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Development and Characterization of Guava Peel Extract Based Nanoparticles

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ABSTRACT

The main objective of the present work was to prepare Guava peel extract based nano particles by chemical complexation method. Ethanolic extracts of Guava peel were prepared by using Soxhlet apparatus and evaluated for phyto-chemical constituents. Qualitative analysis showed that Guava peel extract showed positive results for alkaloids, tannin and saponins. The percentage moisture content and pH of the extract was found to be 72% and 3.6 respectively. A zeta potential and particle size of prepared nanoparticles was found in the range of -24.6 mV to -35.0 mV and 118.6 nm to 231.7 nm, respectively. These range confirms that obtained particles were in nano range, i.e. <500 nm size. SEM results indicated the formation of nanoparticles and were relatively spherical in shape. Energy dispersive spectrometry (EDS) analysis confirms the presence of AgNPs. Further the study will be extended for anti-microbial and wound healing activities.

INTRODUCTION

Nanoparticles are considered the building blocks of nanotechnology. The most important and unique feature of nanoparticles is their larger surface area/volume ratio. Nanoparticles are used in many fields such as biomedicine, tissue engineering and health, and in recent years, research has been conducted on the use of waste materials in many areas. Human life attracted much attention. Discovering additional value by using waste products such as tree bark to identify large biological resources is an area of interest for scientists. Synthesis of metal nanoparticles is one of the places where plant materials such as tree bark, leaves, seeds and roots are widely used today. The use of these waste materials to synthesize nanoparticles is a research based on the direction of green chemistry. Nanoparticles are considered the basis of nanotechnology. In recent years, noble metal nanoparticles have become an important research area due to their excellent electrical, optical, mechanical, magnetic and chemical properties. Metal nanoparticles have broad applications in diagnostics, biology, and catalysis. Ag, Pt, Au and Zn are the main metals used to produce nanoparticles.

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Guava belongs to the family Myrtaceae,. Guava peels have been used as an anthelmintic since ancient times, it fights tracheobronchitis, heals wounds, ulcers, bruises, stomatitis, diarrhea, vaginitis and prevents excessive bleeding.

Guava peel such as curettage, antibacterial, anti-amoeba, anti-bacterial, anti-bacterial, anti-inflammatory, anti-inflammatory, anti-inflammatory, anti-inflammatory, anti-inflammatory. -inflammatory, anti-inflammatory, anti-inflammatory, diuretic, hypotensive, hypoglycemic, hypothermic and antioxidant activity.

Silver nanoparticles are used in various applications such as biomedical devices, biosensors, catalysis, electronics and pharmaceuticals. Many of these materials are not suitable for critical applications such as in medicine. Furthermore, they represent an environmental hazard. Eco-friendly methods for the synthesis of metal nanoparticles are needed to avoid or minimize such problems. Nowadays, metal nanoparticles are synthesized using eco-friendly natural sources such as plant extracts, fruits, fungi, honey and microorganisms.

The recent reports include the biosynthesis of silver nanoparticles by using plant parts like Punica granatum peel, Citrus sinensis peel, Annona squamosa peel, lemon peel, banana peel and mango peel. Silver nanoparticles prepared using biological materials have the properties of a high surface area, smaller in size and high dispersion. Silver particles and fruit peel of Guava it's self have antimicrobial property and when we use combination of silver particles and above mention fruit peel extract may show synergetic effect. So, in this research work our intension is to come out with good economical and highly targeted drug delivery formulation system. In this area as per literature survey and our knowledge nothing more precise and advanced studies are not done. So, here we are representing the advanced research work in relating to the objectives provided.

MATERIAL AND METHODOLOGY:

Material used:

Guava peels were collected from local market, Jaipur. Chemicals like Silver nitrate and Sodium borohydride were obtained from S. D Fine chemicals, Mumbai, India. Milli Q water was used throughout the experiment.

Method used:

Size reduction:

Fruit of Guava were washed well using tap water. The peels are separated and were washed thoroughly with tap water. The washed peels were cut in to small pieces [1-5 cm] and air dried in sunlight for 20 days. The dried fruit peels were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form and then passed through sieve no. 40 to get uniform powder and stored at room temperature. The peel powder was stored separately in air tight bottles in refrigerator.

Preparation of Extract:

100g powdered sample was extracted with 800ml ethanol at room temperature by Soxhlet extraction method for 6 hours. The mixture was filtered through a Whatman filter paper (No.2) for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were filtered and evaporated to dryness under reduced pressure at 60°C by a rotary flask evaporator (Buchi, Japan). The extracts were placed in dark bottles and stored in refrigerator at 4°C until further use.

UV-visible spectrum analysis:

Fruit peel extract were diluted in distilled water and after dilution the samples were observed by UV-visible spectrophotometer (Shimadzu, UV-1800). The sample solution was colored, hence scanning was done in the range of 400-800 nm.

Qualitative and Quantitative analysis of phyto-chemical constituents:

Qualitative analysis:

The obtained peel extract were subjected to variousqualitative analysis.

Test for alkoloids:

A pinch of crude power was mixed with 1% HCl and about 6 drops of Mayor's reagents. A creamish or pale yellow precipitate indicated the presence of respective alkaloids[6].

Test for amino acids:

A pinch of crude extract was treated with few drops of Ninhydrin reagent. Appearance of purple color showsthe presence of amino acids[6].

Test for tannins:

A pinch of crude extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration[6].

Test for anthraquinones (Borntrager's test):

A pinch of crude extract was hydrolyzed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones[6].

Test for saponins:

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins[6].

Test for protein:

A pinch of crude powder was treated with 4% Sodium Hydroxide and few drops of 1% Copper Sulphate was added. Violet or pink colour apper the presence of protein[6].

Test for terpenoids (Salkowski test):

A pinch of crude powder was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids[6].

Test for cardiac glycosides (Keller-Killani test):

A pinch of crude powder was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides[6].

Quantitative analysis:

Equivalent weight determination:

Crude extract (0.5 g) was weighed into a 250 ml conical flask and moistened with 5 ml ethanol, 1.0 g sodium Chloride was added to the mixture followed by 100 ml distilled water and few drops of phenol red indicator. Care was taken at this point to ensure that all the extract had dissolved and that no clumping occurred at the sides of the flask before the solution was then slowly titrated (to avoid possible de-esterification) with 0.1 M NaOH toa pink colour at the endpoint. Equivalent weight was calculated using the equation below:

Equivalent Weight = (Weight of extract/ Volume of Alkali (cm^3) × Molarity of Alkali) × 100%

Ash content determination:

Five grams of each of the sample was accurately weighed into a weighed empty crucible separately. The crucible was transferred to a furnace set at 60°C to burn off all the organic matter. The carbon charred and then burnt off as carbon dioxide, leaving a dark ash; this process lasted for 24 hour. The crucible was taken out of the furnace and placed in a desiccator to cool. The crucible after cooling was reweighed again. This was calculated using:

Ash Content (%) =(Weight of Ash / Weight of Sample) × 100

Moisture content determination:

A dried empty petridish was dried in an oven, cooled in a desiccators and weighed. Five grams of the extract was transferred into the crucibles in the oven which was set at 130°C for 1h thereafter the petridish was removed, cooled in a desiccators and weighed. This process was repeated once (Aina VO et al., 2012). The moisture content was calculated using:

Moisture Content (%) = (Weight of the Residue/ Weight of the Sample) × 100%

pH: pH of obtained crude extract was determined by using calibrated pH meter (Techno scientific products, Bangalore).

Nanoparticles Preparation:

To a 50ml of freshly prepared 0.001M Silver nitrate solution, 5ml of 0.002M Sodium borohydride solution was added with continuous stirring and kept it aside for 15 minutes in a clean 250 ml beaker till a clear and slightly dark solution is obtained. Further, this clear solution is heated and maintained in water bath at 45^{O}C for 30 min (solution A). Guava peel extract in different concentration (200mg, 300mg and 500mg) was dissolved in 4ml of Milli Q water separately in test tube and heated slightly to get Guava yellow colored solution. This solution was added drop wise into solution A with continuous stirring using glass rod for 30-45 min till Guava colour solution is obtained. The obtained solution is cooled to room temperature and 0.5ml of 0.5 mcg/ml PVP solution was added as a stabilizer and filtered to get clear Guava colored solution of silver nanoparticles. This solution was stored in a dark place in a well closed container until further use. Then prepared nanoparticles were evaluated for FTIR, particle size, zeta potential, surface morphology by SEM, X-ray diffractionand energy dispersive spectrometry[7, 8].

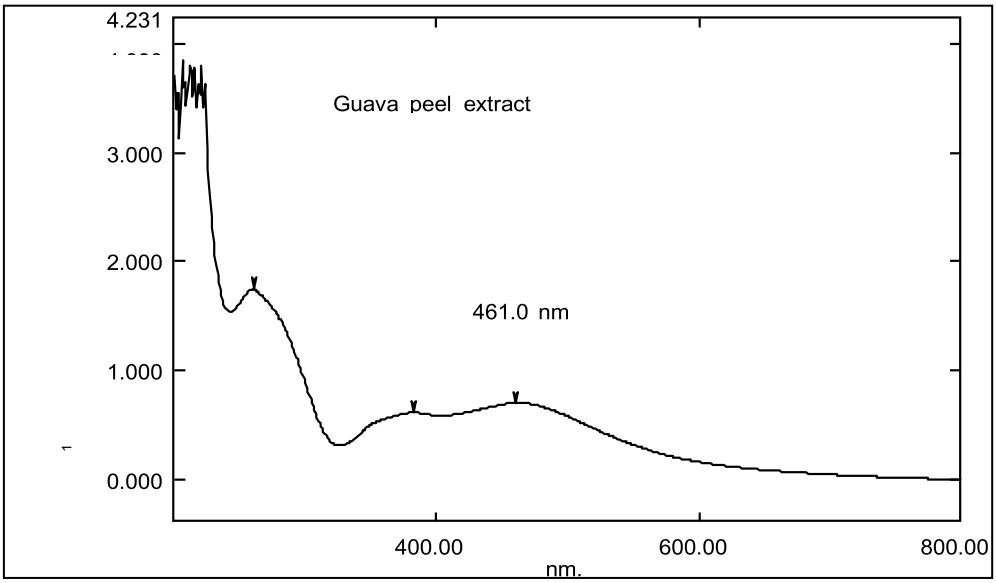

Figure 1: UV-Spectrum of Guava peel extract

Table 1: Phyto-chemical Analysis of Guava peel extract by Soxhlet Apparatus

Sl. No	Phyto-chemicals	Status*
1	Alkaloids	+
2	Amino acid	_
3	Tannin	-
4	Anthraquinones	+
5	Saponins	+
6	Protein	-
7	Terpenoids	+
8	Cardiac glycosides	_

+ = Present, - = Absent

Table 2: Quantitative analysis of Guava peel extract

Sl. No	Parameters	Values
1	Equivalent weight	345.14
2	Ash content	30%
3	Moisture content	72%
4	pH	3.6

The FT-IR spectrum of Guava peel extract showed the distinct peak in the range of 1649, 3390, 2355 and 771cm−1. The absorption peaks located mainly at 3036 cm−1 are generally attributed to aromatic or aliphatic C– H stretching, 2928 cm−1 are generally assigned to the alkyl C–H stretching, whereas peaks at 1734, 1375 and 1332 cm−1 are due to C–O–O stretching bands, 1102 and 1050 cm−1are due to C–C stretching vibrations, 713 and 624 cm−1are due to acetylenic C–H bending vibrations in the region of 40–4000 cm−1. All the spectrum of Guava Peel extracts is present in the Guava Peel extracts nanoparticles. Hence there was no any shift of functional groups are seen in Guava Peel extracts nanoparticles (figure 2 and 3).

The Guava Peel extracts silver nanoparticles were subjected to Zeta Potential analysis to determine the surface charge of the nanoparticles and to find out the aggregation behavior. The values of zeta potential for Guava peel extract silver nanoparticles was found to be– mV to -21.4 mV for Guava O50 and Guava O20, respectively (figure 4a, and 4b). Hence all batches of nanoparticles having higher surface charge which indicates there is least chance of aggregation.

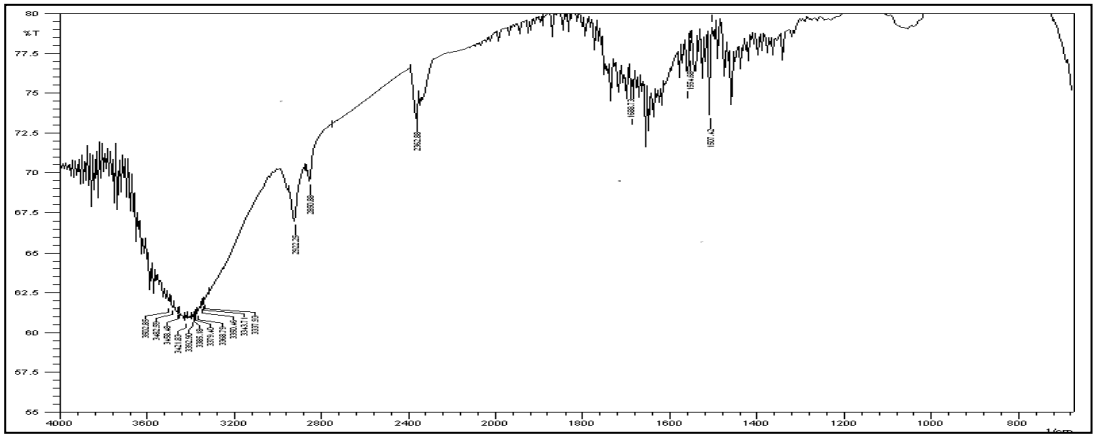

Figure 2: FTIR spectra for Guava peel extract

Figure 3: FTIR spectra Guava peel extract-based silver nanoparticles

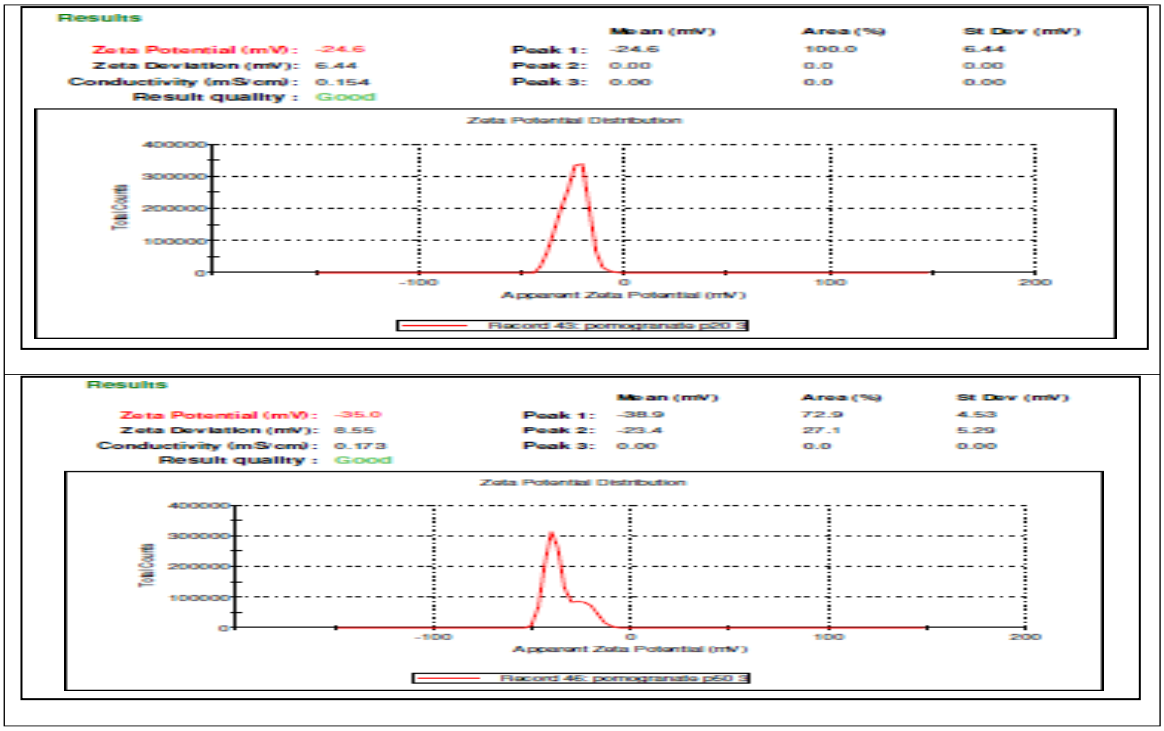

Figure 4a and 4b: Zeta potential of Guava peel extract silver nanoparticles (O50, O20)

The particle sizes of prepared nanoparticles were determined by using Malvern particle size analyzer. The values of particle size for Guava peel extract silver nanoparticles were found in the range of 118.6 nm to 231.7 nm for Guava O50 and Guava O20, respectively (figure 5a and 5b). These range confirms that obtained particles were in nano range, i.e. <500 nm size. To confirm the crystalline nature of Guava peel extract, X- ray diffraction (XRD) patterns were obtained (figure 6). The peaks assigned to the diffraction pattern clearly showed peaks corresponding to 2θ = 11.36°, 16.26°, 19.04°, 27.48°, 49.26°, 59.56°and 64.07°. The surface morphology of prepared nanoparticles was determined by using SEM (Hitachi). SEM results clearly showed the formation of nanoparticles and were relatively spherical in shape and also showed there was only a small degree of agglomeration. The largest size of Guava based nanoparticles peel extracts 74.9 nm. The SEM results were shown in figure 7. Energy dispersive spectrometer (EDS) analysis was performed

for the detection of elemental silver. The EDS microanalysis confirms the presence of AgNPs which is known to provide information on the chemical analysis of the elements or the composition at specific locations. The spectrum analysis reveals signal in the silver region and then confirms the formation of AgNPs. Metallic silver nanocrystals generally showed a typical optical absorption peak at approximately 2 keV due to the surface plasmon resonance. The EDS results were shown in figure 8.

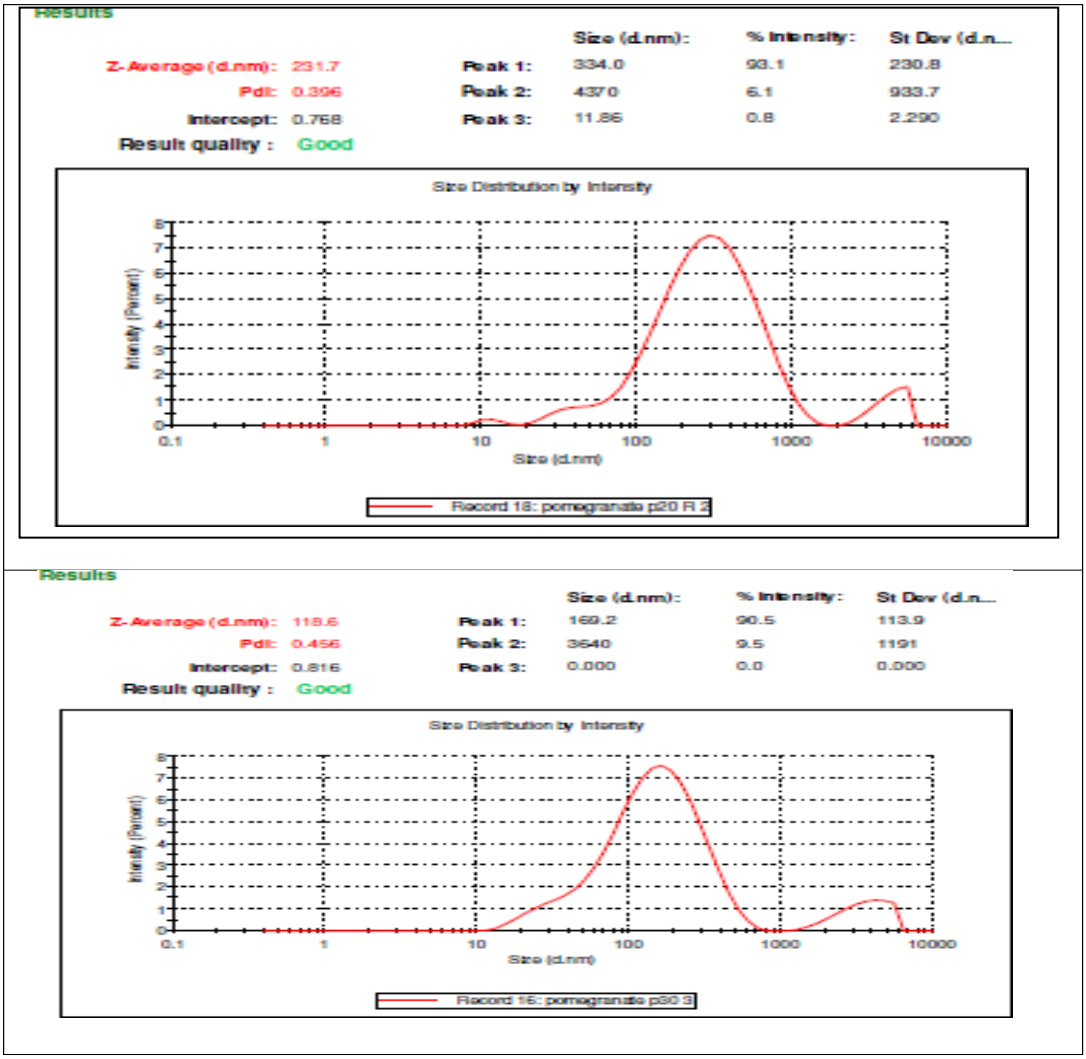

Figure 5a and 5b: Particle size of Guava Peel extracts silver nanoparticles (P20, P50)

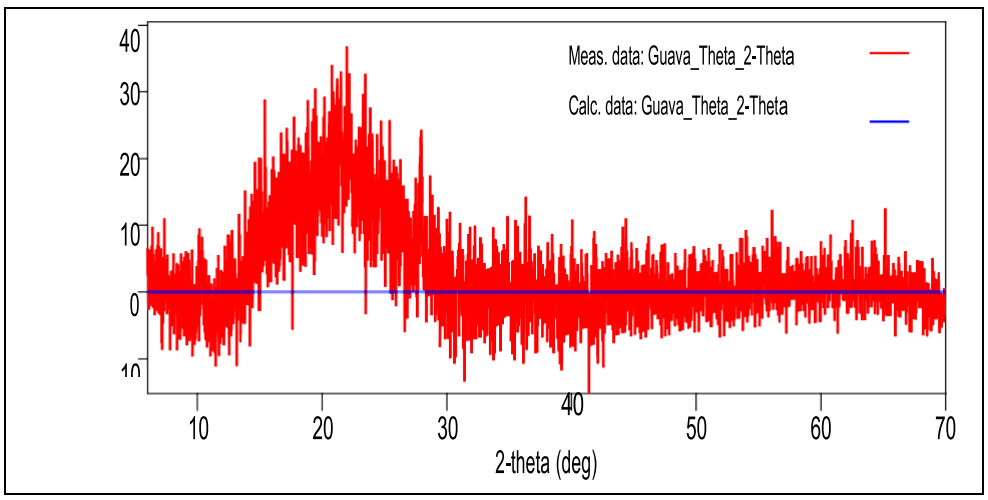

Figure 6: XRD pattern of Guava peel extract

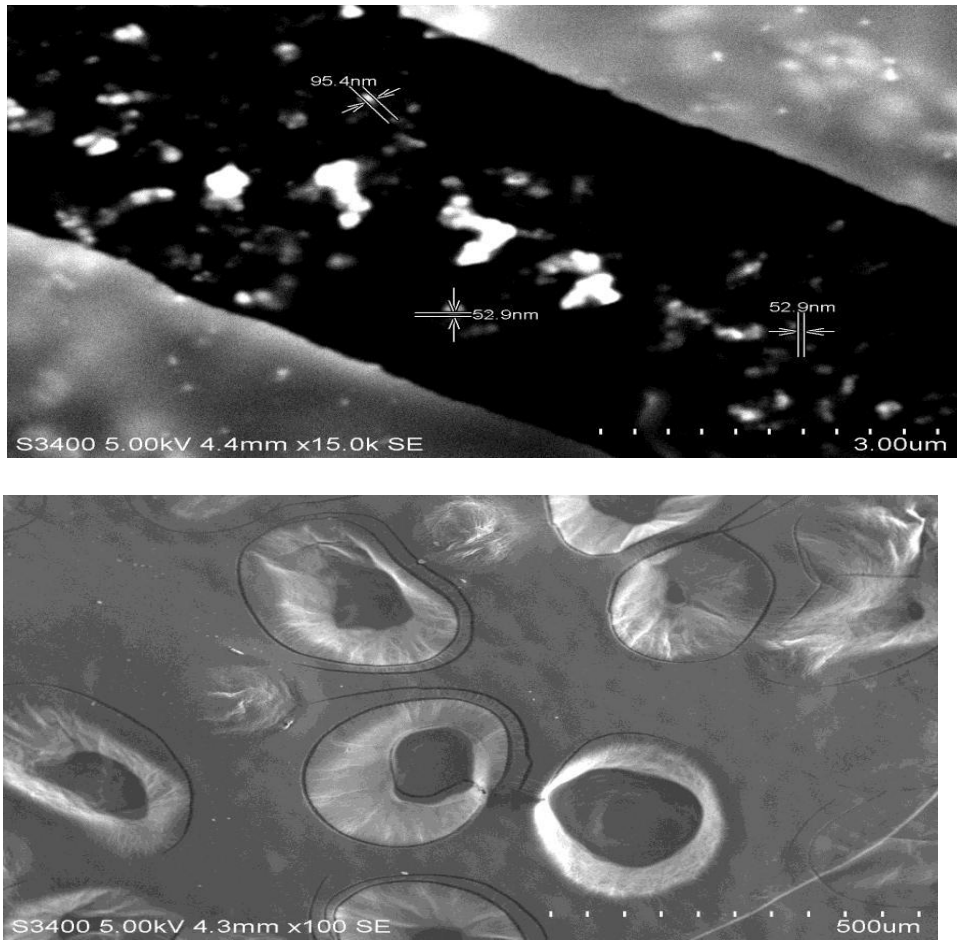

Figure 7: SEM images of Guava peel and AgNPs morphology

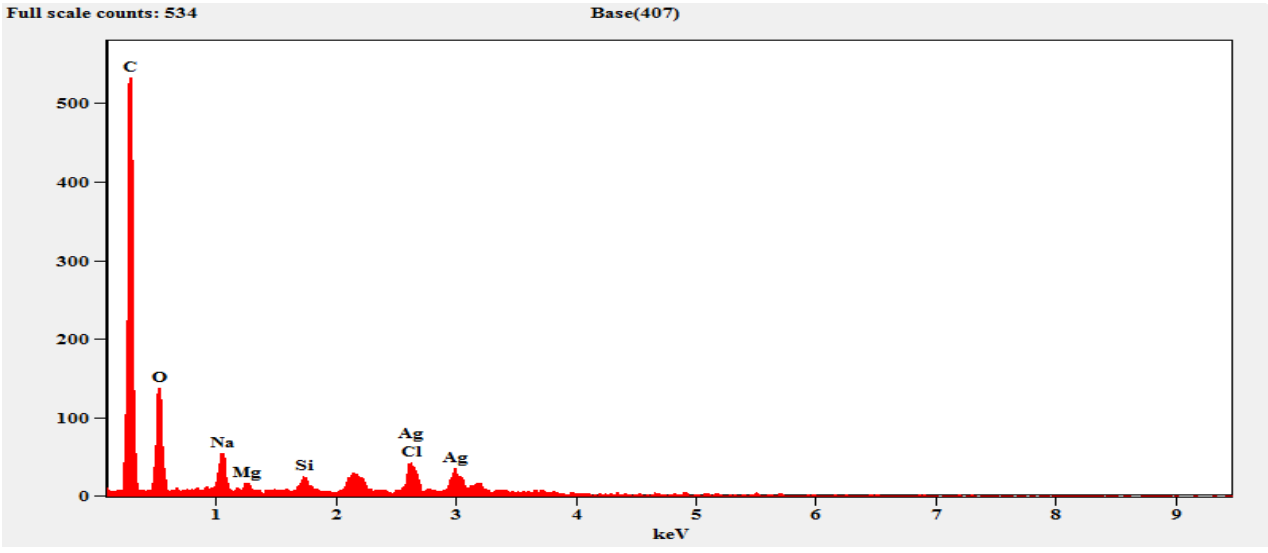

Figure 8: EDS pattern of spherical Guava peel extract AgNPs prepared.

Conclusion

Guava peel extract based nano particles were prepared by chemical complexation method using silver nitrate. Particle size, surface morphology and elemental analysis confirm that prepared formulations were silver particle based nano particle. Further research will be extended for wound healing activity in suitable animal model. Such herbal based nano particles might be safe, economically cheap and user friendly.

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