
[3]Department of Medical Microbiology and Clinical Microbiology University, Near East University, North Cyprus
[4]Department of Medical Microbiology and Clinical Microbiology University, Near East University, North Cyprus
[5]Department of Chemistry, University of Lahore, Pakistan
[6]Department of Microbiology, Women University, Mansehra, Pakistan
[7]Bahria University College of Allied Health Sciences (BUCAHS), Bahria University Health Sciences Campus Karachi
[8]Department of Botany, University of Gujrat Subcampus Satellite Town, Rawalpindi
[9]Department of Allied Health Sciences, Iqra National University, Peshawar Campus

***Corresponding Author:** Farah Siren
*Email: farahshireen@inu.edu.pk
[#]Co-Corresponding Author: Farrukh Hussain
Email: farrukh.biotech@suitmedu.pk

ABSTRACT

Aqueous extracts of *Callistemon viminalis* plant were used to synthesize silver nanoparticles (AgNPs) in this study. The synthesized AgNPs were examined by energy dispersive spectroscopy (EDX), ultraviolet-visible spectroscopy, X-ray diffraction (XRD)and scanning electron microscope (SEM). UV-vis spectroscopy exhibiting peak absorbance at 440nm. XRD exhibiting nanoparticles were polycrystalline in nature. Scanning Electron Microscope concludes nanoparticles were spherical in shape. The green fabricated silver nanoparticles were compared with methanol, ethanol, n-hexane and aqueous extract revealing excellent results against PC3, HeLa cell line, Brine shrimp lethality assays. Significant cytotoxic activity was exhibited by biogenic AgNPs and aqueous extracts (16.66%) at high concentration sample of 1000ml.Biosynthesized AgNPs exhibited (96.4%) antineoplastic activity against PC3- cell line than othercrude extracts Ethanol (32.0%), Methanol (46.2%) and n-Hexane (15.9%) which demonstrated moderate antineoplastic activity. Bioinspired AgNP sex hibited (92.7%) antineoplastic activity against the HeLa cell lines n-Hexane (15.7%), Ethanol (72.5%), Methanol (60.6%) and aqueous extract showed (9.2 %) antineoplastic activity.

Keywords: Plant mediated synthesis, characterization, antineoplastic activity, AgNP.

INTRODUCTION

Nanoparticles are minute substances that range in size from 1-100 nm. They can be categorized indifferent sizes, properties, and morphology. Nano substances possess peculiar physio-chemical properties because of high surface area and miniature size (Khan *et al*., 2019). They act as excellent antimicrobial agents because of their bactericidal activity in farming, industry and health management (HumbertoPalza., 2015). Nanoparticle delivery systems have been extensively studied in clinical practice with many constituents grounded techniques and technologies. The general prospects of nanoparticle drug distribution systems are promising (Anselmo&Mitragotri., 2016). Cancer causes worldwide (about 13% of total deaths) 7.6 million deaths in 2008. Nearly 13 million new cancer cases are diagnosed every year. The deaths are estimated 13.1 million in 2030. Though use of nanoparticles is tremendously promising path for future technological innovations where nanoparticles can act as carriers (Lucky *et al*., 2015). The ecological effect of silver nanoparticles has recently become a matter of concern because they have been involved in numerous consumer goods including fabrics, therapeutic products, domestic appliances, cosmetics, food containers, paints and nano-functionalized plastics. The fabrication, usage and removal of nanoparticles comprising products are potential means for ecological exposure (McGillicuddy*et al*., 2017). Advancement of consistent and ecological friendly approaches for the production of NPs is an important phase in nanotechnology field. The development of green synthesis is ecofriendly, economical, and easily scaled up for massive measure of NPs fabrication (Rafique*et al*., 2017). $AgNO_3$ particles play vital part in nanotechnology in nanomedicine. Though numerous precious metals being used for different initiatives, they have been engrossed on possible applications in analysis and treatment of cancer (Zhang *et al*., 2016). The pharmacological role of Ag nanoparticles is important because of their therapeutic significance (Castro-Aceituno*et al*., 2016). *Callistemon viminalis* belongs to kingdom of Plantae, subkingdom of Tracheobionta, super division of Spermatophyta, division of Magnoliophyta, class of Magnoliopsida, order of Myrtales. *Callistemon viminalis* is a genus of *Callistemon* in the family of Myrtaceae. Its species include *Metrosiderosviminalis* Sol. ex Gaertn., *Callistemon viminalis (*Sol. ex Gaertn.) G. Don, *Melaleucaviminalis*(Sol. ex Gaertn.) Byrnes. *Callistemon*is similar to traditional bottlebrush and is categorized by its cylindrical, brush like flowers comprises of 34 species belongs to family Myrtaceae. *Callistemon viminalis* native to Australia (weeping bottlebrush) is a small tree or shrub, and reaches a height of 4 m where it naturally occurs in temperate areas. Environmentally, for decorative purposes or for weed control and forestry plantations *Callistemon* species are planted. *Callistemon viminalis* leaves contain phenolic substances which have been used as therapeutic agent in conventional medicines. Almost all parts of plant have medicinal applications. This plant has insecticidal, antifungal, antioxidant, molluscicidal, and antibacterial activity. In this context silver nanoparticles and gold nanoparticles are of particular interest for plant-based synthesis (Sharma *et al*., 2018). This study aimed to treat a variety of human health problems using *Callistemon viminalis* as shrubs or natural products which has renewed interest in their usage for cancer treatment.

METHODOLOGY

Plant Collection

In February 2019fresh leaves of *Callistemon viminalis* were collected from Hayatabad Town, District Peshawar, Pakistan. The plant was identified by taxonomist Ghulam Jelani Botany Department, University of Peshawar, Pakistan.

Extraction

Firstly, an electric blender was used to powdered the shade dried leaves of *Callistemon viminalis*. At room temperature 25 °C, 100g of powdered leaves were mixed in 500ml methanol, ethanol and n-hexane for 14 days.25g leaves extract was mixed in 500 ml distilled water to obtain aqueous extract. All the solvents were filtered by filter paper. Methanol, n-hexane and ethanol were allowed to be evaporated at room temperature 25°C and solvent-free crude extracts residues were collected for antineoplastic and cytotoxic assay evaluation in Eppendorf tubes.

Preparation of Silver nanoparticles

In conical flask One mM of $AgNO_3$ solution was prepared. 90 ml $AgNO_3$ was mixed with 10 ml *Callistemon viminalis* aqueous extract. The conical flask was placed in shaking water bath at 75ºC for 1 hour. Color change indicated formation of silver nanoparticles from yellow to brown.Solvent aqueous extract was poured in petri plates then dried over hot plate. The extract was then scratched in petri plate and stored in Eppendorf tubes.

Purification of silver nanoparticles

Distilled water was added in eppendorf tubes, centrifugatedat 2500 rpm for 12 minutes to purify synthesized AgNPs from non-coordinating organic molecules. Purified AgNP pellets were collected at the bottom of eppendorf tubes used for further characterization and supernatantwas removed.

Characterization of silver Nanoparticles

The following methods were used to characterize the chemical and physical properties of silver nanoparticles,

UV-Vis Spectroscopy

UV-Vis spectrometry (Model Shimadzo UV) with maximum absorption within range of 350-500was used for confirmation of AgNPs.

Scanning Electron Microscope

Scanning electron microscope (JEOL, JAPAN, JSM 5910) observedsurface morphological studies and size of silver nanoparticle. A double-sided carbon conductive tape was placed on SEM stub made of brass. A small quantity of AgNPs was placed on upper side of carbon tape with spatula. Then the AgNPs was coated with gold for 40 sec at 35 DC milliampere in SPI Gold Sputter Module. The gold coated AgNPs was placed in SEM machine. When the vacuum was ready, the sample was analyzed through scanning electron microscope at 1000X, 2500X, 5000X magnification.

Energy Dispersive X-Ray Spectroscopy

EDX (UK oxford INCA 200) spectrometer was used for elemental indication of AgNPs. The aggregate indicator of Ag powerfully establishes $AgNO_3$ reduction in AgNPs. A double-sided carbon conductive tape was placed on EDX stub made of brass. A small quantity of AgNPs was placed on upper side of carbon tape with spatula. Then the AgNPs was placed in EDX machine.

X-ray Diffraction Measurement

Properties of AgNPs were analyzed by the crystallographic nature of prepared green AgNPs (JEOL, JAPAN, model JDX 3532) comprises with current supply 30mA/40kv with Cu tube Kα radiation graded with wavelength 1.54A.

Biological Investigations of Silver nanoparticles by comparing Crude plant extracts

The Pharmacological and biological activities of AgNPs and plant extracts (methanol, n-hexane, ethanol and aqueous) were determined for cytotoxic and anticancer activity as follows (Ahmad *et al*. 2016).

Antineoplastic Assay

Test Cancer Cell line

(PC-3) Human prostate cancer cell line,Hela Cell line.

Procedure

Senthilraja&Kathiresan(2015) performed MTT (3- [4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay for survival and cellular growth using 96-well flat-bottomed

micro plates to evaluate antineoplastic activity of compounds.*Callistemon viminalis* plant leaves were used for evaluation of anticancer assay observing AgNPs, n-hexane, ethanol and methanol extracts. 100 µg/ml of streptomycin and100 IU/ml of penicillin with 5% of fetal bovine serum (FBS)and 5% CO_2 incubated at 37 ^oC in 75cm flasks were used for culturing PC3 cells (Prostrate Cancer) in Dulbecco's Modified Eagle Medium. The essential medium was provided for harvesting the growing cells. Then hemocytometer counted the cells. $1x10^5$ cells/ml concentration of cultured cells were added into each (100 µL/well)96-well plates. Medium was removed after incubation at 37 ^oC(1 - 30µM) various concentrations of compounds were added with 200 µL of new medium. (0.5 mg/ml) MTT was added after 48 hours and incubated for 4 hours in each well. Each well was added with 100µL of DMSO. Using (Spectra Max plus, Molecular Devices, CA, USA) micro plate reader, measure MTT reduction to formazan at 570nm absorbance. 50% (IC50) growth inhibition was recorded for evaluation of PC3 cells cytotoxicity (Ahmed *et al*., 2016).

The percent inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{100-(\text{mean of O.D of test compound} - \text{mean of O.D of negative control}}{(\text{mean of O.D of positive control} - \text{mean of O.D of negative control})} \times 100$$

Doxorubicin was used as a positive control while crude plant extract used as a negative control. Soft-Max Pro software (Molecular Device, USA)accessed the percent inhibition results.

Cytotoxic Assay

Test Normal cells

Artimiasalina

Cytotoxic assay conducted for monitoring of physiologically active natural products and screening. It is a general, inexpensive, and rapid bioassay. Brine shrimp *Artemiasalina* eggs are generally used for this purpose available as fish food in pet shops Karachi. The eggs hatched within 48hours when placed in artificial seawater. *Artimiasalina* are considered toxic to bioactive compounds.

Procedure

The hatching tray (a rectangular dish (22 x 32 cm) was half filled with sea water (50mg) eggs of brine shrimp were added in it and incubate at 37^oC. Brine shrimp retain viability at (4^o C) temperature. Crude leaf extracts and AgNPs evaluation was done by lethality assay (brine shrimp)*Artimiasalina*. Cell toxicity was determined by adding brine shrimp eggs to different sample dilution (10, 100, 1000ug/ml) of *Callistemonviminalis* leaves extract. (20mg) test sample was added in 2ml solvent then 5, 10, 500µl from this solution was transferred to each vial. Allowed the solvent to evaporate overnight. Using Pasteur pipette after 2 days when nauplii matured and hatched under illumination place 10 larvae to each vial. Incubate at 25-27^o C for 24 hours. Make the volume 5ml sea water. Data can be read by Finney computer program determining LD_{50} values. After initiation of hatching shrimps can be used for 48-72 hours. Etoposide drug used as a positive control and calculated as follows

$$\text{Percent shrimp lethality} = \frac{\text{No. of dead}}{\text{Total number of shrimps}} \times 100$$

RESULTS

Characterization of Silver Nanoparticles

Different spectroscopic results are analyzed to compare characterization of purified silver nanoparticles and aqueous extracts from *Callistemon viminalis* leaves.

UV-VIS Spectroscopy

UV visible spectra analysis showed maximum absorbance peak for the biosynthesized AgNPs ranging from 350 and 500 nm, while aqueous extract exhibiting asymmetrical absorbance patterns were also in the same range. The absorbance peak for *Callistemon viminalis*AgNPs was displayed at 440 nm, with peak absorbance of 1.70. Spectroscopic results are summarized in (Fig. 4.1 & 4.2).

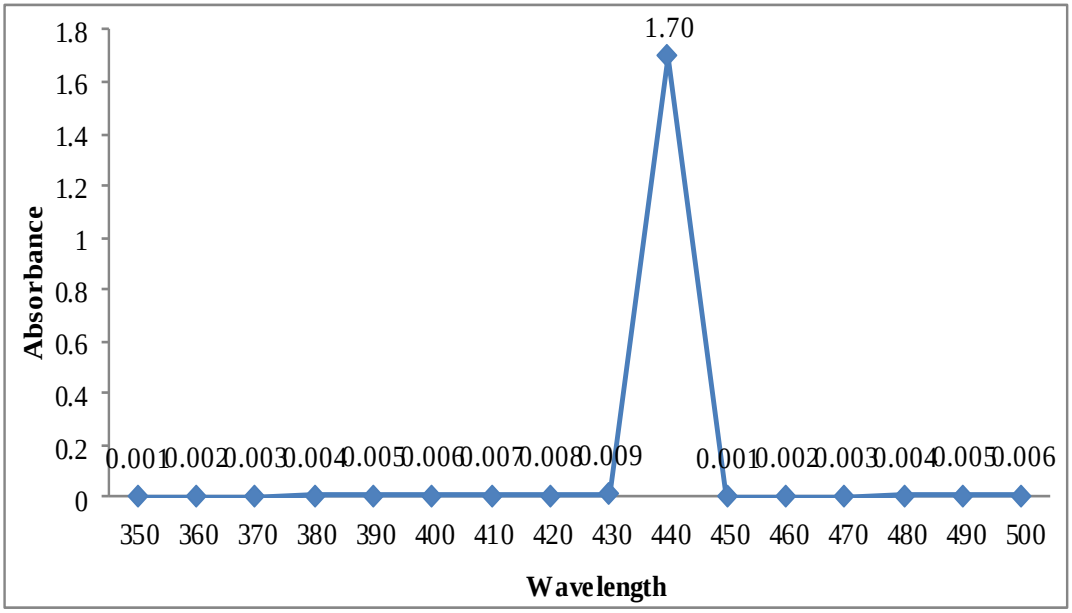

Fig. 4.1 UV absorbance values of *Callistemon viminalis*AgNPs

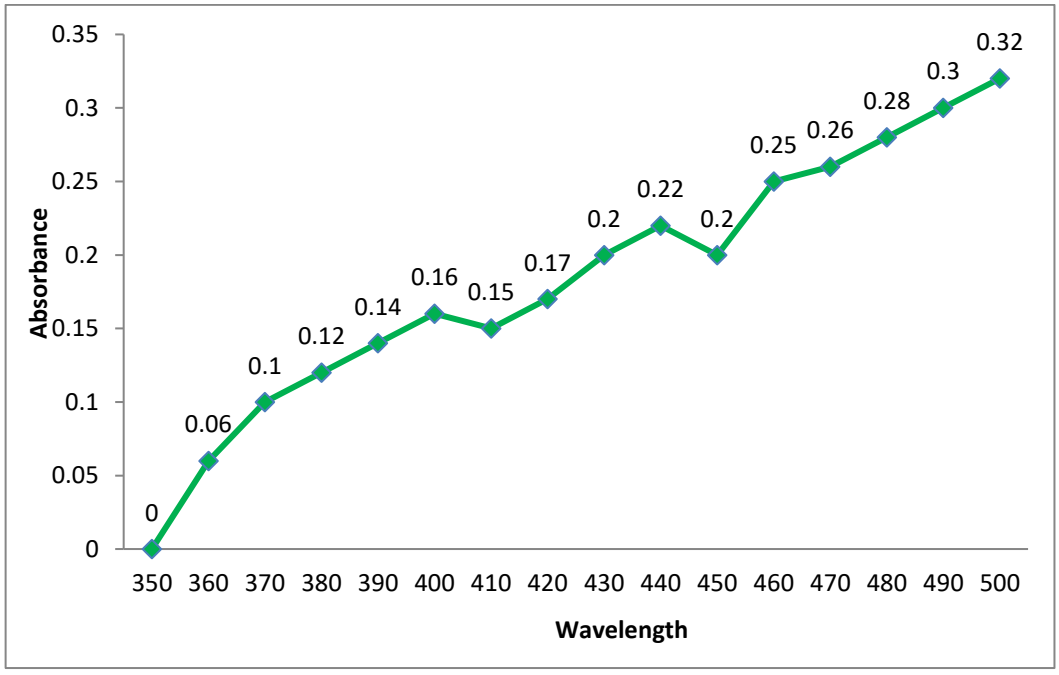

Fig. 4.2. UV absorbance values of *Callistemon viminalis* aqueous extracts

Energy-Dispersive X-Ray Spectroscopy

Energy dispersive X-ray spectroscopy revealed elemental analysis of leaf extracts and green synthesized silver nanoparticles, demonstrated that fabricated AgNPs retained 0.28 % weight of Ag along with other organic elements like magnesium, oxygen, carbon, calcium, potassium, silicon, chlorine, and sulfur. The presence of synthesized Ag nanoparticles was verified by absence of silver in the aqueous extract. Other elemental values of Ag include magnesium 0.40, oxygen 40.70 and silicon 0.31. Similarly, in aqueous extract the elemental values vary calcium 1.0, potassium 0.17 and chlorine 2.43. Spectroscopic results are précised in (Fig. 4.3 &4.4)

Table 4.1. EDX values of *Callistemon viminalis* AgNPs.

Element	Weight%	Atomic%
C K	52.59	61.58
O K	40.60	35.70
Na K	0.31	0.19
Mg K	0.51	0.29
Si K	0.32	0.16
P K	0.15	0.07
S K	0.25	0.11
Cl K	2.02	0.80
K K	2.55	0.92
Ca K	0.42	0.15
Ag L	0.28	0.04
Totals	100.00	

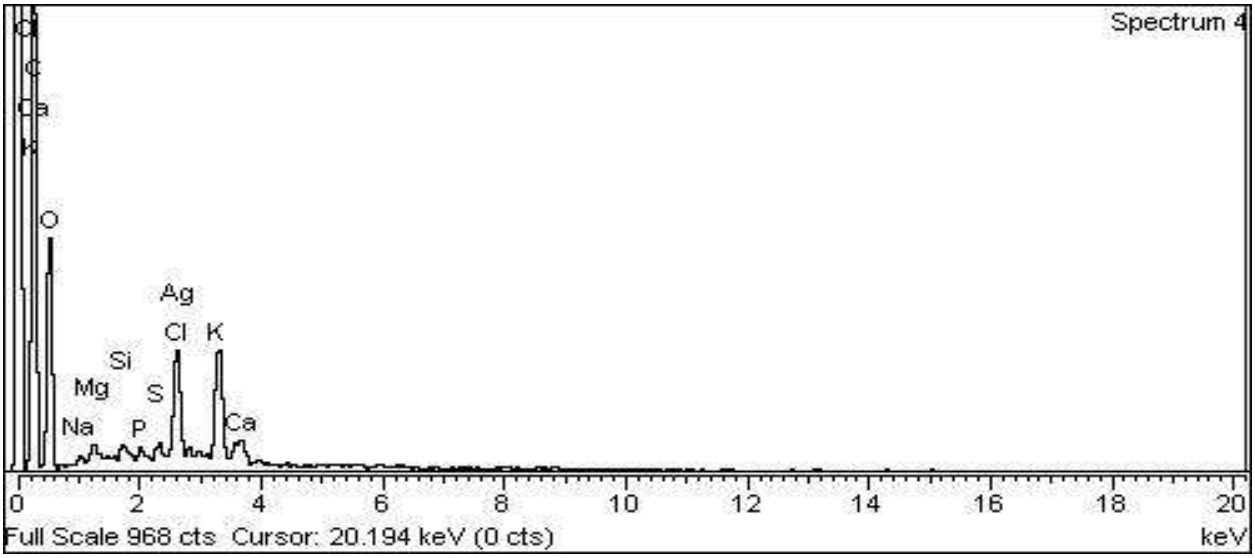

Fig. 4.3. EDX values of *Callistemon viminalis*AgNps

Table 4.2. EDX values of *Callistemon viminalis* aqueous extracts.

Element	Weight%	Atomic%
C K	52.78	62.16
O K	39.21	34.67
Mg K	0.44	0.25
Si K	0.42	0.21
P K	0.16	0.07
S K	0.29	0.13
Cl K	2.39	0.95
K K	3.23	1.17
Ca K	1.09	0.38
Totals	100.00	

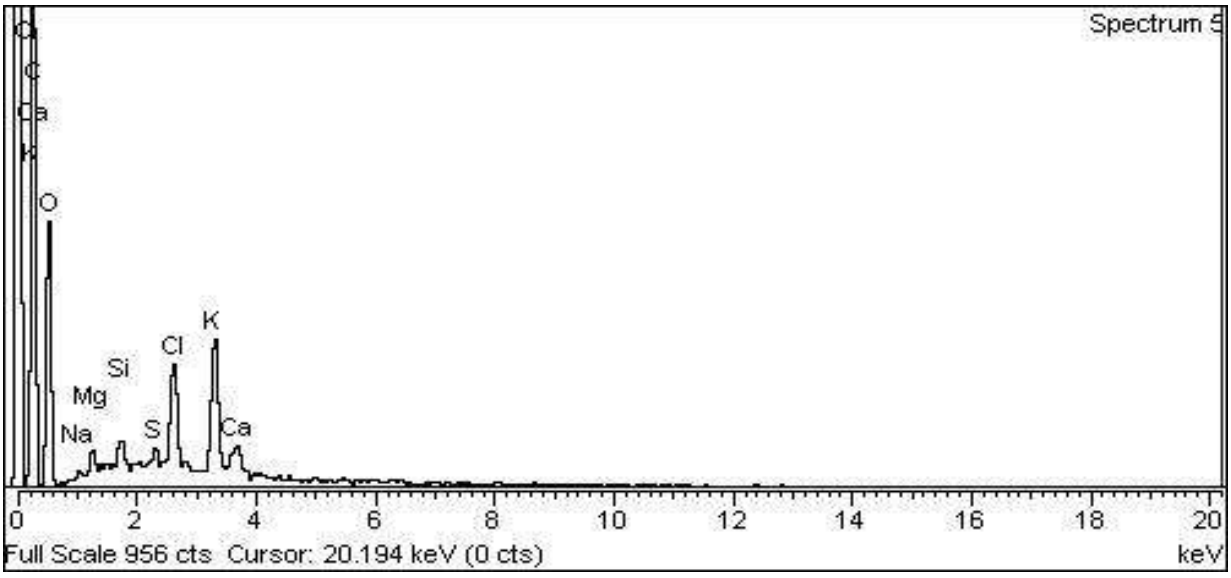

Fig. 4.4. EDX values of *Callistemon viminalis*aqueous extracts

Scanning Electron Microscopy (SEM)

Scanning electron microscopy observedstability of AgNPs and configuration, surface characterizationat 1000X, 2500X, and 5000X magnification. Extractions from plants showed reducing nature which is verified by electron micrographs to provide stable nanoparticles. Furthermore, these investigations were also compared with aqueous leaf extracts, which revealed irregular images. The SEM Micrograph are taken at 1000Xand 5000X different resolution as depicted in the Fig. (4.5, 4.7). The surface morphology of *Callistemon viminalis* mediated synthesized silver nanoparticles and aqueous extract exhibiting spherical shaped particles Fig. 4.5, at 1000X Fig. 4.6, at 2500X Fig. 4.7 at 5000X magnification showing Aqueous sample extract are asymmetrical or irregular in shape whereas Fig. 4.8, at 1000X, Fig. 4.9 at 2500X, Fig. 4.10 at 5000X magnification showing silver nanoparticles acting as a stable, monodispersed nanoparticles. At 5000X high magnification aqueous extract (Fig. 4.7) and silver nanoparticles (Fig. 4.10) clarify the structures of the particles. SEM results are précised in (Fig. 4.5- 4.10).

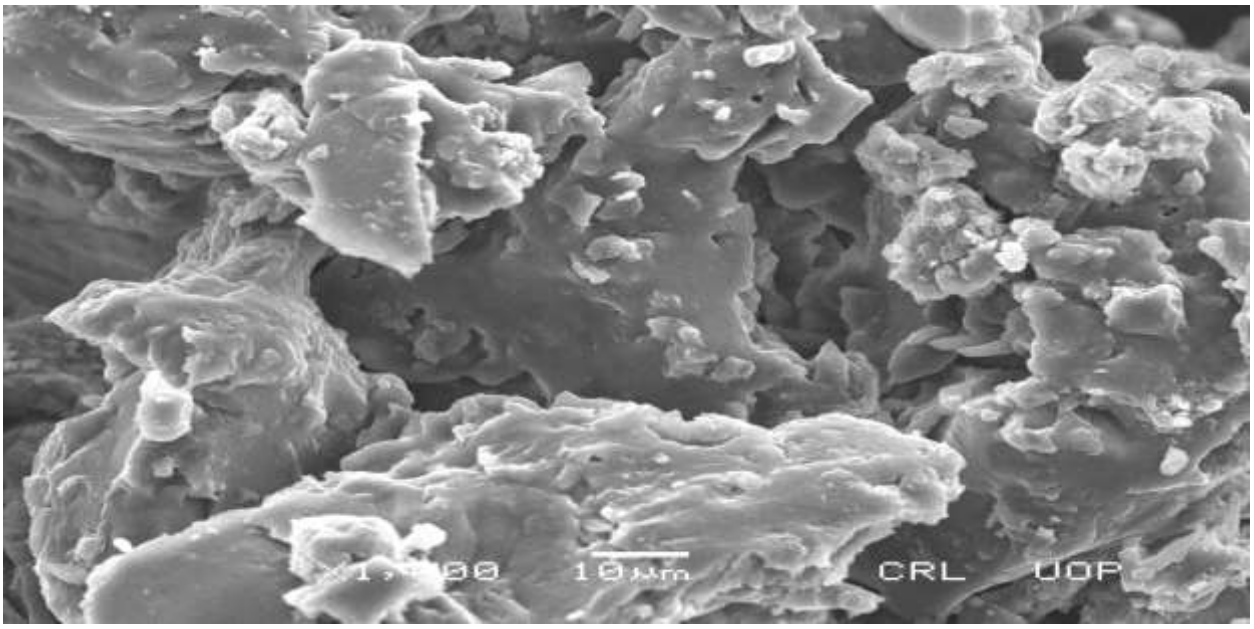

Fig. 4.5. SEM Microscopy of *Callistemon viminalis* aqueous extract at 1000X magnification

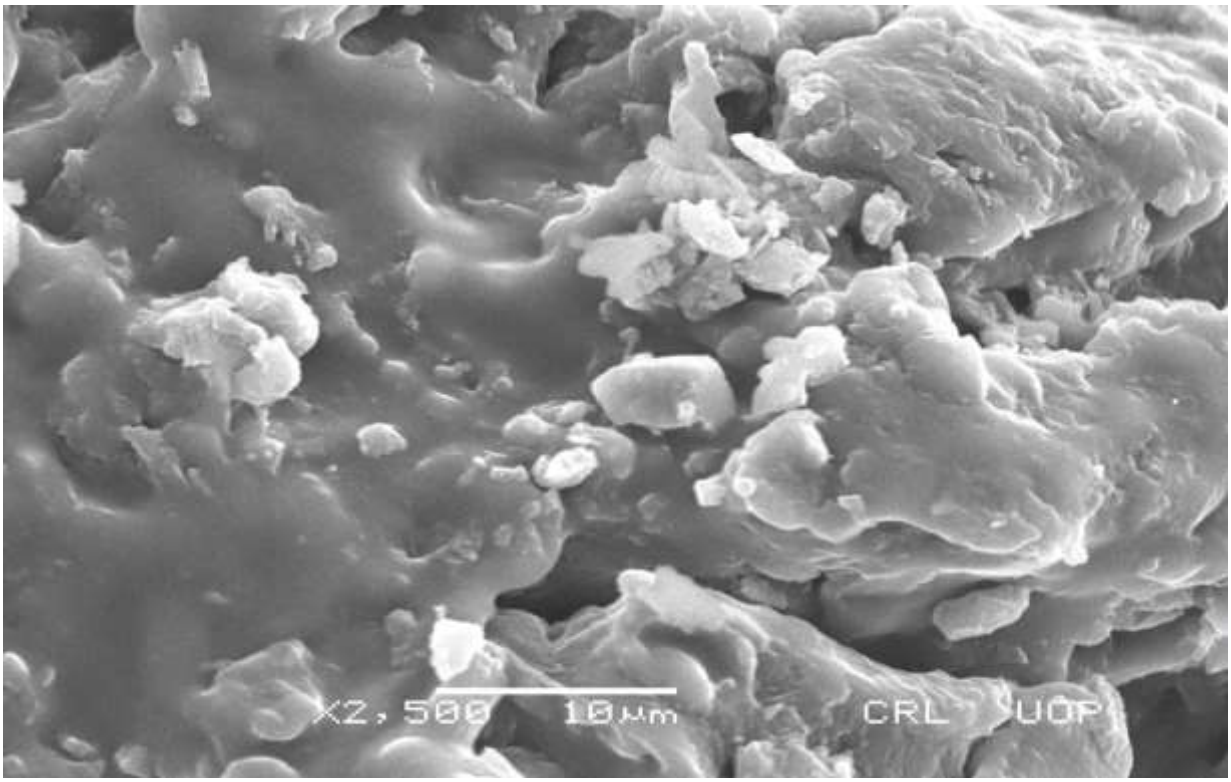

Fig. 4.6. SEM microscopy of *Callistemon viminalis* aqueous extract at 2,500X magnification

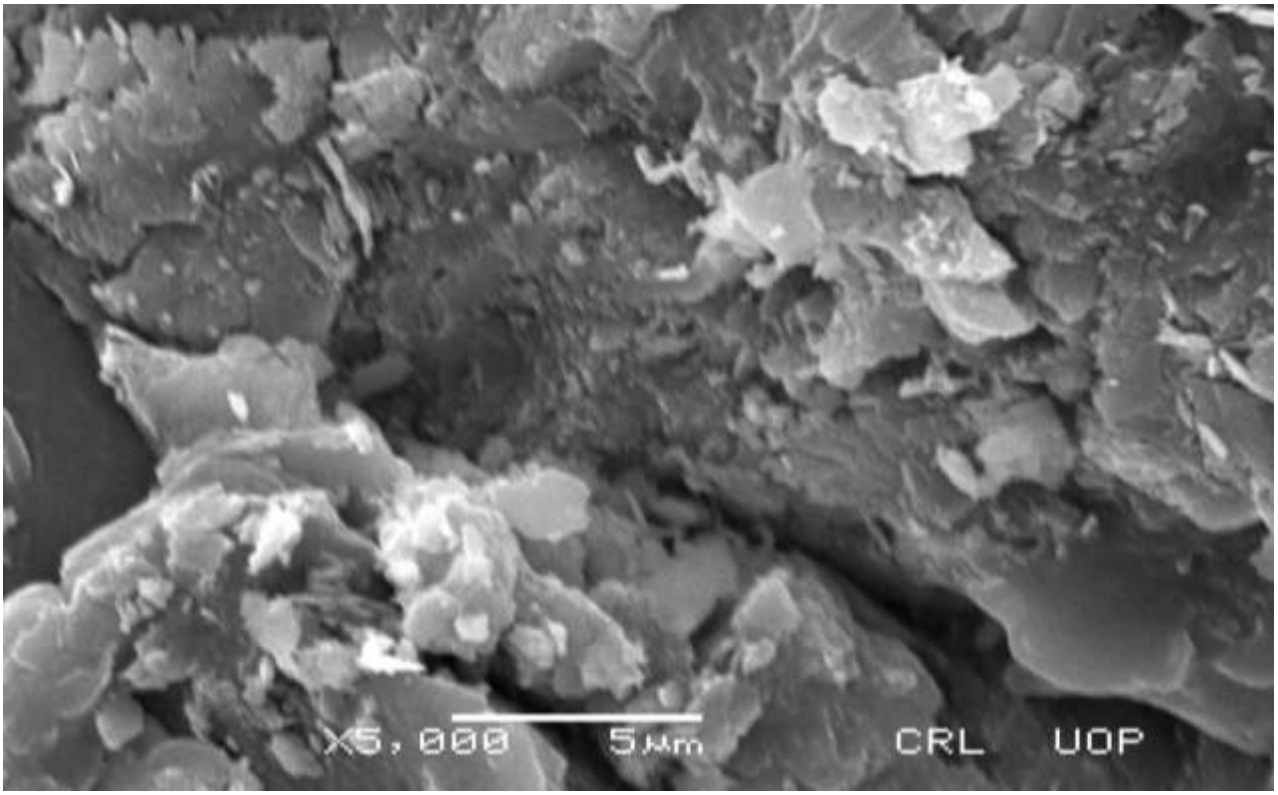

Fig. 4.7. SEM microscopy of *Callistemon viminalis* aqueous extract at 5,000X magnification

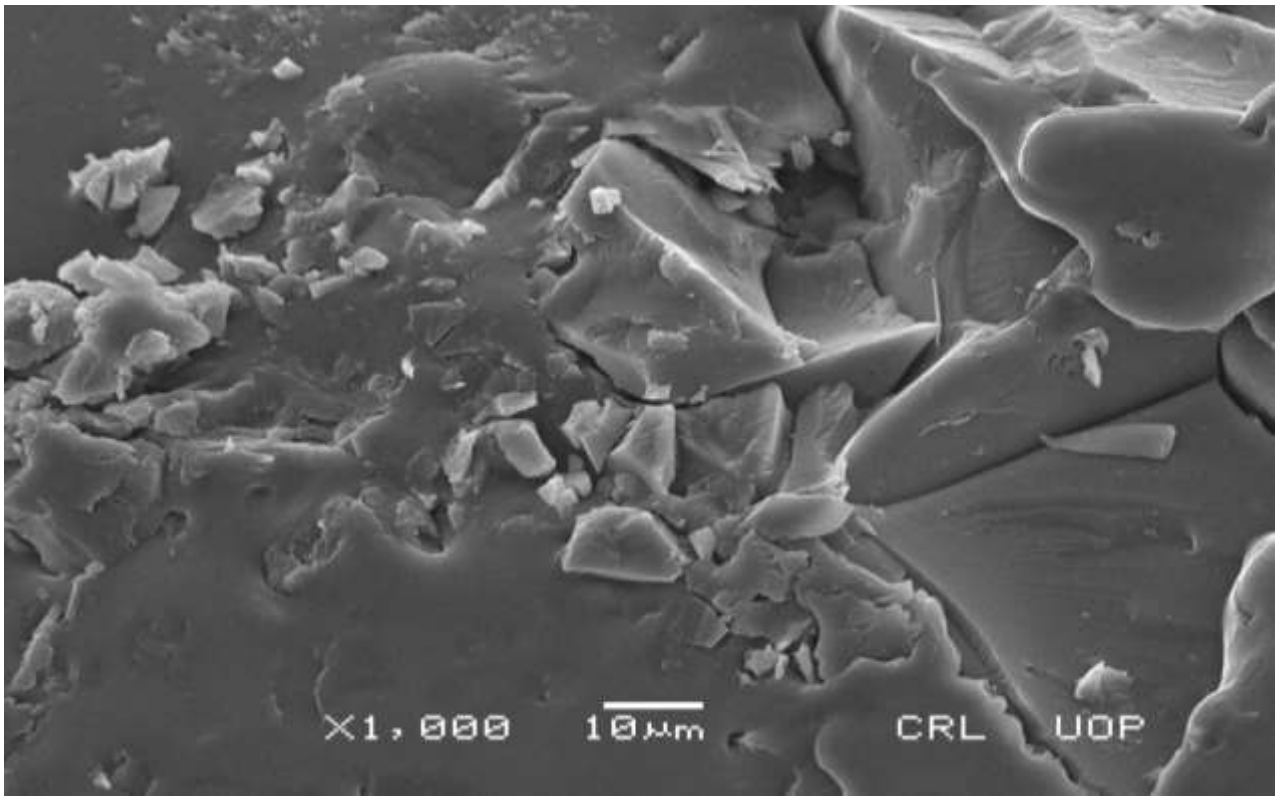

Fig. 4.8. SEM microscopy of *Callistemon viminalis*AgNPs at 1,000X magnification

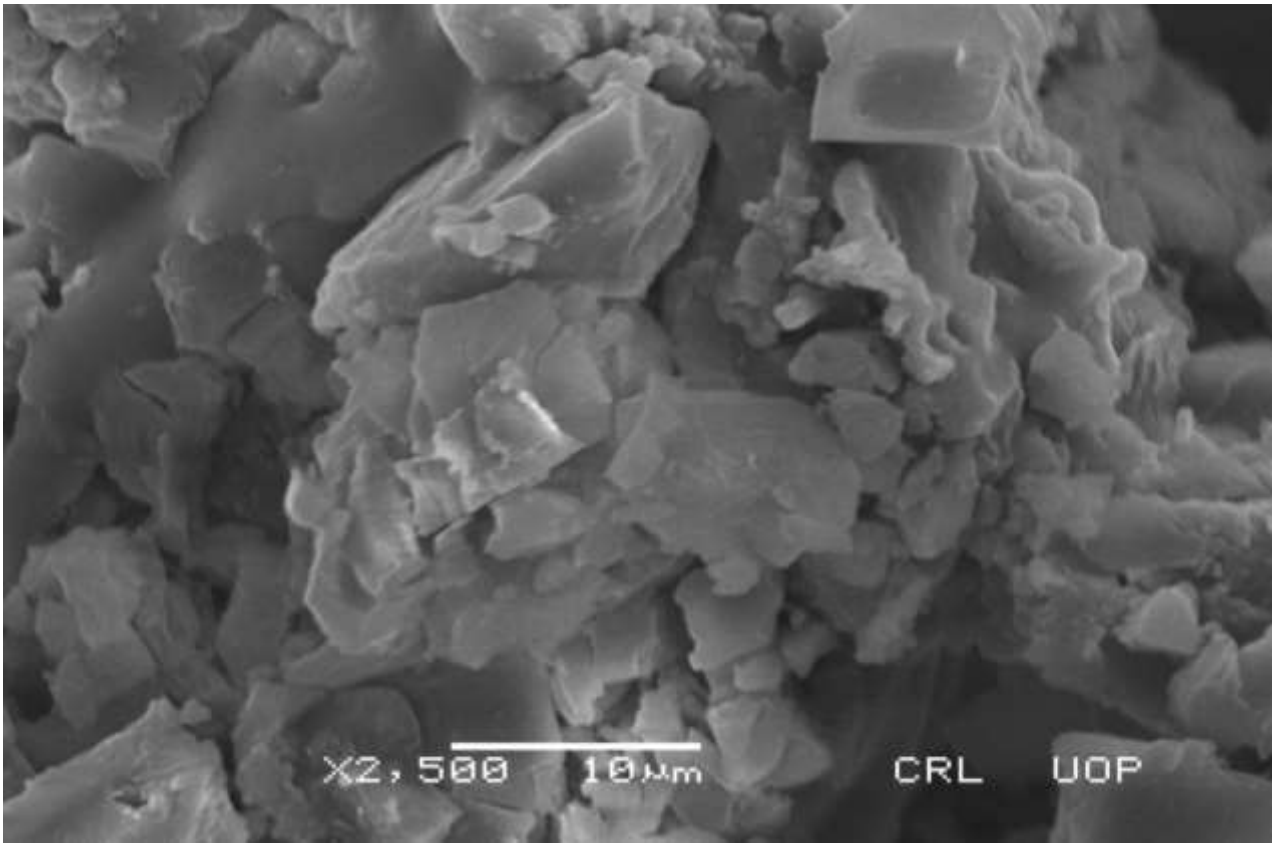

Fig. 4.9. SEM microscopy of *Callistemon viminalis*AgNPs at 2,500X magnification

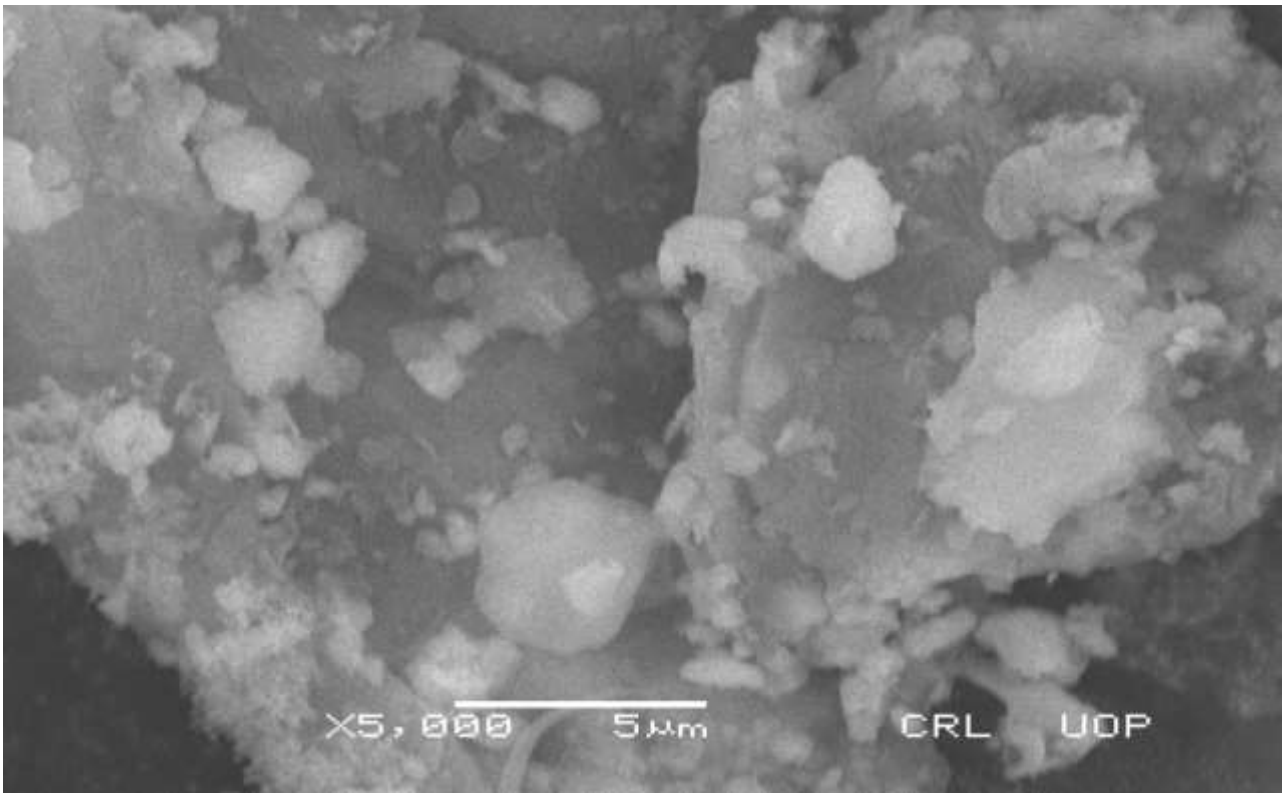

Fig. 4.10. SEM microscopy of *Callistemon viminalis*AgNPs at 5,000X magnification

X- Ray Diffraction Measurements (XRD)

X-ray diffraction measurements (XRD) illustrated crystallographic evaluation of biogenic AgNPs that nanoparticles were polycrystalline in nature and for 2θ readings their whole XRD spectrumlie in the range of 10° to 85°. The extreme peaks for *Callistemon viminalis* at 2θ angles were 17.484, 16.819, 15.629, 14.130 and 10.774, which ranged from 18° to 60°. The investigation was compared with the aqueous showed asymmetrical d values in the same 2θ range. Values for aqueous extract were 15.629, 14.359, 13.587, 12.353 and 9.763. Aqueous extract showed amorphous structure using X-ray Diffraction Measurement. Crystallinity was calculated using the Debye –Scherrer's equation, i.e., 24.84nm. Fig. 4.11 of aqueous extract confirmed that sample analyzed by XRD is amorphous form while Fig. 4.12 of silver nanoparticles showing highest peaks which confirmed sample is polycrystalline.XRD outcomes are summarized in (Fig. 4.11 – 4.12).

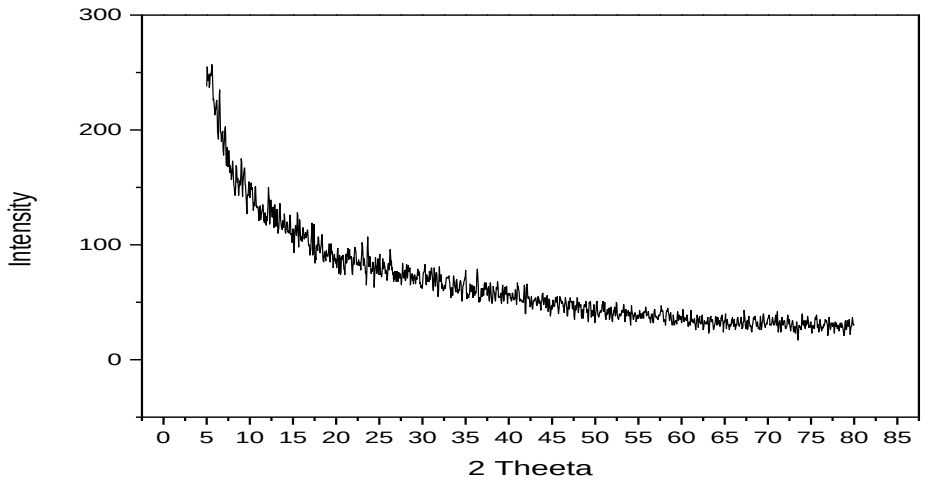

Fig.4.11. XRD of *Callistemon viminalis*Aqueous extract

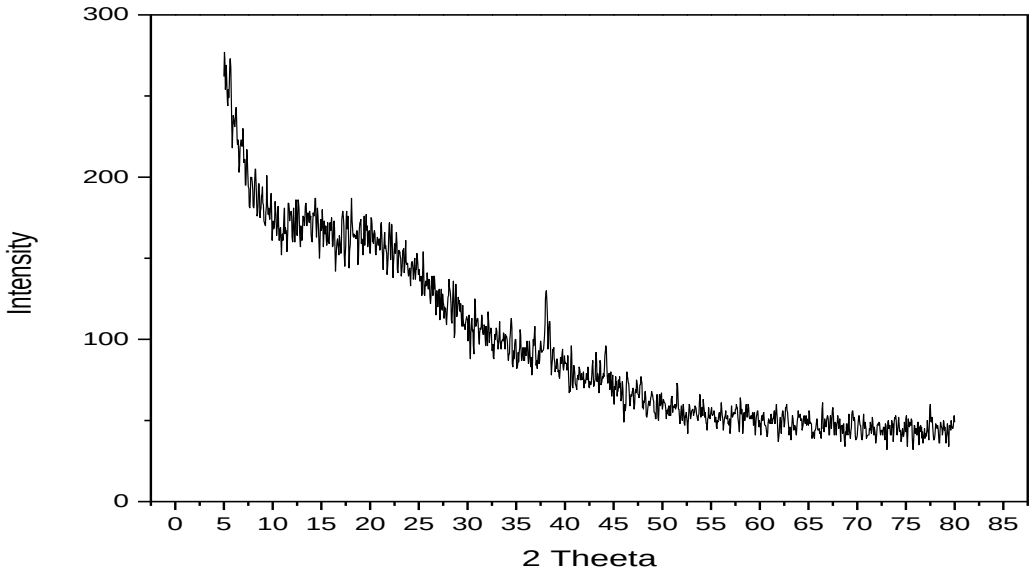

Fig 4.12. XRD of *Callistemon viminalis*AgNPs

Antineoplastic Assay

According to the anticancer evaluation good cancer inhibition was seen by AgNPs of *Callistemon viminalis* 96.4% showing IC50 values as 3.9 or 4 against prostate cancer cell line (PC3). It is defined that crude methanol extract of *Callistemon viminalis*exhibited inhibition of cancer cells having 46.4 percent inhibition. Similarly good 32.0 percent inhibition was exhibited by ethanol. Aqueous extract showing 8.6 percent inhibition and n hexane 15.9 respectively. While in human prostate cancer cell line crude Aqueous extract, ethanol, methanol and n-hexane remained inactive showing low inhibition percentage. Whereas AgNPs exhibit best cytotoxic activity on PC3 cell line (Table 4.3 and Fig. 4.13). It was manifested that AgNPs of *Callistemon viminalis* exhibited 92.7% good inhibition showing IC50 as 5.0 against HeLa cell line. Ethanol exhibiting inhibition concentration 72.5% with IC50 value 20.8ug/ml. Methanol owing good inhibition concentration 60.6% and IC50 value 20.0ug/ml. Aqueous extract 9.2% and n-hexane 15.7% inhibition metastasis and remained inactive. Aqueous and n-hexane crude extract remained inactive against HeLa cell line while methanol and ethanol exhibit low cytotoxicity on HeLa cell line. AgNPs exhibiting best cytotoxic activity against HeLa cell line.

Table 4.3. Antineoplastic activity by *Callistemon viminalis.*

Cancer Cell Line	Control (Doxorubicin) (mg/ml)	Percent inhibition by *Callistemon viminalis* (Leaves)				
		AgNPs	Ethanolic Extract	Methanolic Extract	Aqueous Extract	N-Hexane Extract
PC-3 Cell line	80.9	96.4	32	46.2	8.6	15.9

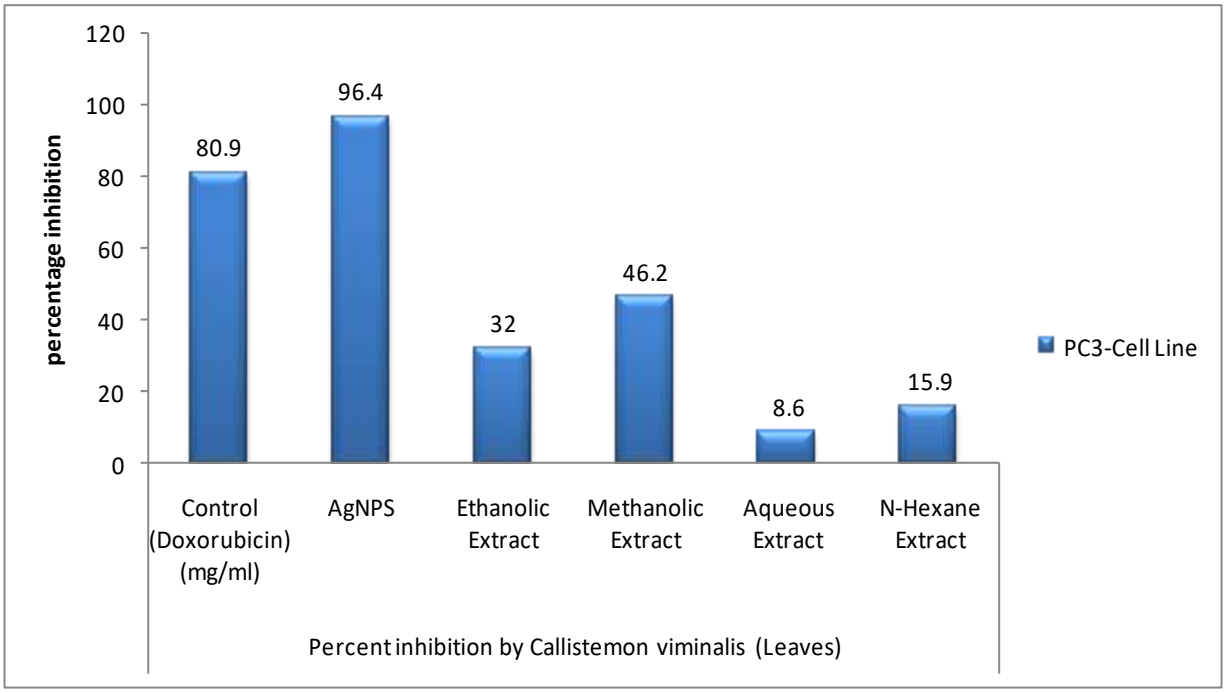

Fig. 4.13. Antineoplastic activity by *Callistemon viminalis*

Table 4.4. Inhibition of HeLa cell line assay by *Callistemon viminalis.*

Cancer Cell Line	Control (Doxorubicin) (mg/ml)	Percent inhibition by *Callistemon Viminalis* (Leaves)				
		AgNPs	Methanolic Extract	Ethanolic Extract	Aqueous Extract	N-Hexane Extract
HeLa (Cell Line)	100	92.7	60.6	72.5	92.7	15.7

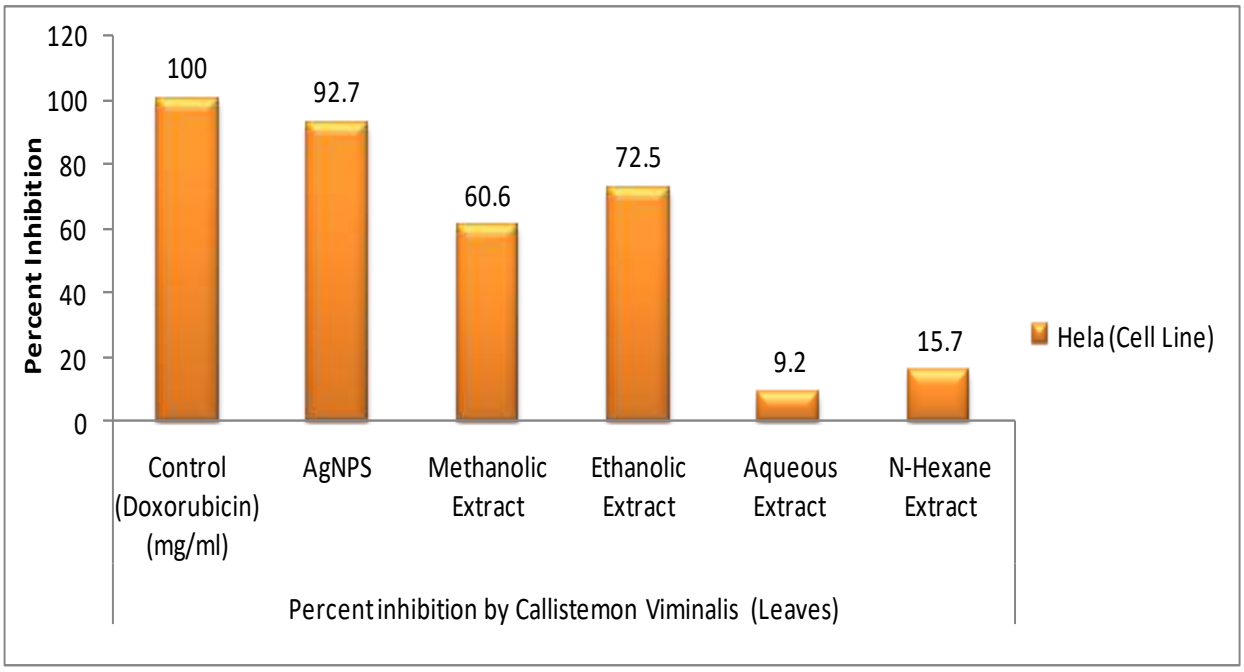

Fig. 4.14. HeLa cell line assay by *Callistemon viminalis*

Cytotoxic Assay

Cytotoxic evaluation concluded that the ethanol and n hexane extracts exhibited 100% mortality at highest concentration of 1000ug/ml. LD50 recorded were 100% for ethanol and 100% for n hexane. Good cytotoxic activity was shown at highest concentration of 1000ug/ml by silver nanoparticles

(16.66) and aqueous extract (16.66) from leaves of *Callistemon viminalis.* LD50 recorded for methanol was 83.33%. At 100ug/ml lower concentration methanol, ethanol, n-hexane showed 76.66%, 83.33%,100% mortality. Silver nanoparticle exhibiting LD50 at lower concentration 100ug/ml 10% while aqueous extract exhibiting 13.33%. At lowest 10ug/ml methanol, ethanol, n hexane showed moderate cytotoxicity range between 13 to 20%. Whereas AgNPs and aqueous extracts showed lowest mortality rate in between 3 to 6% in all the three extracts (Table 4.5 & Fig. 4.15).

Low cytotoxicity exhibited by aqueous extracts and silver nanoparticlesat different concentrations. Moderate activity was revealed by methanolic extract while ethanol and n-hexane manifested excellent cytotoxicity at highest concentration of 1000ul.

Table 4.5. Cytotoxic assay by *Callistemon viminalis.*

Dose µg/ml	Control (Etoposide) (mg/ml)	Percentage of Inhibitions				
		AgNPs	Ethanolic Extract	Methanolic Extract	Aqueous Extract	N-Hexane Extract
10 ml	70	3.33	13.33	3.33	6.66	20
100ml	70	10	83.33	76.66	13.33	100
1000ml	70	16.66	100	83.33	16.66	100

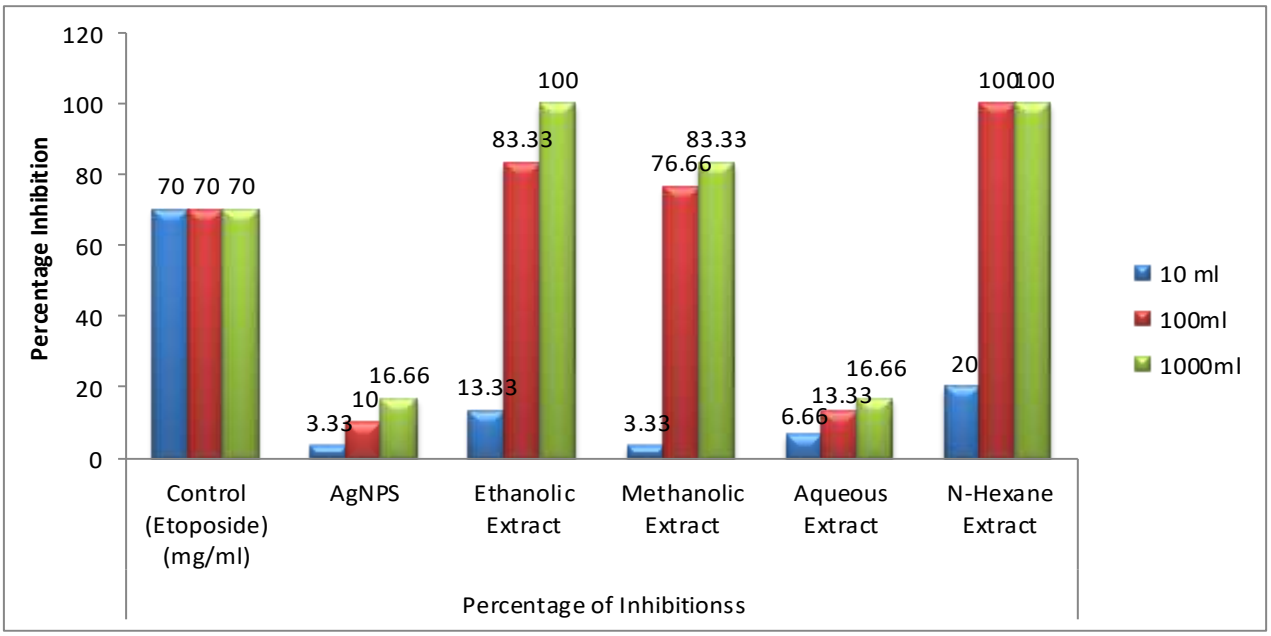

Fig. 4.15. Cytotoxic assay by *Callistemon viminalis*

DISCUSSION

UV-visible spectroscopy of plant mediated AgNPs exhibit maximum absorbance pattern at 350-500. The present study revealed that green biosynthesized AgNPs of *Callistemon viminalis* exhibits maximum absorbance at 440nm with highest peak absorbance at 1.70. Whereas crude aqueous extract maximum reading values at 400-435nm. The aqueous extract comprises irregular absorbance peak distribution. Maximum peak absorbance indicating presence of element silver. Earlier studies reported that *AzadirachtaIndica* aqueous leaf extract UV Visible spectrophotometry showed absorbance peak in the range of 436 -446 nm (Ahmed *et al*., 2016). Chandirika and Annadurai however reported that production of silver nanoparticles by *Abutilumindicum*plant exhibited maximum peak of 440 nm by UV Visible spectral analysis. These two studies have similar findings (Chandirika&Annadurai, 2018). Using energy dispersive X-ray, the *Callistemon viminalis* leaves possess 0. 28% weight of silver along

with other biological elements of the present study. Similar results shown by Fatima *et al*., (2019)concluded that *Saracaasoca* leaves exhibiting strong silver peak 31.69% affirming precise fabrication of biosynthesized silver nanoparticles (Fatima *et al.*, 2019) Various weak signals characterized by Energy dispersive X-ray spectroscopy including elements N, Mg, Na, O, Cl and C. Element silver was confirmed by the spectrumheldat highatomic Percent weight 3.40 KeV of silver (Yasmin*et al*., 2020). AgNPs examined by scanning electron microscopy showed that the silver nanoparticles of *Callistemon viminalis* are spherical in shape, AgNPs are more stable. At 5000X magnification the image of the nanoparticles is more magnified confirming presence of silver. This study shows parallel results with the present study. (Raj *et al*., 2018) revealed that particlesof *Enicostemmaaxillare*were uniformly spherical in shapedwhich strengthen our results by emission scanning electron microscopy. Similarly, *Tinosporacordifolia*confirming spherical shaped and presence of elemental silver (Selvam*et al.*, 2017) as in the present study byenergy dispersive X-ray spectroscopy (SEM-EDS) andelectron microscope. Common grapes (*Vitisvinifera*) were examined by using electron microscope. Showed clearly the existence of spherical shaped and crystal size silver nanoparticles (Acay*et al.*, 2019). These results are parallel with current study findings.

Crystallographic analysis of green AgNPs by X-ray diffraction measurement (XRD) confirmed that the NPs were polycrystalline in nature. Peak reads of *Callistemon viminalis* were 10.774 to 10. The analysis of aqueous leaf extracts of selected plants, characterized as amorphous crystal structure. The range of 10^{o}–80^{o}, using *Anthemisatropatana* plant extract Dehghanizade reported XRD that strengthen the present findings (Dehghanizade*et al.*, 2018). (Anandan*et al*., 2019) *Dodonaeaviscosas*ynthesized AgNPs and various crude extracts methanol, acetone, n-hexane and aqueous extractreported the distinct peaks at 2θ values of 38.12°, 44.31°, 64.45° and 77.41°. The XRD screening and crystalline nature of AgNPs confirmed effective reduction of Ag+ ions to Ag° particles. The findings support the present results. Cytotoxicity activity was evaluated at different sample concentrations resulting 100% mortality at high concentration, except AgNPs exhibiting no cytotoxicity to normal cells.Recent study on zinc oxide nanoparticles (ZnONPs) *Peltophorumpterocarpum* flower extract evaluated its cytotoxic activity and antimicrobial activity. Cytotoxic activity was evaluated showing 50% mortality at 10 µg/ml (Khara*et al*., 2018). Similarly, another study performed cytotoxic effect of silver nanoparticles and MCF-7 human cell lines, resulting a cytotoxic effect against MCF-7 cell lines and HeLa cell lines (Rasheed*et al*., 2017). Best antineoplastic activity was shown by AgNPs of *Callistemon viminalis* against PC3 cell line and HeLa cell lines while other crude aqueous extracts, methanol, ethanol and n-hexane exhibited low inhibition to cancer cells. Earlier studies on silver nanoparticles extract of *Coptischinensis* also showed good inhibition against the elementary mechanism across A549 lung carcinoma cells. Resulting a significant apoptosis in lung adenocarcinoma cells at 10 µg/mL and 25 µg/mL (Pei *et al*., 2019). The findings are in agreement with the present study. FicusKrishnae of aqueous extract and AgFK nanoparticles in ovarian cancer cell line showed remarkable cytotoxicity on ovarian cancer cell lines (Kanjikar*et al*., 2018) and this is line with the present study. Recent study showed ND-AgNPs to treat the cervical cancer cells (Al-Sheddi, 2018). Silver nanoparticles (AgNPs) were synthesized using aqueous extract of the plant *Nepetadeflersiana*.

CONCLUSION & RECOMMENDATIONS

Conclusion

This study represents inexpensive, ecofriendly and a rapid approach to synthesis of silver nanoparticles from*Callistemon viminalis*leaves extract. Green synthesized AgNPs were evaluated by cytotoxic and anticancer assays using methanol, ethanol, n hexane and aqueous leaf extracts of *Callistemon viminalis.* A very good cytotoxicity and anticancer potency was manifested by green AgNPs against HeLa cell line and prostate cancer cell line may help in future researcher in finding and treating various cancer-causing diseases and their treatment by biologically green synthesis of AgNP. AgNPs of *Callistemon viminalis* were characterized and purified resulting maximum absorbance at 440nm. Further characterization of biosynthesized nanostructures characterized as

spherical in shape, amorphous and polycrystalline structures. Excellent cytotoxic values were recorded by crude extract of methanol (83%) ethanol (100%) and n-hexane (100%). Green biosynthesized silver nanoparticles of *Callistemon viminalis* plant by antineoplastic activity can be assisted as a good opportunity for the treatment of various types of cancer therapies.

REFERENCES

1. Acay, H., M. Baran, F. Eren. 2019. Investigating antimicrobial activity of silver nanoparticles produced through green synthesis using leaf extract of common grape (*vitisvinifera*). *ApplEcol Environ Res.,* 17(2):4539-4546.
2. Ahmad, B., F. Shireen, S. Bashir, I. Khan and S. Azam. 2016. Green synthesis, characterization and biological evaluation of AgNPs using *Agave americana*, *Menthaspicata* and *Mangiferaindica*aqueous leaves extract. *IET Nanobiotechnol3.,* 10(5):281-287.
3. Ahmed, S., S, M. Ahmad, B. L. Swami and S. Ikram. 2016. Green synthesis of silver nanoparticles using *Azadirachtaindica* aqueous leaf extract. *J. Radiat*., 9(1):1-7.
4. Al-Sheddi, E. S., N. N. Farshori, M. M. Al-Oqail, S. M. Al-Massarani, Q. Saquib, R. Wahab, J. Musarrat, A. A. Al-Khedhairy and M. A. Siddiqui. 2018. Anticancer potential of green synthesized silver nanoparticles using extract of *Nepetadeflersiana* against human cervical cancer cells (HeLA). *J. Bioinorg. Chem. Appl*., 1-7.
5. Anandalakshmi, K and J. Venugobal. 2017. Green synthesis and characterization of silver nanoparticles using *Vitexnegundo* (KaruNochchi) leaf extract and its antibacterial activity. *Med. Chem*., 7(1):218-225.
6. Anandan, M., G. Poorani, P. Boomi, K. Varunkumar, K. Anand, A. A. Chuturgoon, M. Saravanan and H. G. Prabu. 2019. Green synthesis of anisotropic silver nanoparticles from the aqueous leaf extract of *Dodonaeaviscosa* with their antibacterial and anticancer activities. *Process biochemistry*., 80(1): 80-88.
7. Ankegowda, V. M., S. P. Kollur, S. K. Prasad, S. Pradeep, C. Dhramashekara, A. S. Jain, A. Prasad, C. Srinivasa, P. B. S. Setty, S. M. Gopinath, R. Prasad, A. H. Bahkali, A. syed and C. Shivamallu 2020. Phyto-Mediated Synthesis of Silver Nanoparticles Using *Terminaliachebula* Fruit Extract and Evaluation of Its Cytotoxic and Antimicrobial Potential. *Molecules.,* 25(21): 5042.
8. Anselmo, A. C. and S. Mitragotri. 2016. Nanoparticles in the clinic. *Bioeng.trans Med*., 1(1):10-29.
9. Aritonang, H. F., H. Koleangan and A. D. Wuntu. 2019. Synthesis of Silver Nanoparticles Using Aqueous Extract of Medicinal Plants *(Impatiens balsamina*and *Lantana camara)* Fresh Leaves and Analysis of Antimicrobial Activity. ***Int. J. Microbiol.,***(1):1-8.
10. Benjamin, N. HO., C. M. Pfeffer and A. T. K. Singh. 2017. Update on nanotechnology-based drug delivery systems in cancer treatment. *Anticancer Res.,* 37(11):5975-5981.
11. Castro –Aceituno, V., S. Ahn, S.Y. Simu, P. Singh, R. Mathiyalagan, H. A Lee and D. C. Yang. 2016. Anticancer activity of silver nanoparticles from *Panax ginseng* fresh leaves in human cancer cells**.** ***Biomed.,*** (84):158-165.
12. Chandirika, J. U., &Annadurai, G. 2018. Biosynthesis and characterization of silver nanoparticles using leaf extract Abutilon indicum. *Global Journal of Biotechnology & Biochemistry*, 13(1), 07-11.
13. Dadashpour, M., A. Firouzi-Amandi, M. Pourhassan-Moghaddam, M. J. Maleki, N .Soozangar, F. Jeddi and Y. Pilehvar-Soltanahmadi.2018.Biomimetic synthesis of silver nanoparticles using *Matricariachamomilla* extract and their potential anticancer activity against human lung cancer cells.***Mater. Sci.,*** (92):902-912.
14. Das, A. K., A. Marwal and D. Sain. 2014. One-step green synthesis and characterization of flower extract-mediated mercuric oxide (HgO) nanoparticles from *Callistemon viminalis. Pharm. Nanotechnol.,* 2(2): 25-28.
15. Dehghanizade, S., J. Arasteh and A. Mirzaie. 2018. Green synthesis of silver nanoparticles

using *Anthemisatropatana*extract characterization and in vitro biological activities. *Artif.Cells.Nanomed.Biotechnol* ., 46(1):160-168.
16. Devi, G. K. and Sathishkumar. K. 2016. Synthesis of gold and silver nanoparticles using *Mukiamaderaspatna*plant extract and its anticancer activity. *IET nanobiotechnology*., 11(2):143-151.
17. Elangovan, K., D. Elumalai, S. Anupriya, R. Shenbhagaraman, P. K. Kaleena and K. Murugesan. 2015. Phyto mediated biogenic synthesis of silver nanoparticles using leaf extract of *Andrographisechioides* and its bio-efficacy on anticancer and antibacterial activities. *J. Photochem*., (151*)*:118–124.
18. Fatema, S., M. Shirsat, M. Farooqui and M. A. Pathan. 2019. Biosynthesis of Silver nanoparticle using aqueous extract of *Saracaasoca* leaves its characterization and antimicrobial activity. *Int. J. Nano. Dimens*., 10(2):163-168.
19. Gomathi, M., P. V. Rajkumar, A. Prakasamand K. Ravichandran. 2017. Green synthesis of silver nanoparticles using *Daturastramonium* leaf extract and assessment of their antibacterial activity. *Resource.Efficient.Technologies*., 3(3):280-284.
20. Ibrahim, H. M. M. 2015. Green synthesis and characterization of silver nanoparticles using *banana peel* extract and their antimicrobial activity against representative microorganisms. *J. Radiat*., 8(3): 265–275.
21. Kanjikar, A. P., A. L. Hugar and R. L. Londonkar. 2018. Characterization of phyto nanoparticles from *Ficuskrishnae* for their antibacterial and anticancer activities. *Drug Dev. Ind. Pharm*., 44(3):377-384.
22. Khan, I., K. Saeed and I. Khan. 2019. Nanoparticles: Properties, applications and toxicities. ***Arab. J. Chem.,***12(7): 908-931
23. Khan, I., M. Khan, M. N. Umar and D. H. Oh. 2015. Nanobiotechnology and its applications in drug delivery system a review. *IET nanobiotechnol*., 9(6): 396-400.
24. Khara, G., H. Padalia, P. Moteriya and S. Chanda. 2018. *Peltophorumpterocarpum*flower mediated synthesis characterization antimicrobial and cytotoxic activities of zno nanoparticles. *Arab. J. Sci. Eng.*, 43(7):3393-3401.
25. Khattak, A., B. Ahmad, A. Rauf, S. Bawazeer, U. Farooq, J. Ali, H. Linfang. 2019. Green synthesis characterisation and biological evaluation of plant-based silver nanoparticles using *Quercussemecarpifolia Smith* aqueous leaf extract. *IET nanobiotechnology*., 13(1):36–41.
26. Kummara, S., M. B. Patil and T. Uriah. 2016. Synthesis characterization biocompatible and anticancer activity of green and chemically synthesized silver nanoparticles a comparative study. *Biomed.Pharmacother.*, (84):10-21.
27. Lucky, S. S., K. C. Soo and Y. Zhang. 2015. Nanoparticles in photodynamic therapy. ***Chem. Rev.,*** 115(4):1990-2042.
28. McGillicuddy, E., I. Murray, S. Kavanagh, L. Morrison, A. Fogarty, M. Cormican and D. Morris. 2017. Silver nanoparticles in the environment: Source's detection and ecotoxicology. ***Sci. Total Environ.,*** (575):231-246.
29. Mittal, A. K., D. Tripathy, A. Choudhary, P. K. Aili, A. Chatterjee, I. P. Singh and U. C. Banerjee. 2015. Bio-synthesis of silver nanoparticles using *Potentillafulgens Wall* ex Hook and its therapeutic evaluation as anticancer and antimicrobial agent. *Mater. Sci. Eng.*,(53):120-127.
30. Morais, M. G. D., M. V. G, D. Steffens, P. Pranke and D. C. J. Alberto. 2014. Biological applications of nanobiotechnology. ***J. Nanosci. Nanotechnol.***, 14(1):1007-1017.
31. Moteriya, P., &Chanda, S. 2017. Synthesis and characterization of silver nanoparticles using Caesalpiniapulcherrima flower extract and assessment of their in vitro antimicrobial, antioxidant, cytotoxic, and genotoxic activities. *Artif.Cells.Nanomed.Biotechnol.,* 45(8): 1556-1567.
32. Nasar, M. Q., A. T. Khalil, M. Ali, M. Shah, M. Ayaz and Z. K. Shinwari .2019.Phytochemical analysis, *Ephedra Procera CA Mey*.Mediated green synthesis of silver

nanoparticles, their cytotoxic and antimicrobial potentials.*Medicina*., 55(7):1-369.
33. Nikalje, A. P. 2015. Nanotechnology and its applications in medicine. *Med chem*., 5(2): 081-089.
34. Palza, H. 2015. Antimicrobial polymers with metal nanoparticles. *Int. J. Mol. Sci.,* 16(1):2099-2116.
35. Patra, J. K., G. Das and H. S. Shin. 2019. Facile green biosynthesis of silver nanoparticles using *Pisumsativum L* outer peel aqueous extract and its antidiabetic, cytotoxicity, antioxidant, and antibacterial activity. *Int. J. Nanomedicine.,*14(1):6679–6690.
36. Pei, J., B. Fu, L. Jiang and T. Sun. 2019.Biosynthesis, characterization, and anticancer effect of plant-mediated silver nanoparticles using *Coptischinensis*. *Int. J. Nanomedicine.,* 14(1):1969.
37. Rafique, M., I. Sadaf, M. S. Rafique and M. B. Tahir. 2017. A review on green synthesis of silver nanoparticles and their applications.*Artif.Cells.Nanomed.Biotechnol.*,45(7):1272-1291.
38. Raj, S., S. C. Mali and R. Trivedi. 2018. Green synthesis and characterization of silver nanoparticles using *Enicostemmaaxillare* (Lam.) leaf extract. *Biochem.Biophys. Res. Commun.,* 503(4):2814-2819.
39. Rasheed, T., M. Bilal, H. M. Iqbal and C. Li. 2017. Green biosynthesis of silver nanoparticles using leaves extract of *Artemisia vulgaris* and their potential biomedical applications. *Colloids.Surf. B.,* 158(1):408-415.
40. Salehi, S., S. A. S. Shandiz, F. Ghanbar, M. R. Darvish, M. S. Ardestani, A. Mirzaie and M. Jafari. 2016. Phytosynthesis of silver nanoparticles using *Artemisia marschallianaSprengel* aerial part extract and assessment of their antioxidant, anticancer, and antibacterial properties. *Int. J. Nanomedicine.,* 11(1):1835.
41. Selvam, K., C. Sudhakar, M. Govarthanan, P. Thiyagarajan, A. Sengottaiyan, B. Senthilkumar and T. Selvankumar. 2017. Eco-friendly biosynthesis and characterization of silver nanoparticles using *Tinosporacordifolia* (Thunb.) *Miers*and evaluate its antibacterial, antioxidant potential. *J. Radiat. Res. Appl. Sci.,* 10(1):6-12.
42. Senthilraja, P. and K. Kathiresan. 2015. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *J. Appl. Pharm. Sci*., 5(3):080-084.
43. Sharma, D., N. Thakur, J. Vashistt and G. S. Bisht. 2018. Antibacterial evaluation of cuprous oxide nanoparticles synthesized using leaf extract of *Callistemon viminalis*. *Indian J. Pharm. Educ. Res*., 52(3):449-455.
44. Siegel, R. L., K. D. Miller and A. Jemal. 2019. Cancer statistics.*A Cancer J Clin.,* 69(1):7-34.
45. Singh, D., V. Kumar, E. Yadav, N. Falls, M. Singh, U. Komal and A. Verma. 2018. One-pot green synthesis and structural characterisation of silver nanoparticles using aqueous leaves extract of *Carissa carandas* antioxidant, anticancer and antibacterial activities. *IET nanobiotechnology*., 12(6): 748–756.
46. Singh, H., J. Du, P. Singh and T. H. Yi. 2018. Ecofriendly synthesis of silver and gold nanoparticles by *Euphrasiaofficinalis* leaf extract and its biomedical applications. *Artif Cells NanomedBiotechnol.,* 46(6):1163–1170.
47. Sudha, A., J. Jeyakanthan and P. Srinivasan. 2017. Green synthesis of silver nanoparticles using *Lippianodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. *Resource.Efficient. Technologies*., 3(4):506-515.
48. Vijayakumar, S., B. Malaikozhundan, K. Saravanakumar, E. F. Durán-Lara, M. H. Wang and B. Vaseeharan 2019.*Garlic clove* extract assisted silver nanoparticle Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. *J. Photochem. Photobiol. B. Biol*., 198(1):111-558.
49. Xia, Q. H., Y. J. Ma and J.W. Wang. 2016. Biosynthesis of silver nanoparticles using *Taxusyunnanensis callus* and their antibacterial activity and cytotoxicity in human cancer

cells. *Nanomaterials*., 6(9):160.

50. Yasmin, S., S. Nouren, H. N. Bhatti, D. N. Iqbal, S. Iftikhar, J. Majeed, R. Mustafa, N. Nisar, J. Nisar, A. Nazir, M. Iqbal and H. Rizvi 2020. Green synthesis, characterization and photocatalytic applications of silver nanoparticles using *Diospyros lotus*.*Green Process.Synth.*, 9(1): 87-96.
51. Zhang, X. F., Z. G. Liu, W. Shen and S. Gurunathan. 2016. Silver nanoparticles synthesis, characterization, properties, applications, and therapeutic approaches. *Int. J. Mol. Sci.*, 17(9):1534.