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Anticancer and antioxidant activity of silver nanoparticles of calotropis gigantea

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ABSTRACT:
Natural products, as a pure compound or as a standardized plant extractsProvide unlimited opportunities for new drug lead because of the unmatched availability ofChemical diversity. Curative property of the medicinal plant isattributedtothevariouschemicalsubstancesofdifferentcompositionthatariseas secondary metabolitesandgetlocalizedinoneormorepartsoftheplant.Thesecomplexphytochemicalscouldplayavital roleinminimizingthe development ofdrugresistance. Cancer is an abnormal growth of cells that grows and spreads through uncontrolled celldivision (Fig 2). These 'malignant' cells may invade other tissues and spread (metastasize) to moredistant parts of the body. Cancer is not one disease but a group of more than 100 distinct disorders.Itisthe world's secondbiggestkiller aftercardiovascular disease andwas responsible for thedeath of 7.6 million people in 2005.In our study pharmacological screenings of C. gigantea revealed its medicinal potential and represents as a valuable medicinal plant with several medicinal properties.

1. INTRODUCTION

Nature has bestowed our country with an enormous wealth of medicinal plants; therefore,India has often been referred to as the medicinal garden of the world. The clinical use of plantsdescribed in Indian vedas for curing different diseases. In the present context, the traditionalsystemof medicineis widelyaccepted and practiced bypeople worldwide.

At this stage ,India has a unique position the world where a number of recognized traditional systemofmedicine*i.e.*,Ayurveda,Siddha,Unani,Homeopathy,Yogaand Naturopathy. Medicinal plants have been recognized as potential drug candidates because theypossessdruglikeproperties (Sen*et al.*, 2016).Globally the number ofpeople diagnosed with cancer is estimated at around 11 million people, a figure that is set to rise to16 million by 2020. Of all new cancer cases, it is estimated that one third could be cured if theywereadequatelydiagnosed and treated(Jaiganesh and Arunachalam, 2013).

Cancer may affect people of all ages, but risk tends to increase with age, due to the fact thatDNA damage becomes more apparent in aging DNA. Statistics indicate that men are largelyplagued by lung, colon, rectum, and prostate cancer, while women increasingly suffer from breast,colon, rectal, and stomach cancer. Despite many therapeutic advances in the understanding of theprocesses in carcinogenesis, overall mortality statistics are unlikely to change until, it is believed,there is a reorientation of the concepts for the use

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of natural products as new chemo preventiveagents (Reddy*et al.,* 2003).

Nanotechnology is an important tool in many fields like health and medicine.Nanotechnology is the technology of materials having particle size below hunched nanometers.Nanotechnology isoneoftheactiveresearchareasinmodernmaterialscience.Incurrentscenario the use of nanoparticles in biomedical applications such as drugdelivery(West andHallas 2003; Paciotti et al., 2004), Cancer-cell diagnostics (Wu et.al., 2013; Chan et al., 2002).Biomoleculeshavebeenused fornanomaterial synthesis.

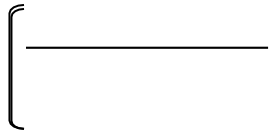
Silver nano particles (AgNPs) among all noble metals have been widely used in many pharmaceutical and biological applicationbecauseofitsuniqueproperties.Silvernanoparticles were able to exert inhibitory effect at a concentration that is below their cytotoxiclimits. The scientific and practical interest in silver nanoparticles was exclusively caused by thepossibility of their use as highly dispersed supports for enhancing the signals from organicmolecules in the Raman spectroscopy (Lee and Meisel, 1982).In recent years, there is an upsurge in the areas related to newer developments in prevention of disease,especiallytherole of free radicals and 'antioxidants' in its prevention. Antioxidants are substances neutralize freeradicals, or their actions (Sies, 1996).Many products with antioxidant properties, mainly ofsyntheticorigins, arewidelyused toincreasethe shelf lifeof foods(Liet al., 2011b).

2. MATERIAL AND METHODS

*Invitro*antioxidantassays

DPPH˙radicalscavengingassay

The antioxidant activity of water dissolved methanol extract of latex of *C.gigantea*wasmeasured on the basis of stable DPPH free radical reduction method (Khalaf NAet.,al 2008).One mL of 0.1mM DPPH solution in methanol was mixed with 1 mL of various concentrations(20-120µg/mL) of leaves extract. The mixture was then allowed to stand for 30 min incubation indark. One mL methanol and 1mL DPPH solution was used as the control. The decrease inabsorbancewasmeasuredusingUV-VisSpectrophotometerat517nm.Ascorbicacidwasusedasthe standard reference . The percentage of inhibition was calculated as:



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$$\% \text{ of } DPPH^{\cdot} \text{ radical inhibition} = \left(\frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100$$

Superoxideradical($O_2^{\bullet -}$)scavengingassay

Superoxide radical scavenging activity was carried out by the method of (Ravishankaret.,al 2014) . Different concentrations of water dissolved methanol extract of latex (20-120µg/mL) of *C.gigantea* was mixed with 50mM of phosphate buffer (pH 7.8), 1.5mM of riboflavin, 12mM ofEDTA and 50mM of NBT solutions and added in that sequence. The reaction was started byilluminating the reaction mixture for 15min. After illumination, the absorbance was measured at590nminUV-VisSpectrophotometer.Ascorbicacidwasusedasstandardreference.Thepercentageof

$$\left(\frac{\qquad\qquad}{} \right)$$

inhibition was calculated as:

$$\%\text{of superoxide radical inhibition} = \left(\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \right)$$

Ferric(Fe^{3+})reducingpowerassay

The reducing power of water dissolved methanol extract of latex *of C. gigantea* wasdetermined by Fe^{3+} reduction method with slight modification (Oyaizu. M 1986). OnemL oflatex extract of different concentrations (20 - 120µg/mL) was mixed with 1mL of phosphatebuffer (0.2M, pH 6.6) and 1mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixtureswere then incubated at 50°C in water bath for 30min.

$$\left(\frac{\qquad\qquad}{} \right)$$

One mL of trichloroacetic acid (10 % w/v)was added to each mixture. Then 1mL of freshly prepared $FeCl_3$ (0.1% w/v) solution was addedandtheabsorbancewasmeasuredat700nminUV-V is spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:% of

$$Fe3+\text{reduction} = \left(\frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100 \right)$$

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Phosphomolybdenumreductionassay

The antioxidant capacity of water dissolved methanol extract of latex *of C. gigantea* wasassessed by Mo^{6+} reduction method (Prieto. P et.,al 1999). The latex extract with concentrationsrangingfrom20to120µg/mLwascombinedwith1mLofreagentsolutioncont ainingammonium molybdate (4mM), sodium phosphate (28mM) and sulphuric acid (600mM). Thereaction mixture was incubated in water bath at 95ºC for 90min. The absorbance of the colouredcomplex was measured at 695nm in UV-Vis spectrophotometer. Ascorbic acid was used as thestandardreference. Thepercentageof reduction was calculated as:

$$\%\text{ofphosphomolybdenumreduction} = \left[\frac{\text{Sample–Controlx100}}{\text{Sample}}\right]$$

Anticanceractivity

CellgrowthinhibitionstudiesbyMTTassay

Cellviabilitywasmeasuredwiththeconventional MTTreductionassay,asdescribedpreviously withslightmodification.Briefly,MCF7cellswereseededatadensity of5×10^3cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatantwas removed and RPMI containing various concentrations (5-160 µg/mL) of test compound wasadded and incubated for 48 h. After treatment cells were incubated with MTT (10 µL, 5mg/mL) at37ºC for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm onascanning multi-well spectrophotometry(Mosmann, 1983)

Cellviability(%) =Mean OD/ControlOD ×100

Synthesisofnanoparticles

Source of AgNo3 Silver Nitrate (AgNo3) analytical grade was purchased from sigma Aldrichchemical Pvt. Ltd. Source of latex Crude latex was obtained cutting the green stems of *Calotropisgigantea.* Milky white latex was stored at - 20º c until use. All the aqueous solutions were preparedusingtriplydistilledde-ionizedwater.SynthesisofAgnanoparticlesinatypicalreactionprocedure.3mlcrudelatexwasdil utedto100mlusingtriplydistilleddeionizedwatertomakeit3%and25mlofthislatexsolutionwast akeninaR.B.Flaskandheatedat60ºCwithconstantstirringfor15minin oil bath. Then latex solution mixed with it and heated at 80ºC for 30 to 45 mins and silver nanoparticles were obtained gradually. This naturally occurring nanoparticles are generated by the erosionandchemical degradation ofplants.

Characterisationofnanoparticles

UV-Visiblespectroscopy

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TheUVspectraofthereactionsolutioncontainingsilvernanoparticlesweredeterminedusing the SJIMMADZU spectrophotometer (Model UV-3150PC) operated at a resolution of 1 nm.Thereductionofsilverionsinthesolutionwasscannedatarangeof200-600nminthespectrophotometer using a quartz cuvette with water as reference. Aliquots of the reaction solutionwereremovedat different timeintervalsand theabsorption weremeasured.

FourierTransformInfraredSpectroscopy

Fourier Transform Infrared Spectoscopy (FT-IR) deals with the vibration of chemical bonds ina molecule at various frequencies depending on the elements and types of bonds. After absorbingelectromagneticradiation,thefrequencyofvibrationofabondincreases,leadingtotransitionbetween ground state and several excited states. These absorbant frequencies represents excitation ofvibration of the chemical bonds and thus are specific to the type of bond and the group of atomsinvolved in the vibration. The energy corresponding to these frequencies correspond to the infraredregion (4000-4oo cm^{-1}) of the electro magnetic spectrum. The term Fourier Transform (FT) refers tothe manner in which the data are collected and converted from an interference pattern to an infraredabsorptionspectrum thatis likeamolecular“fingerprint”(Griffithsand deHaseth, 1986).

ScanningElectronMicroscopy

SEM (scanning electron microscope) image uses the electron reflected from a specimen. TheimageofSEM,lookedmorelikeanormalphotograph.ThisSEMhasanFEIQuanta200environmentalscanningelectronmicroscope(ESEM)withEDAXEDSsystem.SEMproduces images of a variety of specimens, achieving magnifications of over 100000xproviding highresolutionimagingindigitalformat.TheEDSsystemattachedwiththeSEMenablestheelementaryanalysis of thesamples.

The SEM has a tungsten gun which is capable of imaging the samples under different vacuumregimessuch as High – vacuum (10-2 to 10.-4 Pa).

HiVac: Conductivesamples

LoVac: Nonconductive or contaminatingsamples

ESEM: Wet samples(useH_2O gasmedium)

The scanning electron microscope (SEM) is a type of electron microscope that images thesample surface by scanning it with a high energy beam of electrons. These electrons interact withthe atoms that make up the sample producing signals that contain about the sample’s surfacetopography, composition and other properties. The type of signals produced

by an SEM caninclude secondary electrons (SE), back scattered electrons (BSE), characteristics X-rays and light.These signals are captured by various detectors such as an overhead Thornely Detector (TD),Large Field Detector (LFD)and Gaseous SecondaryElectron Detector(GSED). The reducing agents involved include the various water soluble plant metabolites (e.g.alkaloids,phenoliccompounds, terpenoids) and co-enzymes (Amit Kumar Mittal et al., 2013). Nano-size particles ofless than 100 nm can exhibit properties that differ substantially from those of bulk materials, as aresult of small particle dimension, high surface area, quantum confinement and other effects likedispersing without agglomeration. the electromagnetic, optical and catalytic properties of silvernanoparticles are strongly influenced by shape, size and size distribution, which are often variedby varying the synthetic methods, reducing agents and stabilizers (Abou El-Nour, et al., 2010)

Thesilvernanoparticlesisacquiredfromaqueoussilverionswhenexposedto*Garciniamangostana* leafextractwerecharacterizedbyUV–Visible,Fouriertransforminfra-redspectroscopy (FT-IR) and scanning electron microscopy (SEM) techniques(Veerasamy, et al.,2011). In the present study antioxidant, anticancer activity and synthesis of silver Nano particlesandcharacterization of *Calotropis giganteanwerestudied in vitro*.

3. RESULTS AND DISCUSSION

DPPH radical scavenging activity of stem, leaf, flower, latex and root of methanol extract of *Calotropis gigantea* was studied. Maximum percentage of inhibition was observed at highest concentration (120 µg/ml) of all the extracts and it was found to be 74.13±0.15 (stem) 40.59±0.15 (leaf) 44.24±0.15 (flower) 72.27±0.15 (root) 78.72±0.15 (latex) respectively. Methanol extract of latex of *Calotropis gigantea* showed the maximum level of DPPH radical scavenging activity when compared to other extracts. And the percentage of inhibition was dose and time dependent (Table 1 and Fig 1).

Superoxide radical scavenging activity of latex of methanol extract of *Calotropis gigantea* was studied. Maximum level of superoxide radical scavenging activity was observed at 120µg/ml and it was found to be 68.85±0.20 and lowest level was observed at 120µg/ml and it was found to be 50.45 ± 0.36 respectively(Table 2 and Fig 2).

Ferric (Fe^{3+}) reducing power activity of latex of methanol extract of *Calotropis gigantea* was studied *in vitro*. Maximum level of Ferric (Fe^{3+}) reducing power was observed at 120µg/ml and it was found to be 88.13±0.15 and lowest level was observed at 120µg/ml and it was found to be 36.77± 0.16 respectively(Table 3 and Fig 3).

Phosphomolybdenum reduction activity of latex of methanol extract of *Calotropis gigantea* was studied *in vitro*. Maximum level of Phosphomolybdenum reduction was observed at 120µg/ml and it was found to be 97.86±0.36 and lowest level was observed at 120µg/ml and it was found to be 80± 0.29 respectively(Table 4 and Fig 4).

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Methanol extracts of latex of *Calotropis gigantea* exhibited Cell growth inhibition activity in a dose dependent manner(Table 5 and Fig 5). The maximum level of percentage of cell viability was observed at 160 µg/ml of methanol extract of latex of *Calotropis gigantea* in MCF 7 and it was found to be 48.76 %. The percentage growth inhibition was found to be increasing with increasing concentration of both plant extracts. Statistical analysis revealed *In vitro* cytotoxicity assaymethanol extracts of latex of *Calotropis gigantea* were significantly different ($P<0.05$) (Fig 6).

Nanoparticles from latex of *Calotropis gigantea* was synthesized and it was characterized by following methods such as UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy.In recent years, ethno medicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of plant origin.

UV-visible Spectroscopy

Bell shaped spectrum curve was obtained from UV-Vis analysis. Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 425 to 475 nm due to surface Plasmon resonance of AgNPs(Fig 7).

The UV –Vis spectra recorded, implied that most rapid reduction was achieved using latex which was denoted by broadening of the peak which indicates the formation of polydispersed large nanoparticles due to slow reduction rate.

FourierTransformInfraredSpectroscopy

The band intensities in different regions of the spectrum for latex of *Calotropisgigantea* and silver nanoparticles were analysed. FTIR spectrum shows different majorpeakpositionsat3464, 2922,2886,2073,1670,1493,1452,1290,1029,757,700 and 628 cm (Fig.18).FTIRhasbecomeanimportanttoolinunderstandingtheinvolvementof functional groups in relation between metal particlesand biomolecules which is usedto search the chemical composition of the surface of the silver nanoparticles and identifythebiomoleculesforcappingandefficientstabilizationofthemetalnanoparticles(Padalia et.al., 2014).There were many functional groups present which may have beenresponsible for the bio-reduction of Ag ions. The similarities between the spectra withsome marginal shifts in peak position, clearly indicate the presence of the residual plantextract in the sample as a capping agent to the silver nanoparticles. The broad and intensepeak at 3464 cm- corresponds to OH stretching vibrations of phenol carboxylic grouppresent in extract. A peak observed at 2922 and 2886 cm is due to C-IH stretching ofalkanes(Sivakumar,2014).Thepeakat1452cmassignedtonitroN-Obendinganda peak at 1029 cm to C-O-C stretching aromatic ring. showed peak in the range of 700 cmrelatingtothealkylhalidesbandespeciallytheC-Clbond(Sadeghietal.,2015).Therefore, it may be inferred that these biomolecules are responsible for capping andefficientstabilizationofsynthesizednanoparticles(Fig 8).

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ScanningElectronMicroscopy

ScanningElectronMicroscopy-(SEM)Biogenicsilvernanoparticlesaspolycrystalline structure were revealed. From SEM result, latex from *Calotropis gigantean* has shown the agglomerates of biogenic silver nanoparticles. It was shown that relativelyspherical and uniform AgNPs were formed in the nanometer range. Due to interactions ofhydrogen bond and electrostatic interactions between the bioorganic capping moleculesboundtothesilvernanoparticlesappearlargerasaresultoftheaggregationofthesmaller ones. The UV- Visible spectrum of silver nanoparticles was recorded at thewavelength rangefrom100 to 200nm(Fig 9).

4. CONCLUSION

Antioxidants are added as redox system possessing higher oxidative potential than the drugthey are designed to protect or as chain inhibitors of radical inducted decomposition. In general,the effect of antioxidants is to break up the chains formed during the propagation process byprovidinga hydrogen atom or an electron atom to the free radical and receivingthe excess energy possessed by the activation of molecules. It is evident that the formulation of latex based nano particle with all anticancer phyto compounds such as 3,5-dehydro-6-methoxy-pivalate;4,4,6a,6b,8a,11,11,14b-octamethyl; 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2; urs-12-en-24-oicacid,3H-1,5-Benzodiazepine, 2-(1H-Benzoimidazol-2-In which Benzoimidazole is regarded as heterocyclic motif proved to be anticancer agents have been identified which had uncovered novel compounds from ***c. gigantea*** which has more potent and capacity by exhibiting diversities in their chemical constituents and secondary metabolites even within different parts of the plant and may be responsibly difference in their antioxidant activity.

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Table 1: DPPH radical scavenging activity of stem, leaf, flower, latex and root of methanol extract of *Calotropis gigantea*

S. No	Conc µg/mL	Root	Stem	Leaf	Flower	Latex
1	20	23.61±0.32	9.070±0.32	15.90±0.32	24.55±0.32	32.70±0.32
2	40	44.35±0.67	15.65±0.67	17.74±0.67	33.60±0.67	48.34±0.67
3	60	61.20±0.51	24.04±0.51	19.34±0.51	40.63±0.51	52.60±0.51
4	80	64.89±1.03	26.08±1.03	22.12±1.03	53.63±1.03	55.69±1.03
5	100	69.61±0.92	34.01±0.92	24.19±0.92	63.67±0.92	63.51±0.92
6	120	74.13±0.15	40.59±0.15	44.24±0.15	72.27±0.15	78.72±0.15

Fig 1: DPPH radical scavenging activity for stem, leaf, flower, latex and root extracts of *Calotropis gigantea*

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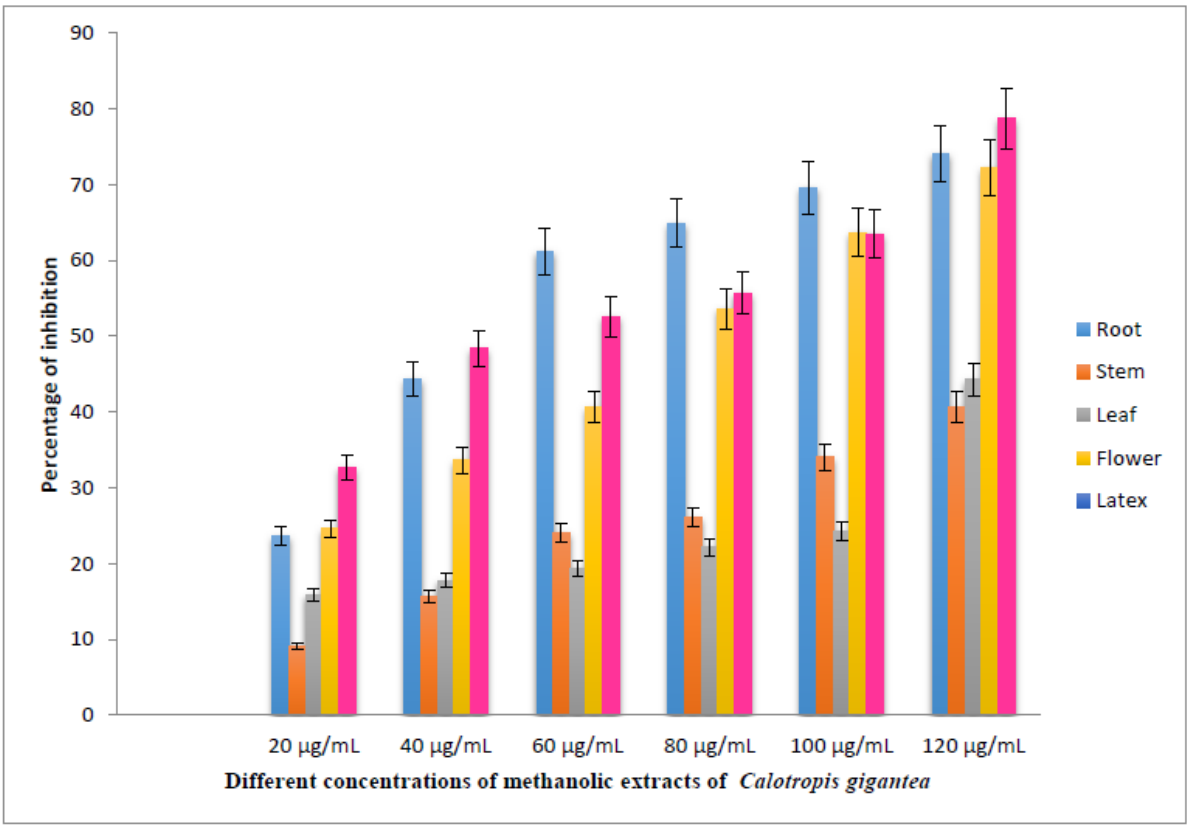

Table 2: Superoxide radical scavenging activity of latex of methanolic extract of *Calotropis gigantea*

S. No	Concentration µg/mL	Superoxide Radical scavenging activity
1	20	50.45±0.36
2	40	55.12±0.49
3	60	62.99±0.19
4	80	67.13±0.47

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5	100	67.24±0.12
6	120	68.85±0.20

Fig2: Superoxide radical scavenging activity of latex of methanolic extract of *Calotropis gigantea*

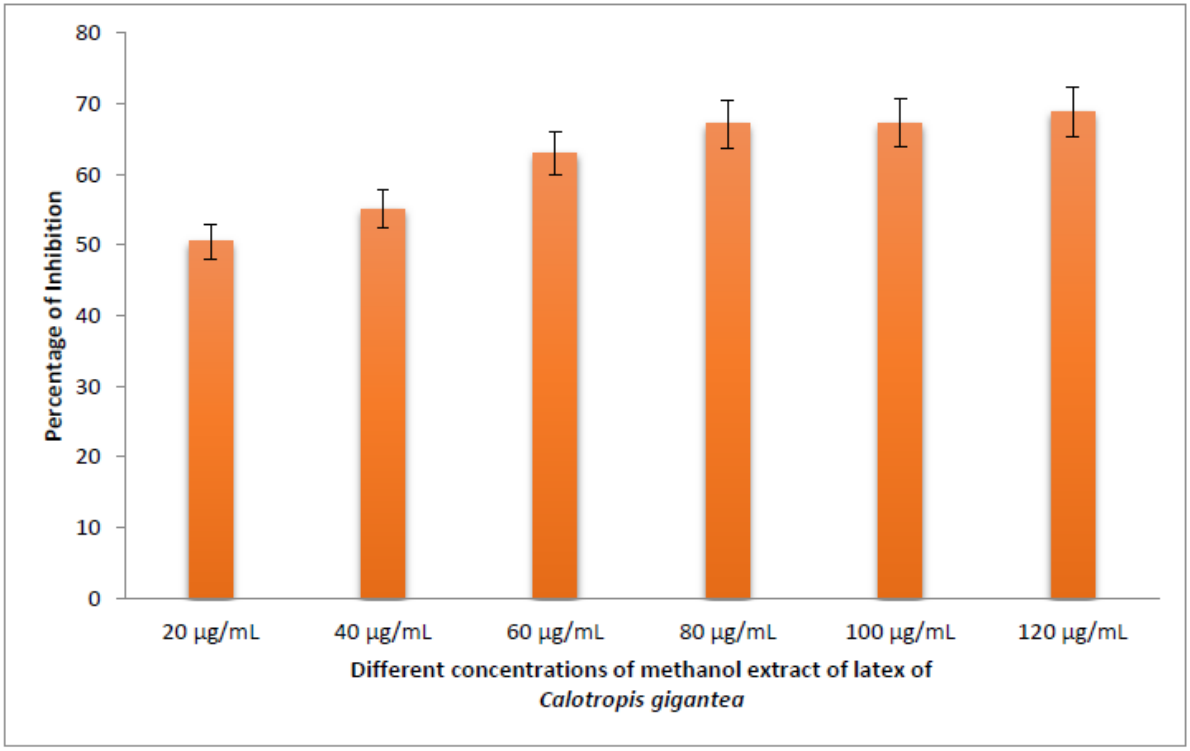

Table 3:Fe $^{3+}$reduction activity of latex of methanolic extract of *Calotropis gigantea*

S. No	Concentration µg/mL	Fe 3+ reduction
1	20	36.77± 0.16
2	40	59.56±0.23
3	60	63.34±0.19
4	80	74.33±0.11
5	100	79.25±0.21
6	120	88.13±0.15

Fig 3:Fe $^{3+}$reduction activity of latex of methanolic extract of *Calotropis gigantea*

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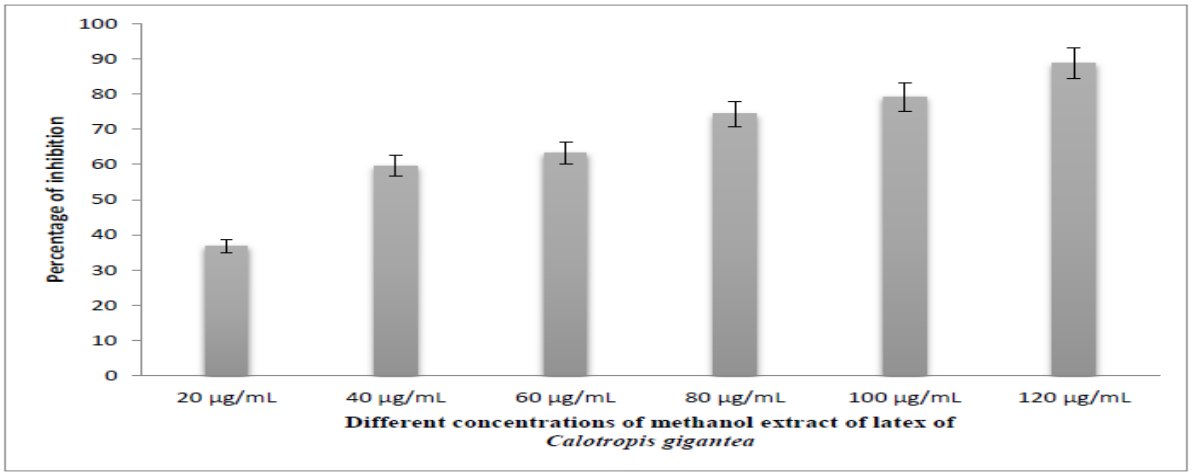

Table 4:Phosphomolybdenum reduction activity of latex of methanolic extract of *Calotropis gigantea*

S. No	Concentration µg/mL	Phosphomolybdenum reduction
1	20	80± 0.29
2	40	86.31±0.57
3	60	89.65±0.65
4	80	91.12±0.67
5	100	93.05±0.58
6	120	97.86±0.36

Fig 4: Phosphomolybdenum reduction activity of latex of methanolic extract of *Calotropis gigantea*

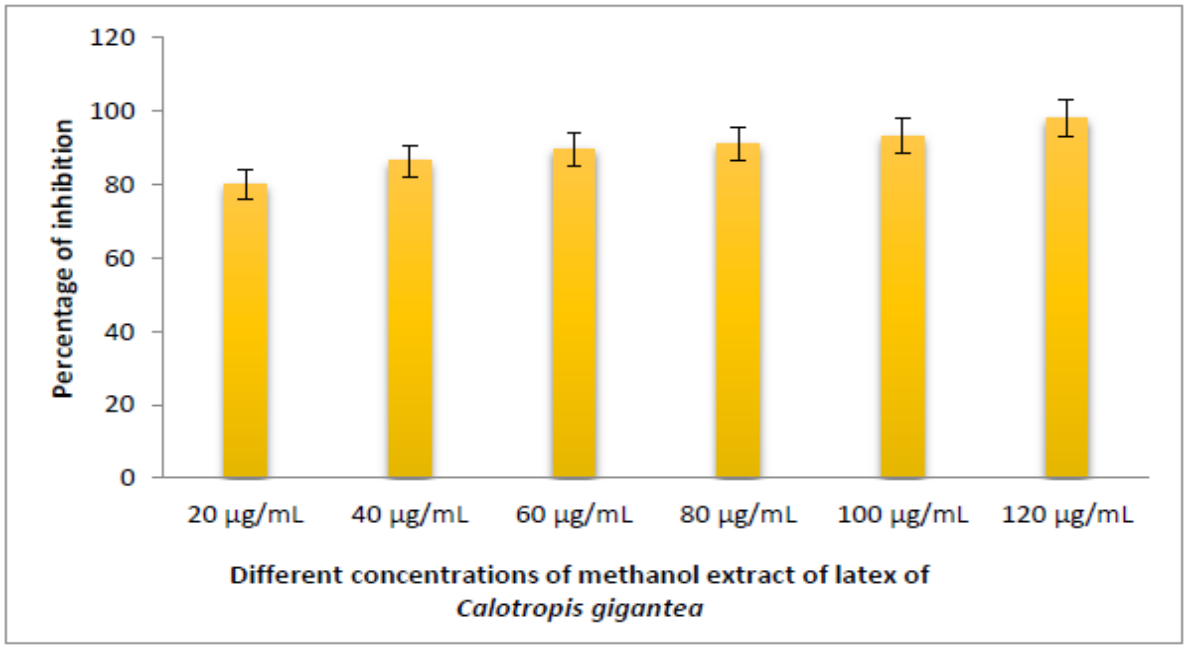

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Table 5: Anti acncer activity of latex of methanolic extract of *Calotropis gigantea* on MCF -7 (Breast Cancer Cell Line)

S. No	Concentration µg/mL	% Cell viability
1	5	98.04 ± 0.16
2	10	94.57 ± 0.23
3	20	84.60 ± 0.33
4	40	75.59 ± 0.20
5	80	60.62 ± 0.4
6	160	48.76 ± 0.36

Fig 5: Anticancer activity of latex of methanolic extract of *Calotropis gigantea* on MCF -7

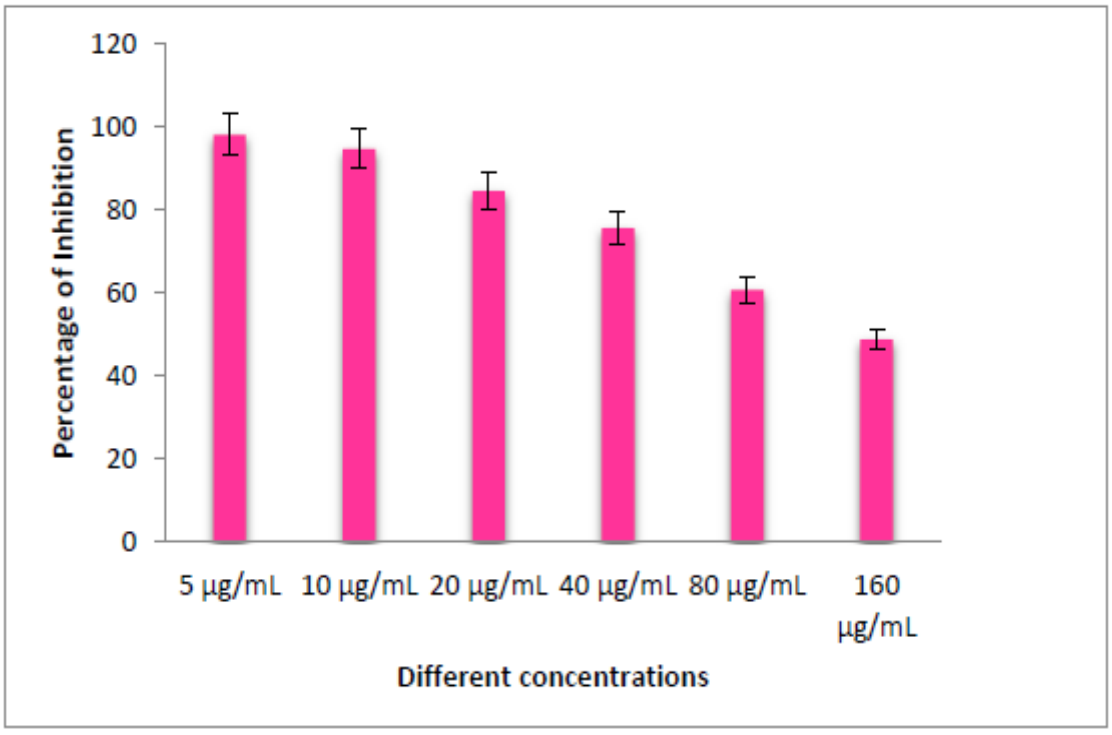

Fig 6: Anticancer activity by MTT Assay Method

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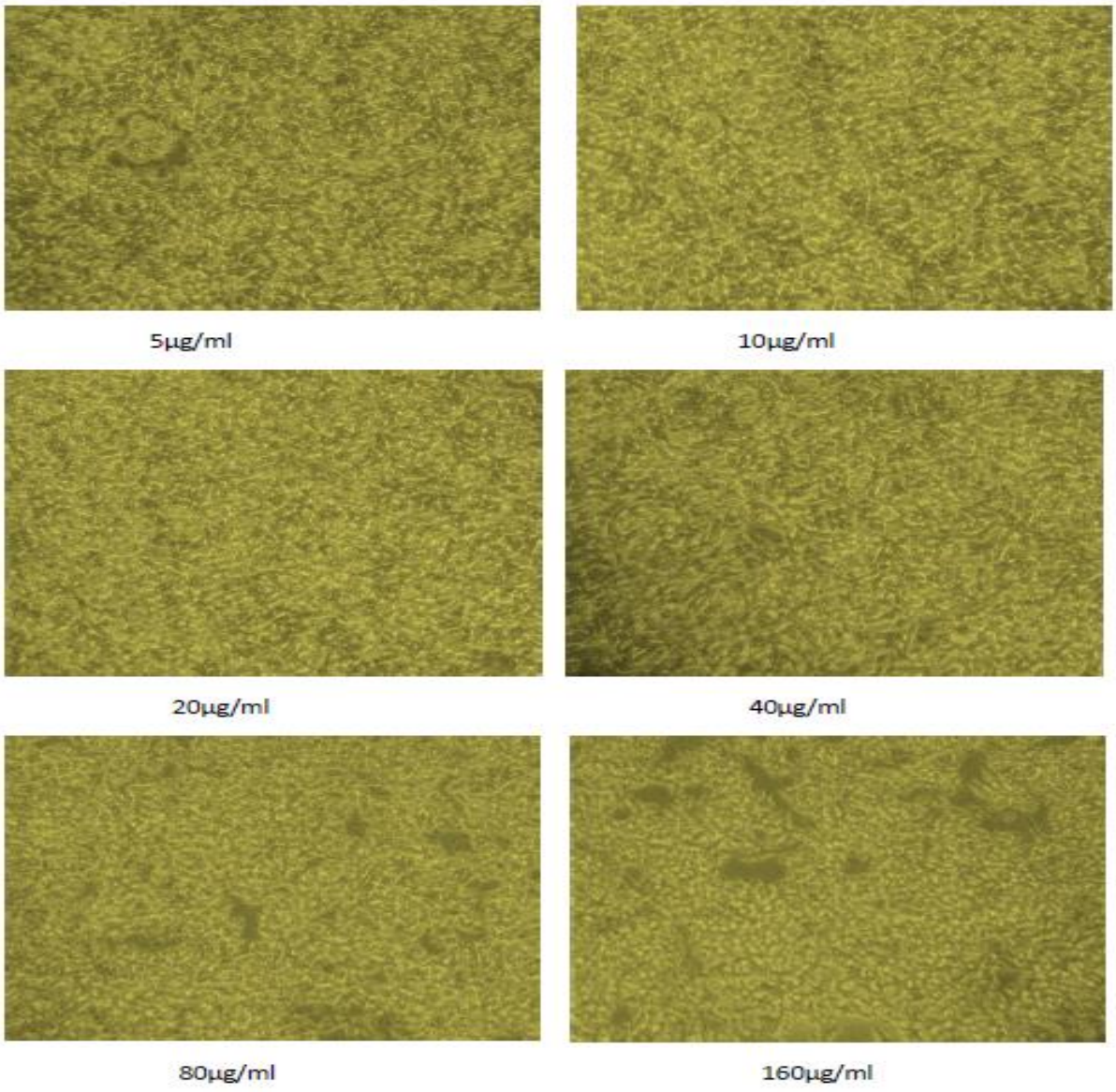

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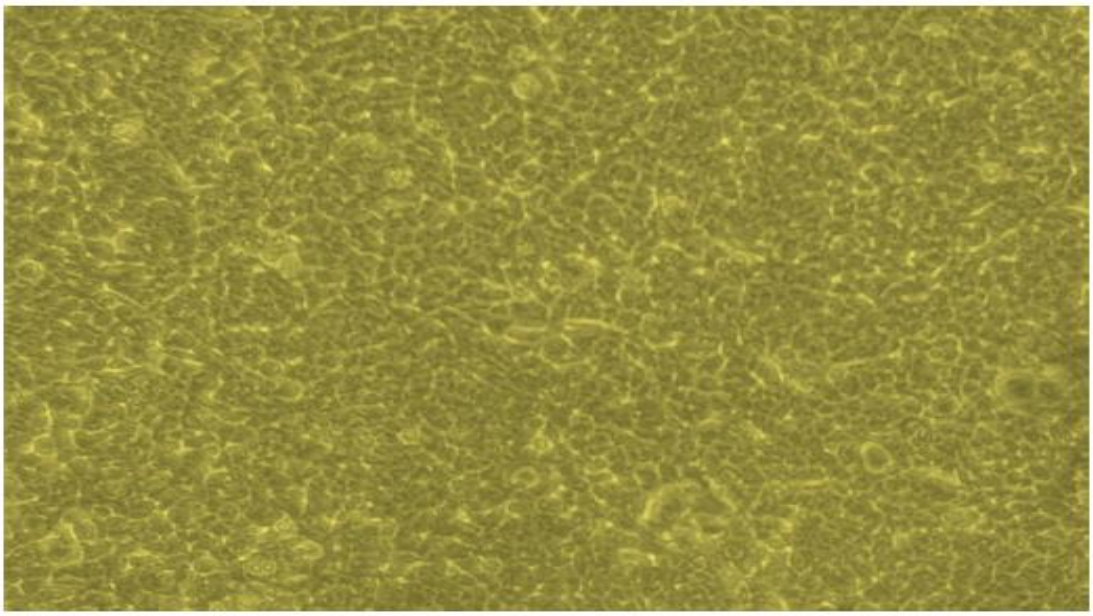

Anticancer activity control

Fig 7: UV–Vis absorption spectra of silver nanoparticles synthesized by latex of *Calotropis gigantea* extract during reaction

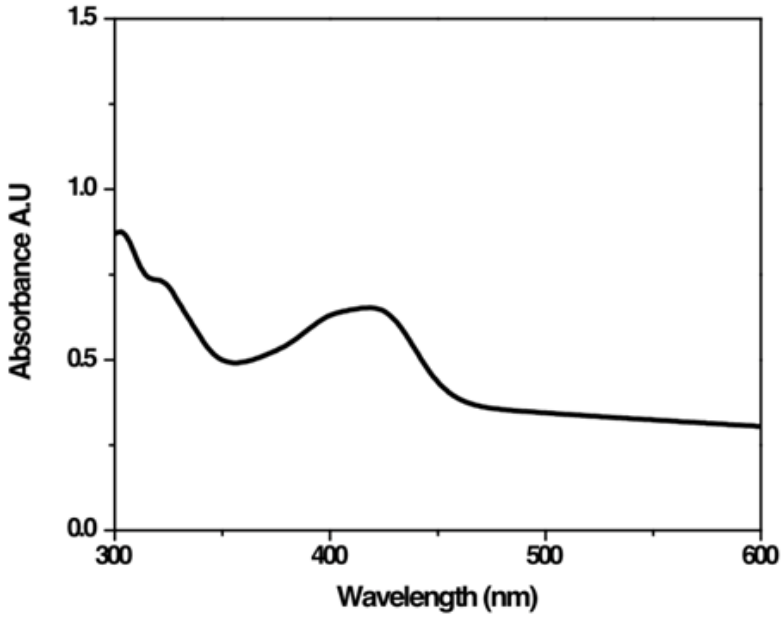

Fig 8: FTIR spectra of synthesized Ag NPs by latex of *C. gigantea* extract

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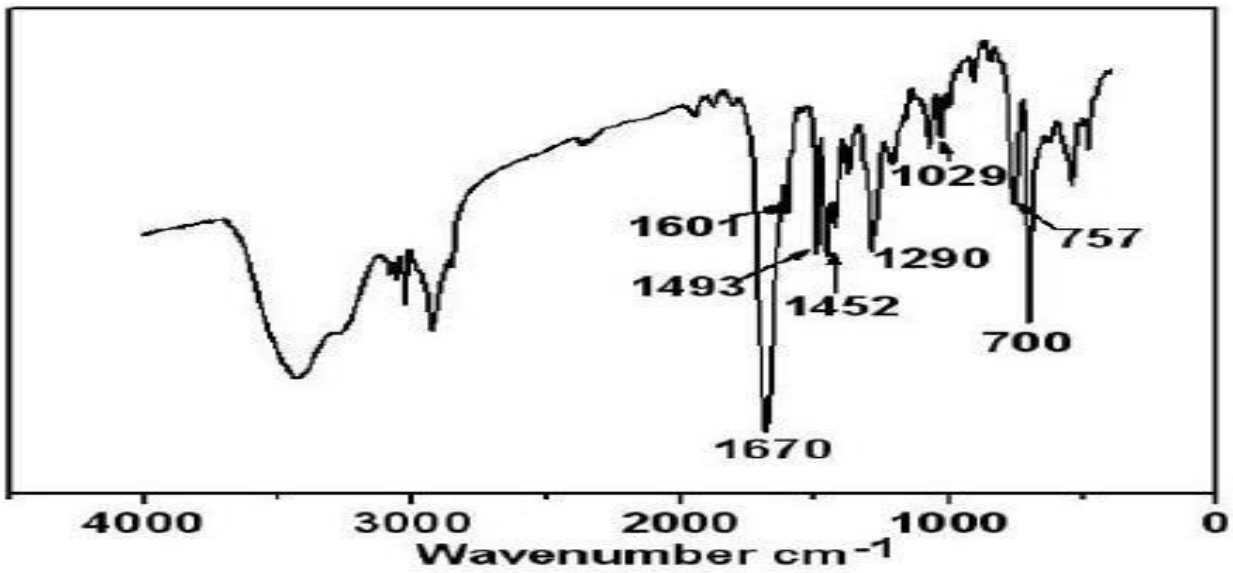

Fig 9: SEM analysis of Ag NPs synthesized by latex of *C. gigantea* extract