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Synthesis and Characterization of Silver Nano Particles from *Semecarpus Anacardium* Seeds

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Abstract
The plant *Semecarpus anacardium* is a well-known ayurvedic medicinal plant and is called as "Marking nut". This has prompted the screening of this plant for anti-angiogenic activity. The main aim of this study was to isolate compounds from seeds and characterization of silver nanoparticles. According to previous studies, *Semecarpus anacardium* has been extensively investigated for the treatment of anticancer, antibacterial, anti-inflammatory, anti-arthritic properties of plant extracts. The nuts or seeds contain a variety of biologically active compounds such as bhilwanols, flavonoids, minerals, vitamins, and amino acids which shows various medicinal properties. The characterization of HMSNPs, HMSCNPs was done by using U.V, Zeta potential, X-ray diffraction technique, Fourier Transform Infrared Spectroscopy (FTIR), Scanned Electron Microscope (SEM) parameters.

Keywords
Semecarpus anacardium, Silver Nanoparticles, Silver Colloidal Nanoparticles, Scanned Electron Microscope.

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INTRODUCTION
Nanotechnology is coined as "intentional design, characterization and production of materials, structures and systems by controlling their size and shape in nanoscale range 1 to 100nm" (Kaviya S, et al., 2011). The field of nanomedicines aims to use the properties and physical characteristic of nanomaterials for their treatment of diseases at molecular level. The field of nanotechnology is one of the upcoming areas of research in modern field of material science. Nanoparticles have shown completely new or improved properties such as size, distribution, and morphology of the particles etc. New applications of nanoparticles and nanomaterials are emerging rapidly in individual research areas such as pharmacy and medicine etc. (Catauro M, et al., 2005).

Nanoparticles have high specific surface area and unique properties of size and shape. Because of their unique physicochemical characteristics including anticancer, anti-diabetic, antibacterial properties and magnetic properties, they are gaining interest of researchers for novel methods of synthesis. (Zhao G, et al., 1998).

Application of Nanoparticles:
From the past decade, the synthesis of metal nanoparticles is a important topic of research in modern material science and medicine nanotechnology. Nano-crystalline silver particles have been providing application in the fields of high sensitivity biomolecular detection, diagnostics. However, there is need for economical and easily available route to synthesize the silver nanoparticles. Silver is well known to possess an inhibitory effect

towards many bacterial strains and microorganisms commonly present in medical and strains and microorganisms commonly present in medical and industrial processes (Jiang H et al., 2005), medical devices and implants prepared with silver-impregnated polymers (Rao B et al, 1999). In textile industry, silver-embedded fabrics are now used in sporting apparatus. According to present research scenario especially the silver nanoparticles have been playing an important role in herbal nanotechnology to prevent chronic diseases like cancer and diabetes etc.

GREEN SYNTHESIS:

Green synthesis is the process of synthesizing desired size and bio compatible silver nanotechnology using indigenous medicinally active plants for better pharmacological activity. It states that the plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an crucial role in metal ions reduction into nanoparticles and helps in supporting their subsequent stability. Properties of the nanomaterials atoms are quite different from those of the bulk materials. Conventionally, synthesizing nanoparticles by chemical method in which chemicals are used because of their general toxicity; endangering the serious concern to grow environment friendly processes. (K Rajaram et al., 2015)

Silver is the basic interesting elements with particular properties in the multidisciplinary fields of research. It occurs naturally, slightly harder than gold and very ductile and malleable. Pure silver shows highest electrical and thermal conductivity of all metals and has lowest contact resistance. Silver can be present in four different oxidation states: AgO, Ag2+, Ag3+. The former two are most abundant ones, the latter are unstable in aquatic environment (M Ramya et al., 2012). Metallic silver itself is insoluble in water, but metallic salts such as AgNo3 and NaCl are soluble in water (WHO, 2002). Metallic silver is used for surgical prosthesis and splints, fungicides and coinage. Soluble silver compounds such as silver salts, have been used in treating mental illness, epilepsy, nicotine addition, gastroenterities and infectious diseases including syphilis and gonorrhoea. Although acute toxicity of silver in the environment is dependent on avaliability of free silver ions, investigations have shown that these concentrations of Ag+ ions are too low to lead toxicity (WHO, 2002). Metallic silver appears to show minimal risk to health, whereas soluble silver compounds readily absorbs and have the potential to cause adverse effects. A variety of use of silver allows exposure through various routes of entry into the body. Ingestion is the primary route for the entry of silver compounds and colloidal silver proteins. Dietary intake of silver is estimated at 70-90µg/day, since silver in any form is not expected to be toxic to the immune, cardiovascular, nervous or reproductive system and is not considered to be carcinogenic, hence silver is relatively non-toxic (Chen X et al., 2009). Silver demand rises as it finds new applications especially in medical, textiles and plastic fields, by changing the pattern of silver emission as these technologies and products diffuse through the global economy.

Traditional system of medicine in Nanotechnology such as Ayurveda can serve as an excellent tool for human in nanomedicine category. A research work has shown that the traditional medicines such as Ayurveda Bhasma may hold strong relevance in the emerging area of nanomedicine. The purposeful advantage shows that the traditional system of medicine combines with metal-based nanomedicine (Sanjeetha Paul et al., 2011). Recent study reveals that Ayurvedic Bhasma are alike the nano crystalline material. This serves the usage of Bhasmas targeted drug delivery, the reason behind they are biocompatible and non-toxic (Chaudary AK et al., 2011, Garima Singhal et al., 2011).

In medicines, silver and silver nanoparticles have ample of applications which includes skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers. Present study was conducted to assess herbal mediated silver nanoparticles for anti-angiogenic activity.

Nanopowders can be defined as powdered materials with individual particles in nanometer scale or materials with crystalline in nanometer scale i. e, having a size under 100nm. Colloidal nanoparticles are nano-meter sized particles that can be uniformly dispersed within a solution.

Nanoemulsions are heterogeneous but clear and transparent dispersions of two or more immiscible liquids, stabilized by an interfacial film of surfactant molecule in which internal phase is present in nano size range.These are isotropic in nature and thermodynamically stable. “Nanoemulsions are also referred to as miniemulsions, ultrafine emulsions and submicron emulsions”.

Semecarpus anacardium also known as Bhallatakah, aruskarah(Sanskrit) *(*Anacardiaceae) is a deciduous tree, distributed in kanara forest of Tamilnadu state and at the outer Himalayas from Sutlej to Sikkim and fairly at hotter parts of India as far as east of Assam.

The traditionally plant Marking nut is effective for improving sexual power and increases sperm count, helps in curing diseases which relates to digestive system, balancing kapha dosha in the body is its main aim. Bhallataka is used for hair care in traditional system of medicine. It is used for dyeing and promoting hair growth in folk medicine. It also shows activities like anti-inflammatory, antioxidant, anti-reproductive, anti- carcinogenic, anti-rheumatic, CNS stimulant.

MATERIALS AND METHODS

Plant materials

The plant material of *Semecarpus anacardium* (seeds) were purchased in bulk quantity from local market in Hyderabad then Authenticated by Dr. Nirmala Babu Rao, Botanist, Osmania University, Hyderabad as *Semecarpus anacardium* seeds. The freshly collected *S. anacardium* seeds were identified dried in shade for 15days and is powdered. The dried seeds were then pulverised into coarse powders. Accurately weighed 5gms of the crude drug of *S. anacardium* seed powder which was placed in a glass stopper dry conical flask and macerated with 100 ml of solvent for about 24 hours and then Shake frequently for the first 6 hrs and allowed to stand for 18hrs. The resultant mixture was filtered rapidly by taking care not to lose any solvent. 25 ml of filtrate was transferred to an empty tarred flat-bottomed porcelain dish and evaporated to dryness. The residue was dried at 105° for 6hrs, cooled in desiccators for 30minutes and weighed.

The coarsely powdered seed material was extracted in maceration jars with ethanol and ethyl acetate. Then the extract solution filtered and further concentrated under reduced pressure using rota evaporator, a specially designed instrument for the evaporation of solvent (single-stage or straight distillation) under vaccum.

GREEN METHOD SYNTHESIS OF HERBAL MEDIATED SILVER NANOPARTICLES (HMSNPs):

About 0.16gms, silver nitrate solution was prepared and kept in brown bottles. Then 2ml of silver nitrate solution and 100ml distilled water were taken in 250ml conical flask. Then allow the conical flask for stirring for about 30minutes. To it add 2ml of herbal extract then allow it for stirring for about 4hrs. To this solution add hydrazine hydrate which acts as a reducing agent at 2 to 30rpm. The colour change was observed from white to brown. Keep it aside for aging (room temperature). Now the liquid which was suspended at the top was transferred into another flask then washed thrice with distilled water. Then heated at 80°c for 7-8hrs. The obtained powder material is preceded for characterization.

GREEN SYNTHESIS OF HERBAL MEDIATED SILVER COLLOIDAL NANOPARTICLES (HMSCNPs):

To 2ml of silver nitrate solution, add 100ml distilled water into 250ml conical flask. Then allow the conical flask for stirring for about 30minutes. To it add 2ml of herbal extract then allow it for stirring for about 4hrs. The obtained colloidal material is carried for characterization. X-ray diffraction technique is not possible for colloidal nanoparticles.

CHARACTERIZATION OF SILVER NANOPARTICLES:

Green synthesized NPs were confirmed by taking the serial dilutions of samples at regular intervals and the absorption maxima was scanned at 350-800nm by using Schimadzu UV-Visible spectrophotometer. The size and shape of HMSNPs were measured using Scanning electron microscopy (SEM) images. The crystalline structure of the particles was measured by X-ray diffraction instrument (XRD). The functional groups involved in the reduction of silver were detected using Fourier Transform Infrared Spectroscopy (FTIR). Particle size of HMSNPs was measured by Particle size analyzer.

Phytochemical screening

The crude Methanolic extract was analysed for the presence of important phytochemicals following the protocol. Briefly, tannins and flavonoids were determined by boiling 0.5 g of extract with 20 ml of distilled water for 5 minutes in a water bath. Several drops of 0.1% ferric chloride and hydroxide solutions were added to 2 ml of the filtrate, respectively. Brownish green or blue-black colouration indicated the presence of tannins, while yellow colouration showed the presence of flavonoids. Presence of saponins was determined by frothing upon boiling of 2 g of crude extract with 20 ml of distilled water in a water bath for 10 minutes. The sample was filtered and allowed to cool. The filtrate (10 ml) was diluted with distilled water (1:1 v/v) and shaken vigorously until formation of froth, which was stable for a few minutes. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion. Alkaloids were determined by boiling 1 g of extract with distilled water and acidified with 5 ml of 1% HCl in a water bath. Several drops of Meyer's reagent were added to 2 ml of the filtrate. The formation of creamy white/turbid precipitate indicated the presence of alkaloids. Terpenoids were determined by mixing 5 ml of extract solution with 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was then carefully added to form layers. A reddish-brown precipitate at the interface indicated the presence of terpenoids. Presence of cardiac glycosides was determined by dissolving 5 mg of extract in 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. The mixture was then layered with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycosides. Presence of phlobatannins was determined by deposition of a red precipitate when several drops of dilute HCl were added to 2 ml of extract[5,6]

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the crude extract revealed the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides.

Table no 1: preliminary phytochemical screening of *S.anacardium* seed extract

S.NO	CHEMICAL CONSTITUENTS	ETHANOL	CHLOROFORM	N-HEXANE	ETHYL ACETATE
1.	Alkaloids	+ve	+ve	+ve	+ve
2.	Flavanoids	+ve	-ve	-ve	+ve
3.	Tannins	+ve	+ve	+ve	+ve
4.	Phenols	+ve	-ve	+ve	+ve
5.	Glycosides	+ve	-ve	-ve	+ve

The percentage (%) yield of crude plant extracts are:

Table no. 2: Percentage yield of extracts

S.NO	EXTRACT	CONSISTENCY	CONSISTENCY	% YIELD
1.	Ethanolic	Dark brown	Semi solid	20%
2.	Ethyl acetate	Dark brown	Semi solid	16%

By comparing the extractive values of different solvents, ethanol and ethyl acetate are preferred and the phytochemical analysis of *Semecarpus anacardium* seeds showed the presence of alkaloids, flavanoids, tannins, glycosides, phenols.

CHARACTERISATION OF HMSNPs:

After successful synthesis of herbal mediated silver nanoparticles, the nano powder form and colloidal form were confirmed by absorbance between 400-480nm using Schimadzu UV-Vis spectrophotometer. The crystalline structure of the particles was measured by X-ray diffraction instrument (XRD). The size and shape of HMSNPs were measured using Scanning electron microscope (SEM). The surface area of nanoparticles was measured by zeta potential.

Determination of particle size distribution

The determination of particle size distribution is provided for SAEPE, SAECE, SAEAPE, SAEACE samples . The results obtained are:

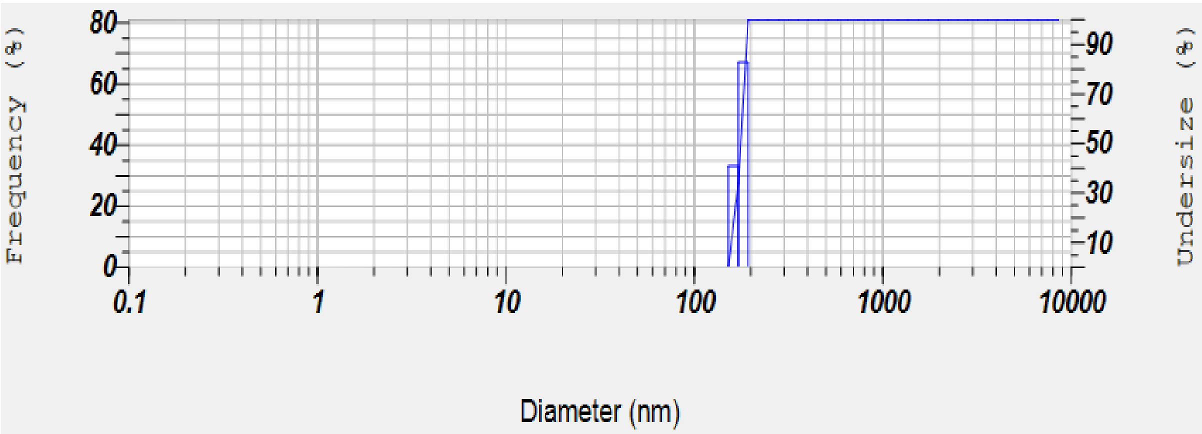

Fig. 1: Dynamic light scattering measurements were done for SAEPE to determine the size of Ag NPs formed. The particle size distribution curve of the synthesized Ag NPs is shown. It showed an average particle size of 26.3 nm respectively.

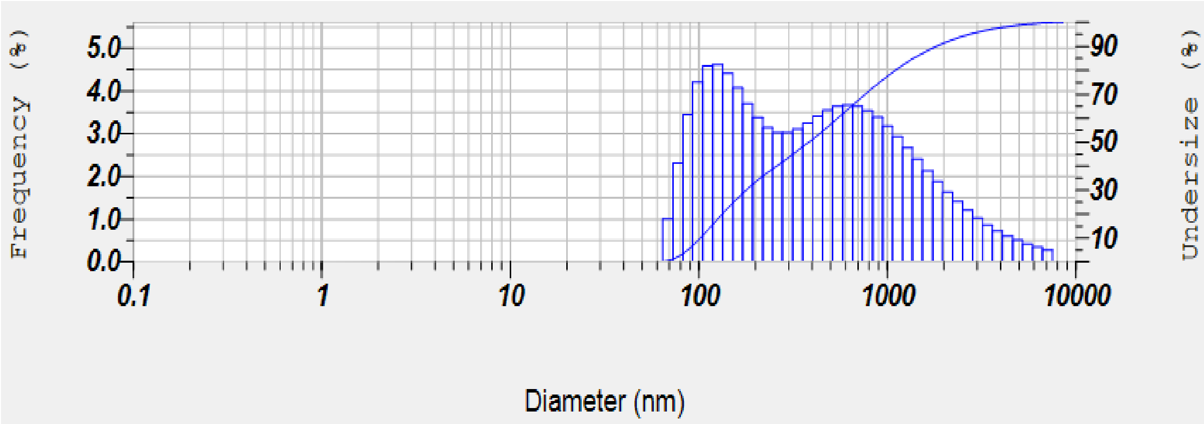

Fig.2: Dynamic light scattering measurements were done for SAECE to determine the size of Ag NPs formed. The particle size distribution curve of the synthesized Ag NPs is shown. It showed an average particle size of 14.9 nm respectively.

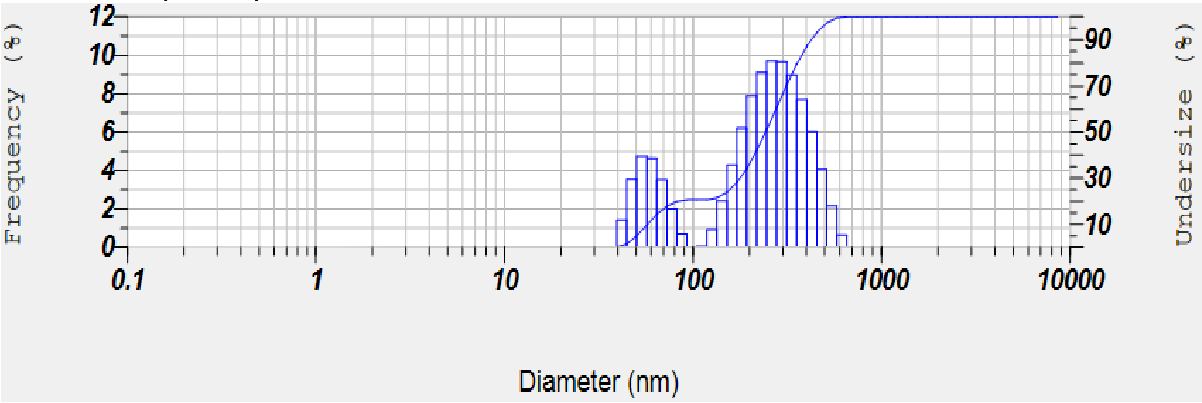

Fig.3: Dynamic light scattering measurements were done for SAEAPE to determine the size of Ag NPs formed. The particle size distribution curve of the synthesized Ag NPs is shown. It showed an average particle size of 48.6 nm respectively.

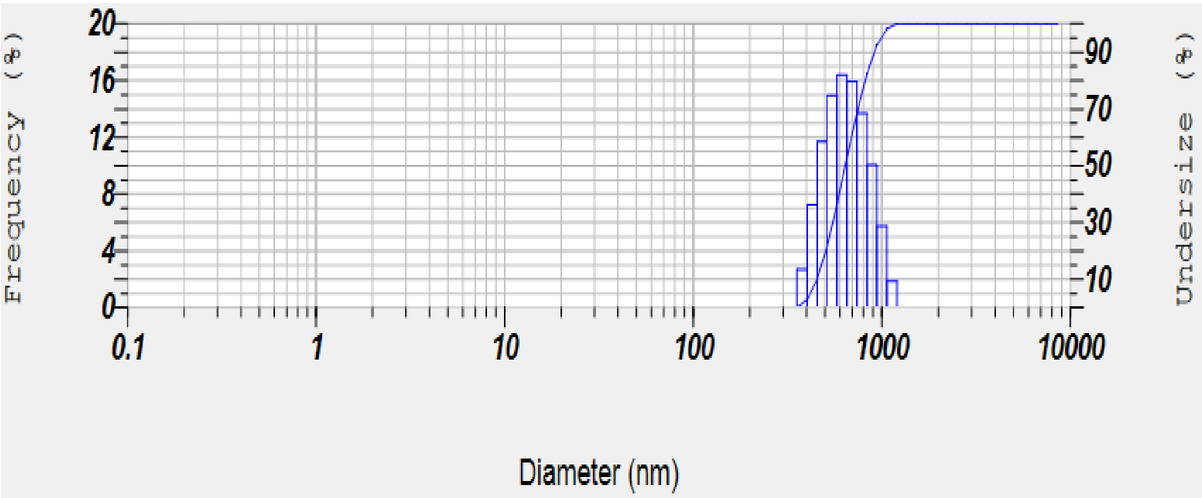

Fig.4: Dynamic light scattering measurements were done for SAEACE to determine the size of Ag NPs formed. The particle size distribution curve of the synthesized Ag NPs is shown. It showed an average particle size of 35.7 nm respectively.

UV –vis spectrophotometry

It is well known that the size and shape of the Nano particles are related to the wavelength of absorption and the form of the band of the UV –vis spectrum due to resonance of the surface. The images of reveal that the UV –vis spectra is obtained for four samples

(SAEPE, SAECE, SAEAPE, SAEACE) with the absorptivity peak between 190 to 400 nm.

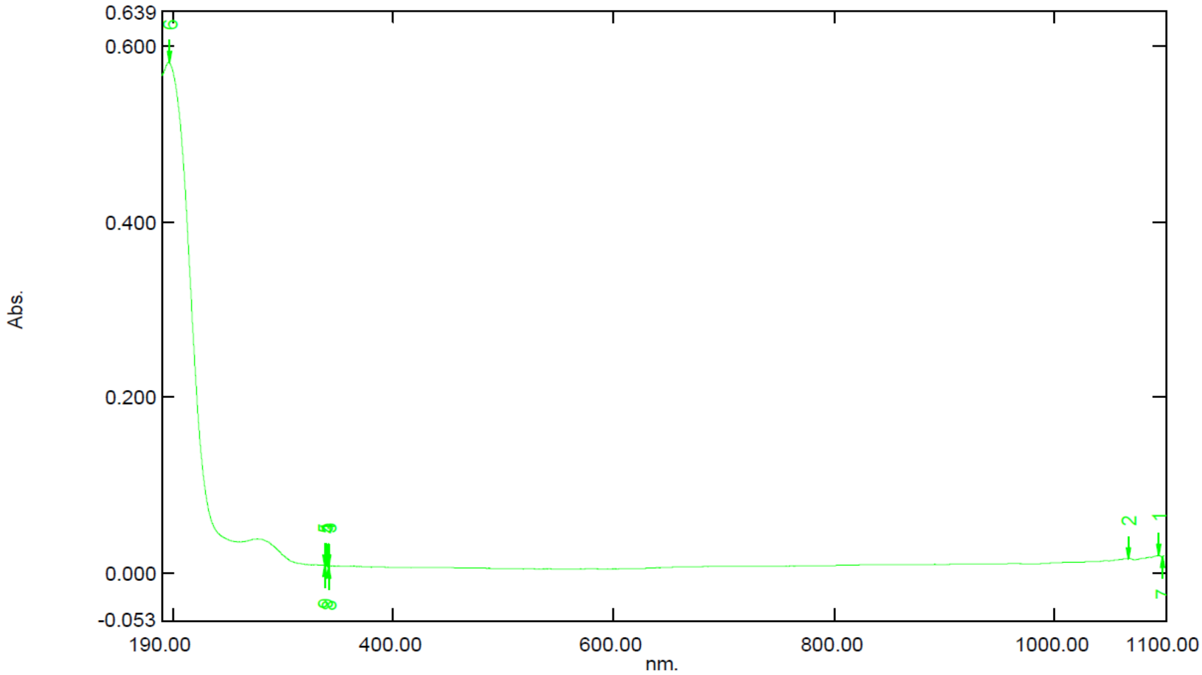

Fig.5: UV –vis spectrum of the Ag NPs synthesized using SAEP extract

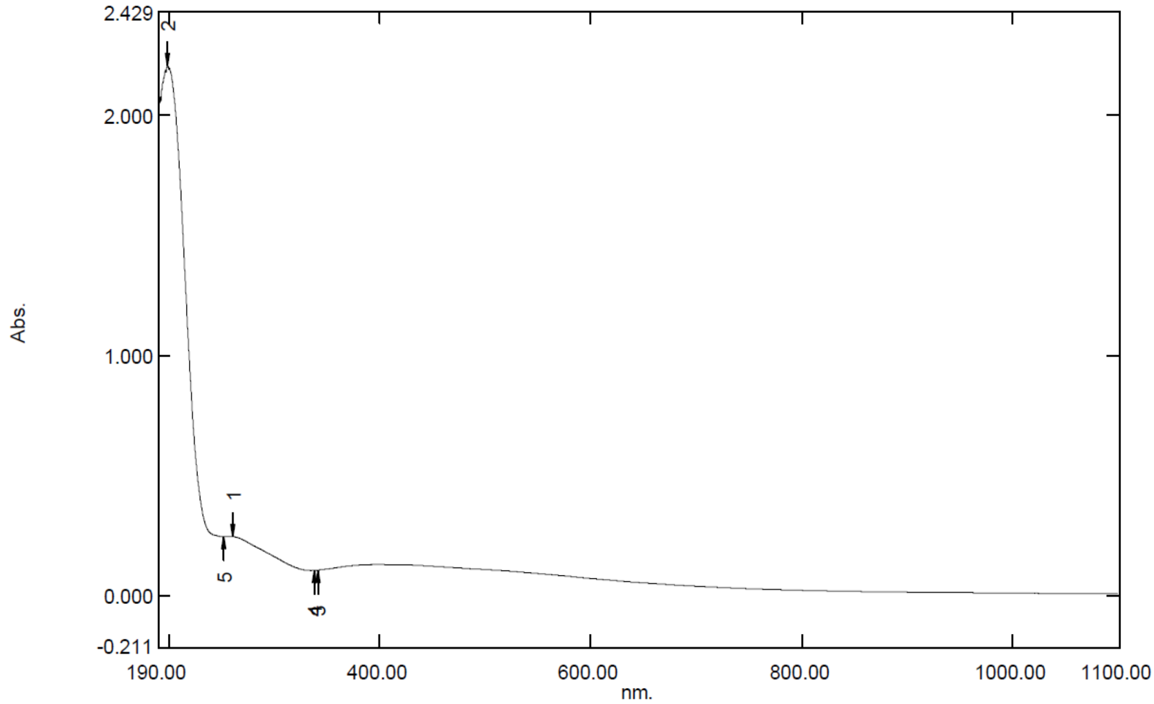

Fig.6: UV –vis spectrum of the Ag NPs synthesized using SAEC extract

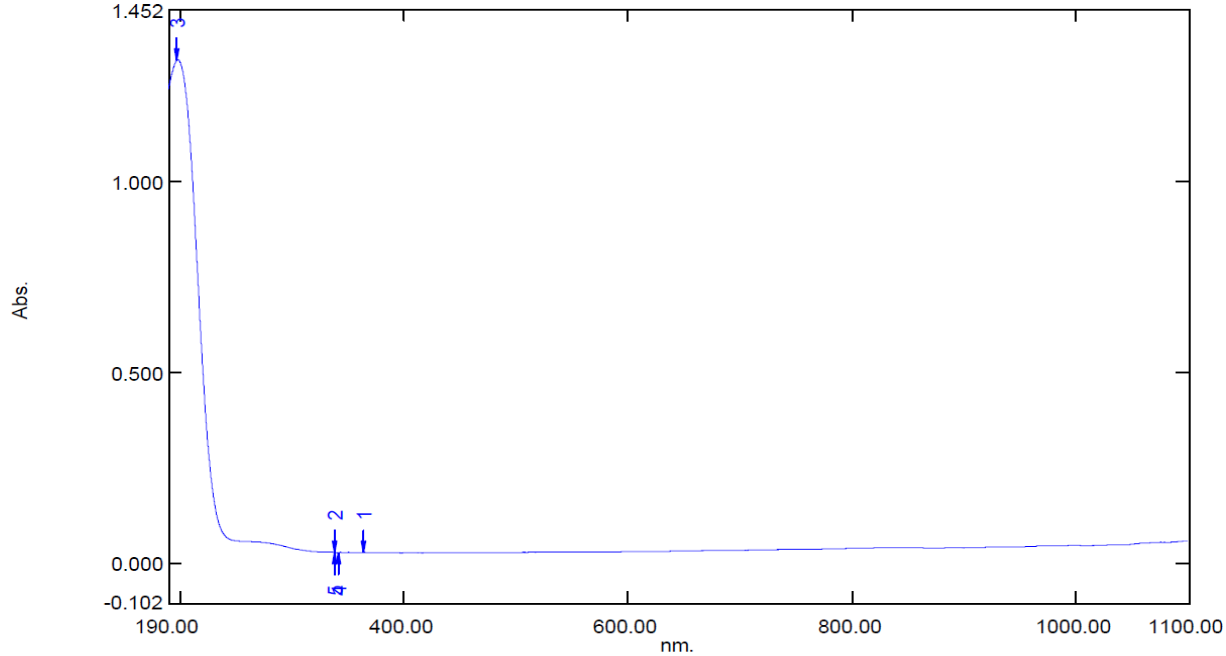

Fig.7: UV –vis spectrum of the Ag NPs synthesized using SAEAP extract

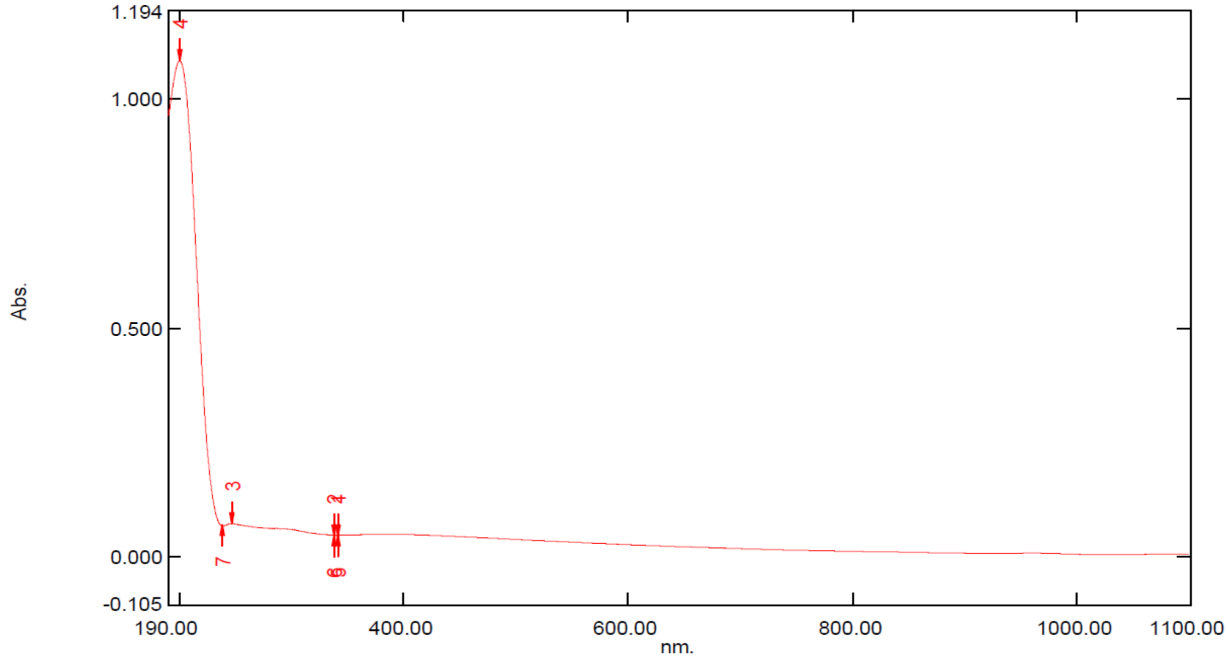

Fig.8: UV –vis spectrum of the Ag NPs synthesized using SAEAC extract

Zeta potential

Zeta potential states the electric potential on NPs boundary and can be used to determine the NPs surface charge. It was found that the zeta potential of the $AgNP_s$ of SAEPE, SAECE, SAEAPE, SAEACE extracts of biomolecules present in the system, are negatively charged. These negative charges give stability to the Nano particles, preventing aggregation and agglomeration.

Table.3: Zeta potential data of SAEP extract

E-P--1-Zeta.nzt

Measurement Results

Date	:	20 September 2019 11:08:33
Measurement Type	:	Zeta Potential
Sample Name	:	E-P--1-Zeta
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.895 mPa.s
Conductivity	:	0.121 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-58.9 mV	-0.000456 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-58.9 mV
Electrophoretic Mobility mean	:	-0.000456 cm^2/Vs

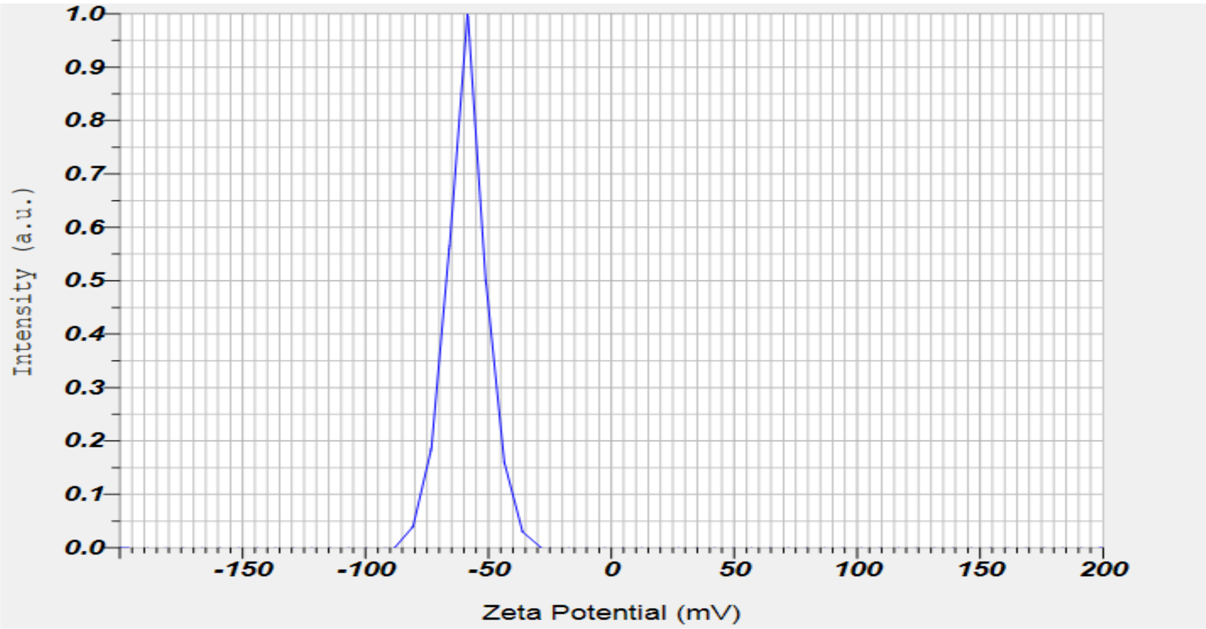

Fig.9: The Zeta potential value of dispersed biosynthesized Ag NPs of SAEPE was found to be -58.9 mV, typically have degree of stability.

Table 4: Zeta potential data of SAEC extract

E-C-3-Zeta.nzt

Measurement Results

Date	:	20 September 2019 11:20:11
Measurement Type	:	Zeta Potential
Sample Name	:	E-C-3-Zeta
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.895 mPa.s
Conductivity	:	0.126 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-56.3 mV	-0.000436 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-56.3 mV
Electrophoretic Mobility mean	:	-0.000436 cm²/Vs

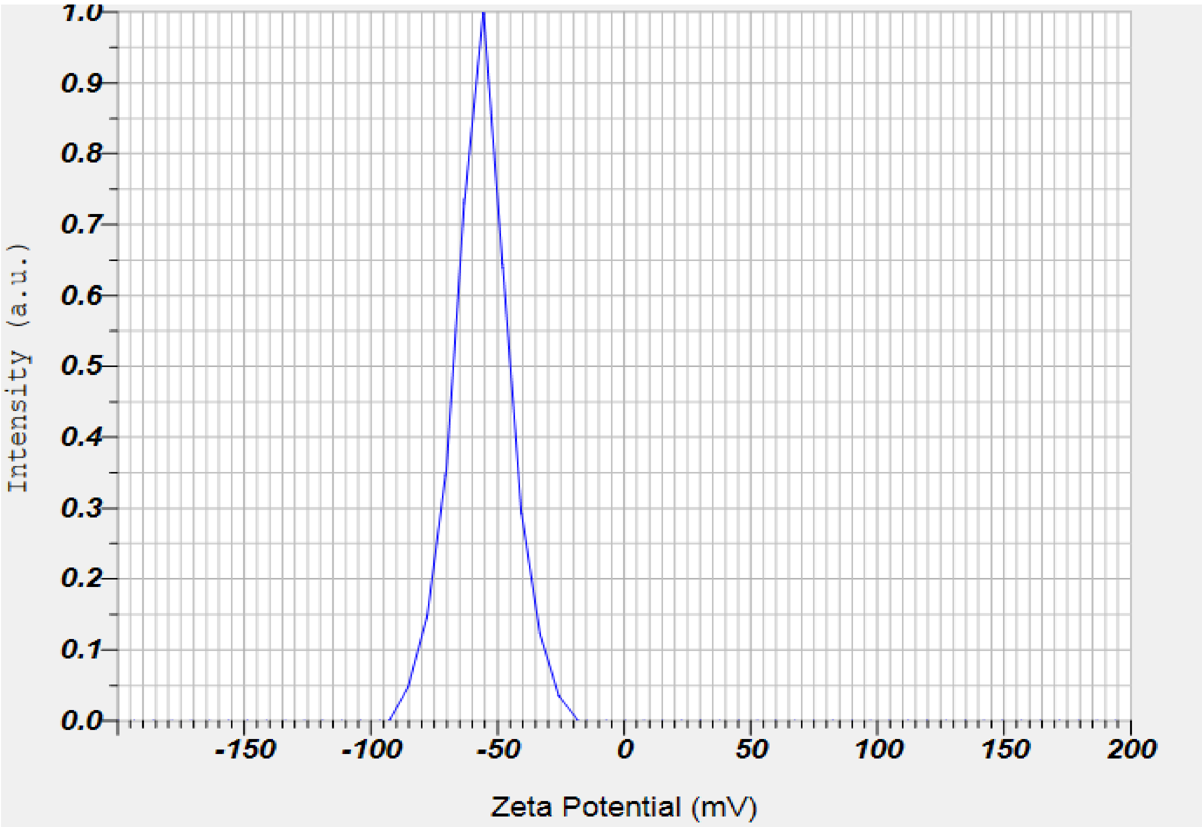

Fig10: The Zeta potential value of dispersed biosynthesized Ag NPs of SAECE was found to be -56.3mV, typically have degree of stability.

Table 5: Zeta potential data of SAEAP extract

E-A-P-2-Zeta.nzt

Measurement Results

Date	:	20 September 2019 11:12:14
Measurement Type	:	Zeta Potential
Sample Name	:	E-A-P-2-Zeta
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.895 mPa.s
Conductivity	:	0.123 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-60.5 mV	-0.000468 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-60.5 mV
Electrophoretic Mobility mean	:	-0.000468 cm^2/Vs

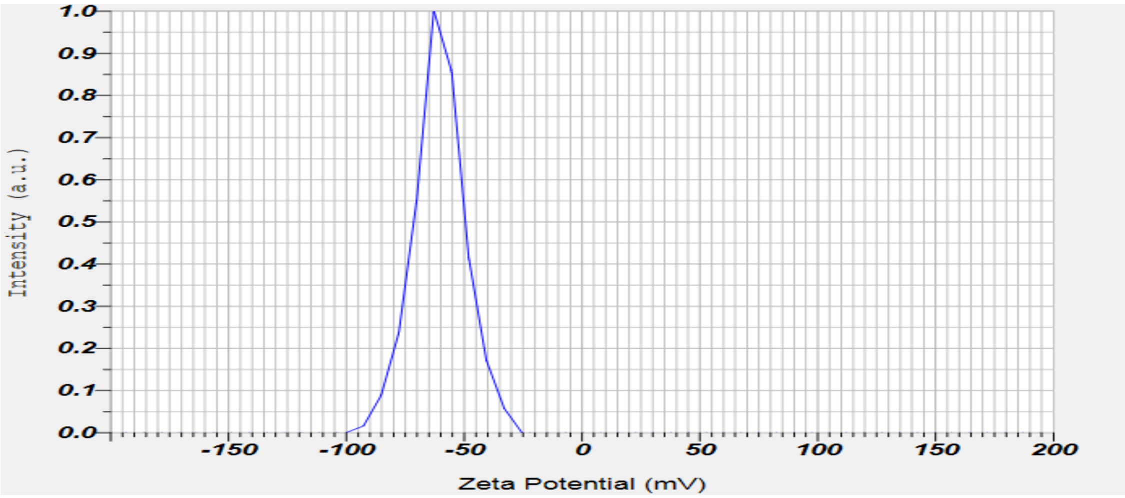

Fig.11: The Zeta potential value of dispersed biosynthesized Ag NPs of SAEAPE was found to be -60.5mV, typically have degree of stability.

Table 6: Zeta potential data of SAEAC extract

E-A-C-4-Zeta.nzt

Measurement Results

Date	:	20 September 2019 11:16:38
Measurement Type	:	Zeta Potential
Sample Name	:	E-A-C-4-Zeta
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.894 mPa.s
Conductivity	:	0.125 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-56.1 mV	-0.000435 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-56.1 mV
Electrophoretic Mobility mean	:	-0.000435 cm^2/Vs

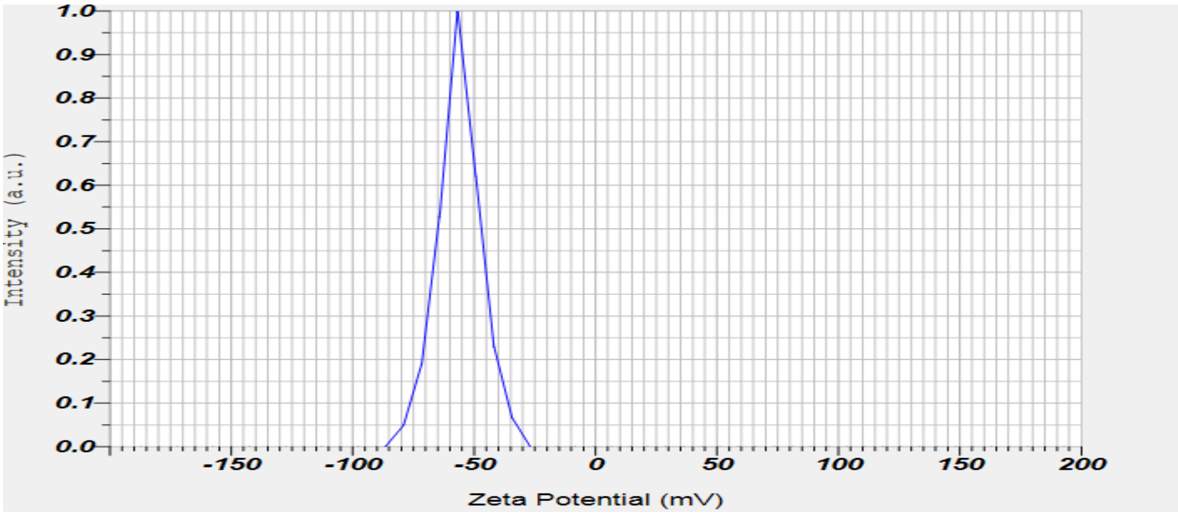

Fig.12: The Zeta potential value of dispersed biosynthesized Ag NPs of SAEACE was found to be -56.1 vM, typically have degree of stability.

Scanning electron microscopy (SEM)

The particle size, morphology of the drug and Ag NPs of powder and colloidal form can be investigated by SEM. The SEM pictures of formulation shows the shape and size of the suspended particles which are appeared to be spherical and the maximum number of particles were found to be in Nano size range which indicates Nano is formed.

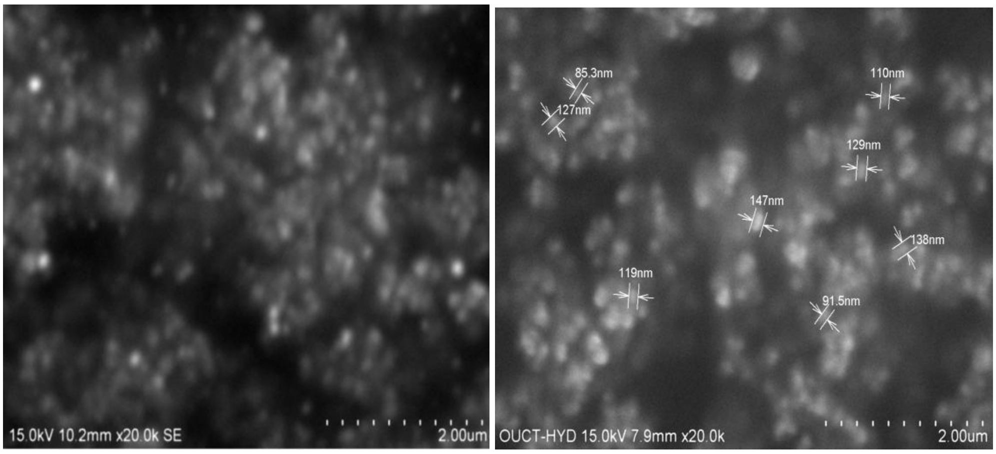

Fig. 13: SEM image of Ag NPs synthesized using SAEP extract

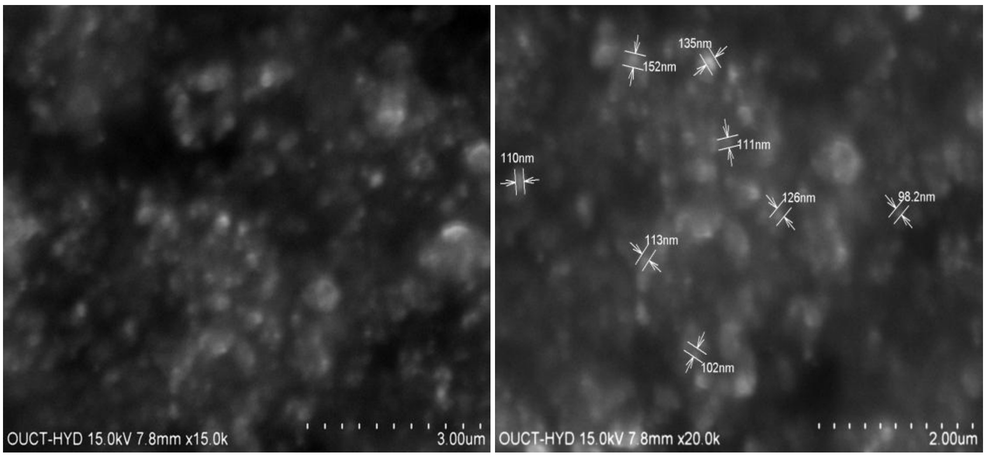

Fig.14: SEM image of Ag NPs synthesized using SAEC extract

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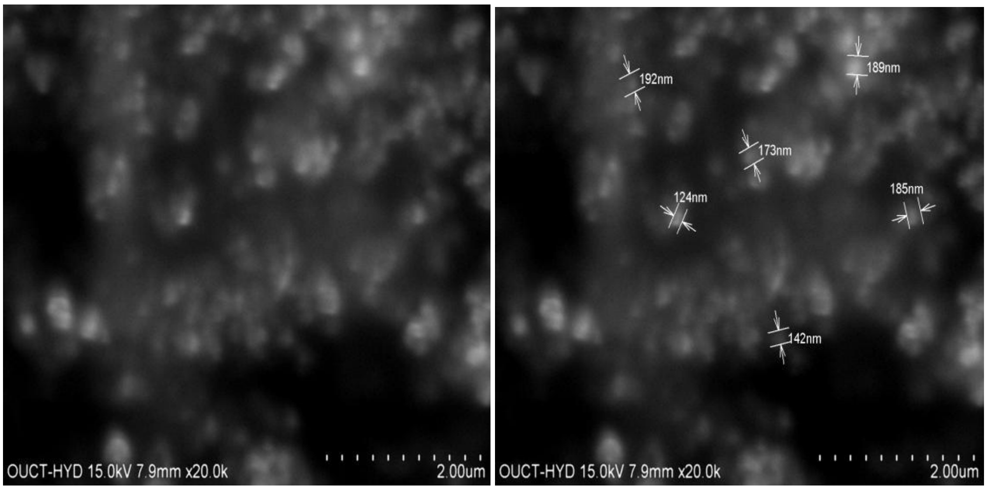

Fig.15: SEM image of Ag NPs synthesized using SAEAP extract

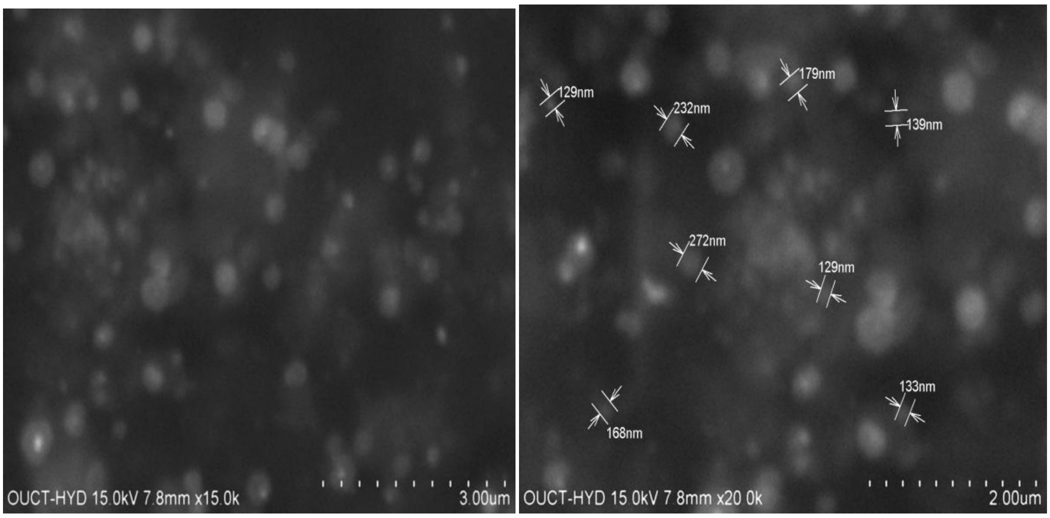

Fig.16: SEM image of Ag NPs synthesized using SAEAC extract

X-ray diffraction (XRD)

Crystalline nature of Ag nanoparticles determines the important properties such as solubility, bioavailability, rate of dissolution and stability. XRD studies were used to confirm the crystalline nature and other excipients used in the formulation of SAEP and SAEAP extract. XRD results are show that exhibited crystalline nature with characteristic peaks at 2Theta positions are 118, 64, 47 in SAEP extract and also SAEAP extract shows crystalline nature with characteristic peaks at 2Theta positions are 117, 66, 48, which confirms the presence of pure Ag NPs.

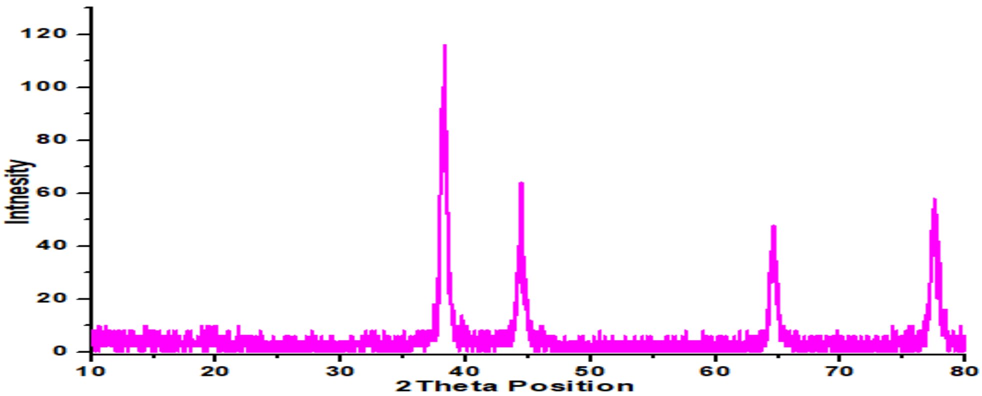

Fig.17: XRD image of Ag NPs synthesized using SAEP extract

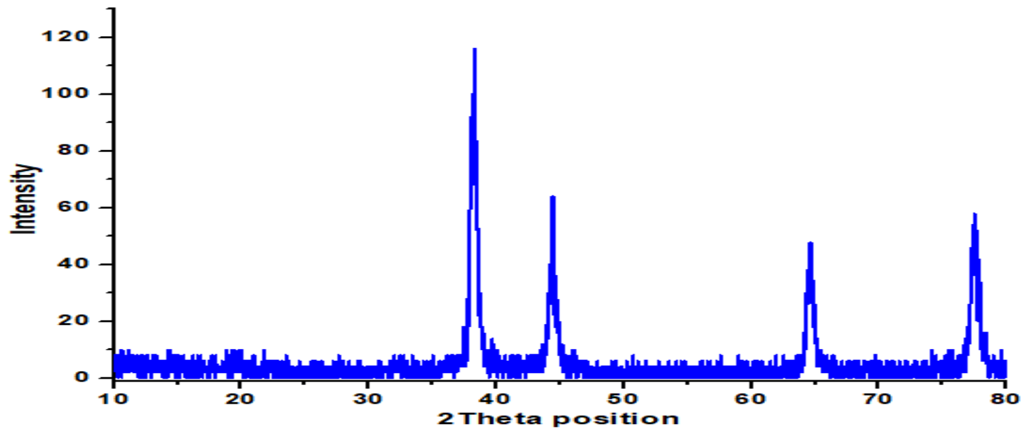

Fig.18: XRD image of Ag NPs synthesized using SAEAP extract

Fourier-transform infrared spectroscopy (FTIR)

FTIR play an important key role in the reduction of metal ions and stabilization of nanoparticles. Fourier transform infrared spectra showed the interactions between SAEP, SAEC, SAEAP, SAEAC extracts and synthesized Ag NPs. The significant shifting of peaks provides information about the functional groups involved in the reduction of silver nitrate to silver nanoparticles.

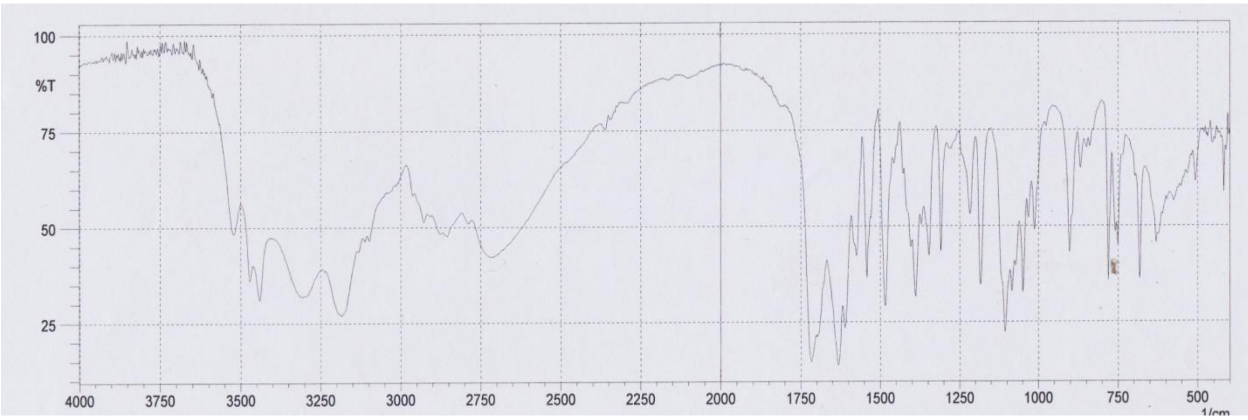

Fig.19: FTIR image of Ag NPs synthesized using SAEP extract

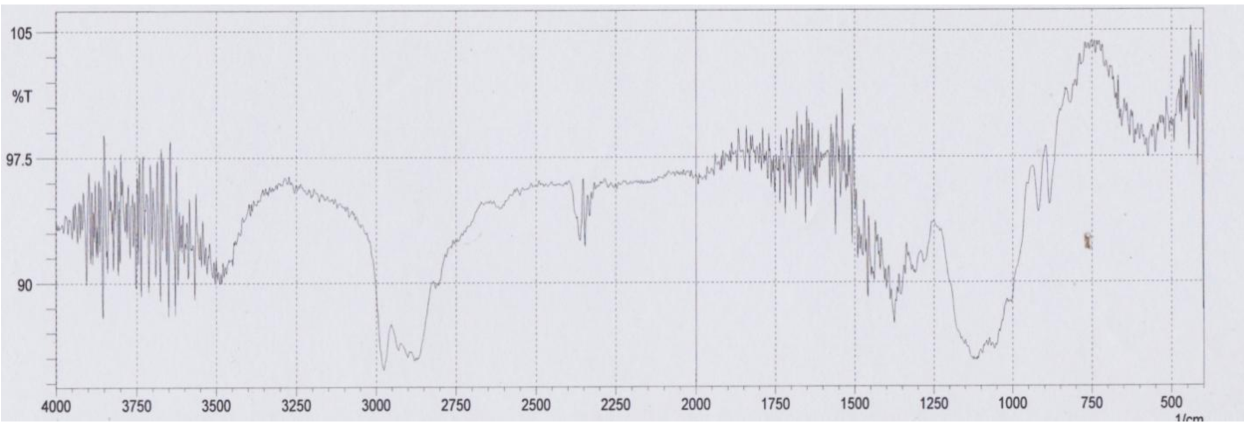

Fig.20: FTIR image of Ag NPs synthesized using SAEC extract

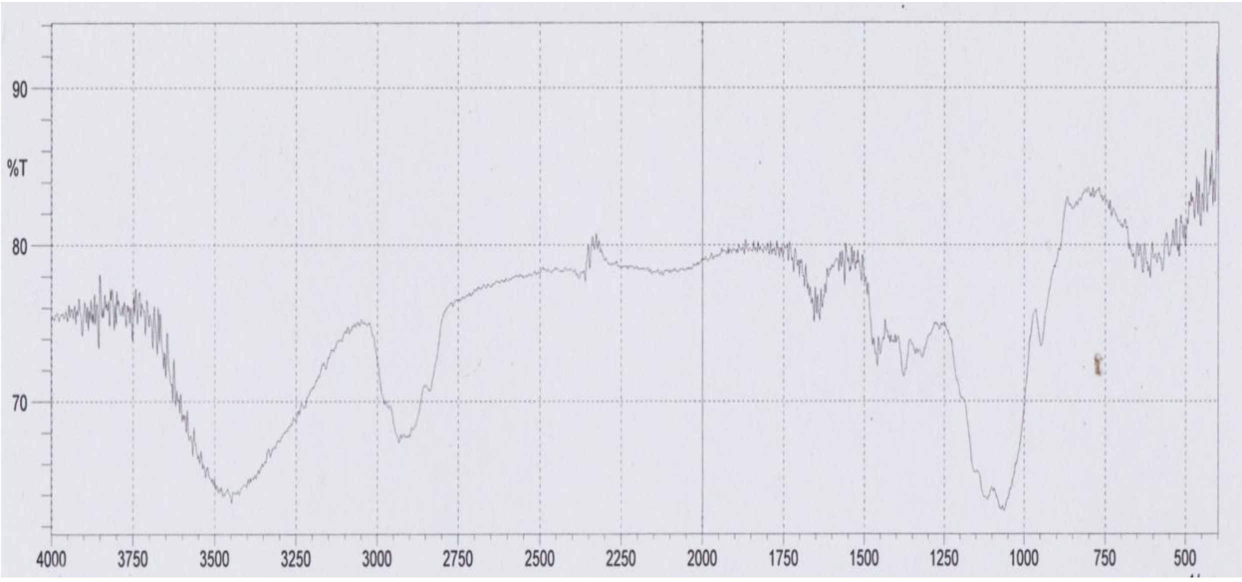

Fig.21: FTIR image of Ag NPs synthesized using SAEAP extract

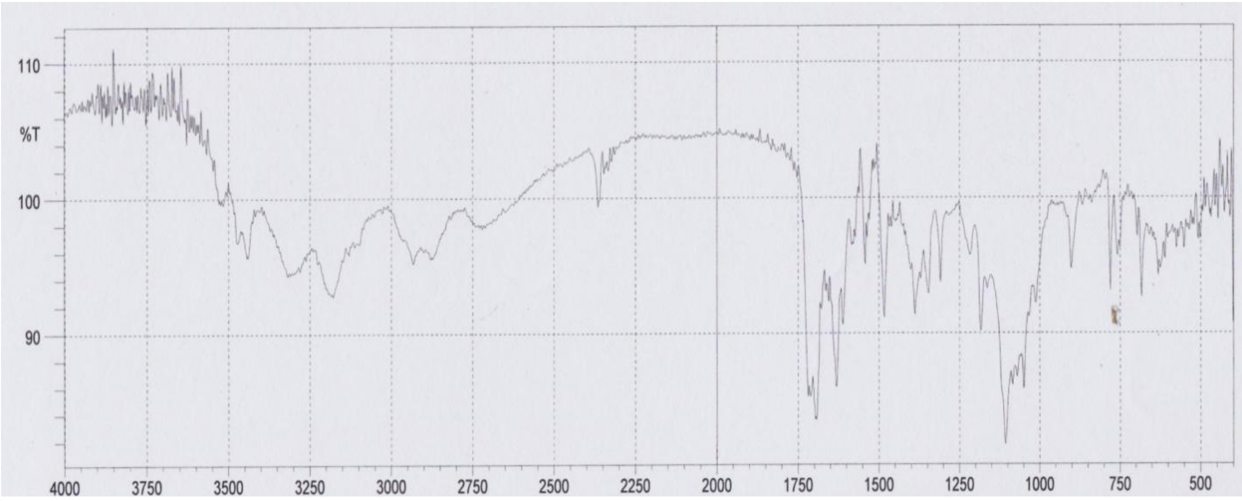

Fig.22: FTIR image of Ag NPs synthesized using SAEAC extract

CONCLUSION:

The silver Nanoparticles and Silver Colloidal Nanoparticles were suceesfully prepared from the extracts of *Semecarpus anacardium* seeds. The characterization of HMSNPs, HMSCNPs was done by using U.V, Zeta potential, X-ray diffraction technique, Fourier Transform Infrared Spectroscopy (FTIR), Scanned Electron Microscope (SEM) parameters.from thses studies it was evident that the prepared HMSNPs, HMSCNPs were of nano size and can be further studied for their In-Vivo activities.

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