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Green Synthesis of Silver Nanoparticles Using *Actinidia deliciosa* Extracts.

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ABSTRACT

In the present study silver nanoparticles were synthesized in a single step by a green biosynthetic method using an extracts and juice of *Actinidia deliciosa* as a reducing and capping agents from 3 mM aqueous silver nitrate. The current research aimed to optimize the best conditions for synthesis silver nanoparticles like time of storage, temperature, pH, silver nitrate concentration and the mixing ratio of the reactants on silver nanoparticles synthesized. The nanoparticles were characterized using UV-Visible, X-ray diffraction and scanning electron microscope. The prepared silver nanoparticles showed surface plasmon resonance centered at 440, 435, 425, 420 nm for ethanolic, hot, cold extracts and juice respectively. The XRD pattern showed the face centered cubic structure of silver nanoparticles. While the results of scanning electron microscope show the size of nanoparticles prepared 76, 78, 83, 85 nm for ethanolic, hot, cold extracts and juice respectively.

Keywords: *Actinidia deliciosa*, Silver Nanoparticles, Green Biosynthetic.

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INTRODUCTION

The term nanotechnology is known as the creation, exploitation and synthesis of materials at a scale smaller than 100 nm. The word "nano" is derived from a Greek word called dwarf or extremely small. The concept of nanotechnology was first begun by a lecture delivered by Richard Feynman in 1959 [1]. The domain of nanotechnology is one of the most active areas of research in the new materials science. Nanoparticles show fully new properties based on fixed characteristics such as size, shape and distribution [2].

Metal nanoparticles are intensely studied due to their unique optical, electrical, and catalytic properties [3]. Among the noble studied metals, nanometric silver is a large choice because silver nanoparticles are nontoxic to human cell at low concentrations and most effective versus bacteria, virus and other eukaryotic microorganisms [4]. Optical properties of silver nanomaterial allow for their used in different scientific applications like sensors, nanophotonics, cancer treatment, photothermal therapy, diabetic healing, biological activity and medicine [5].

Different physical, chemical and biological methods have been developed for synthesis of nanoparticles [6] .Biological methods using fungi, bacteria, algae and plant extracts have benefit over other methods because of their systems are single step in nature, Eco friendly (Green Chemistry), no need to use toxic chemicals, high pressure, energy and simply scaled up for large –scale synthesis [7].

Using plant parts for nanoparticles synthesis can be advantageous over other biological methods because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for mass-scale synthesis of nanoparticles under natural environment [8]. In the plant, the main compounds which act as the reduction and the capping of the nanoparticles are biomolecules such as phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids and alcoholic compounds [9].

The medicinal plant used in this research is *Actinidia deliciosa* (Kiwifruit) which is famous as a king of fruits because of its remarkable highest vitamin C content and balanced nutritional composition of minerals, dietary fibre and different health beneficial metabolites [10]. Kiwifruit is one of the most public fruits worldwide and it has different biological properties including antioxidant, anti-allergic and cardiovascular defensive effects. The peel of kiwi fruit, which is a secondary of processing is a good source of flavonoids [11]. Kiwifruit is a highly nutritious fruit due to its high content levelof vitamin C and its strong antioxidant including carotenoids, phenolics, flavonoids and chlorophyll. Kiwifruit is a wealthy source of fructose, galactose and minerals, it contains isoflavones, flavonoids [12].

MATERIALS AND METHODS

Collection of Plant Samples:

The plant materials were taken from local markets at Hilla that native to southern China. The fruit plants were washed in tap water and the peels were removed, then cut into small pieces used to make the ethanolic extract, aqueous extracts and juice that analyzed.

Preparation of the Extracts:

The ethanolic extract, aqueous extracts were made according to [13].

Preparation of the Juice:

The juice was made by weighing 450 g of the fruit with using an electric blender to make mixture, then separated out by using filter paper. The filtrate was kept in the refrigerator to use afterwards.

Synthesis of Silver Nanoparticles:

Silver nitrate, aqueous solution 3 mM was prepared and utilized for the synthesis of silver nanoparticles from *Actinidia deliciosa* extracts and juice. Preparing of Silver nitrate with slight modificationaccording to [14].

Synthesis of Nanoparticles from Ethanolic and Hot Extracts:

Five mL of *Actinidia deliciosa*extracts were added to 95 mL of aqueous solution of 3 mM $AgNO_3$ and heated with stirrer at 70 °C. The appearance of brown color indicated compilation of silver nanoparticles formation.

Synthesis of Nanoparticles from Cold Extract and Juice:

Five mL of *Actinidia deliciosa*ofcoldextract and juice were added to 95 mL of an aqueous solution of 3 mM $AgNO_3$ and heated with stirrer at 45°C. The appearance of brown color was indicated compilation form of silver nanoparticles.

Study of the Optimum Conditions for Silver Nanoparticles:

To examine the optimum conditions for silver nanoparticles synthesis. The experiments were carried out in different conditions. These were a silver ion concentration (1, 3, 5 mM), pH (4, 6, 8, 10, 12), temperature (60, 70, 80, 85, 90 °C) for ethanolic and hot extract, but cold extract and juice (35, 45, 55, 60, 65 °C), time (1 day - 2 months) and the *Actinidia deliciosa*extracts and juice to silver nitrate ratio (5:95, 10:90 and 15:85).

The pH of the solution was modified by using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. The impression of these parameters on the synthesis of silver nanoparticles was observed by UV-Visible spectrophotometer.

Characterization of Green Synthesis of Silver Nanoparticles:

Color Change

The color change in the reaction mixture was recorded through visual observation. The color change from green pale or yellow to brown indicated that the silver nanoparticles were formed.

UV-Visible Spectral Analysis

The bio reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) was confirmed by measuring the sample in UV-visible spectrophotometer at different wavelength after dilution with a constant volume of deionized water to avoid a large value of absorbance. Using deionized water as a blank .

X- Ray Diffraction Studies (XRD)

The silver nanoparticles formed were centrifuged at 15,000 rpm for 15 min and the pellet was collected. The pellet was washed with distilled water to clear any purity and dried to obtain the powder. The X-ray diffractions check was performed to find out of the crystalline kind of the metal nanoparticles was done by X-ray diffractometer Shimadzu XRD-6000 AS (3K .NOPC).

Scanning Electron Microscopy Analysis (SEM)

The formation of silver nanoparticles were centrifuged at 15,000 rpm for 15 min and collect the pellet. The pellet was washed with distilled water to clear any purity. The pellet was carefully placed on a glass cover slip followed by drying. The cover slip itself was used during scanning electron microscopy (SEM) analysis .

RESULTS AND DISCUSSION

Characterization of Green Synthesis of Silver Nanoparticles:

Color Change

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The checking of formation of AgNPs was primary well known bythe color change from greenish pale to brown color in cold extract and juice, but ethanolic extract and hot extract exhibit yellow to browncolor as shown in Figure (1).

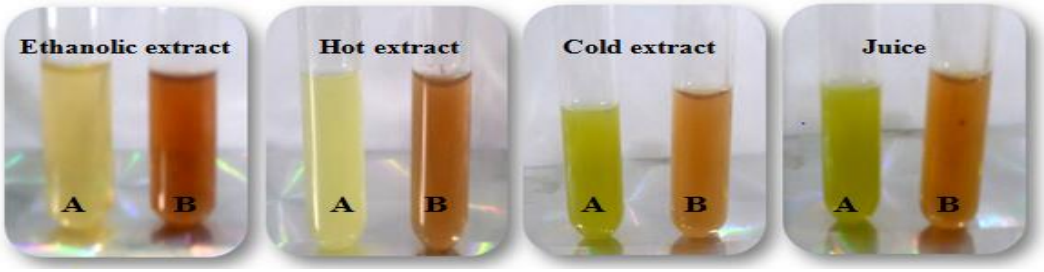

Figure (1): Color Change of Samples Indicates the Formation of Silver Nanoparticles
A: *Actinidia deliciosa* Extracts and Juice
B: Brown Color Indicates the Formation of Silver Nanoparticles

The time duration of change in color differs fromextracts to juicedue to the excitation of free electrons, which gives surface plasmon resonance absorption band due to the combined vibration of electrons of AgNPs in resonance with light wave [15]. Silver colloids show various colors due to light absorption and scattering in the visible area based on plasmon resonance. The resonance wavelength depends on particle size and shape [16].

UV-Visible Spectral Analysis

Silver nanoparticles showed important optical properties directly associated with localized surface plasmon resonance which is strongly dependent on the morphology of the nanoparticles. Reduction of Ag^{+} ions through exposure to the extracts and juice of *Actinidia deliciosa* fruit was easily followed by UV-Vis spectroscopy. Maximum Absorbance peak at 440, 435, 425 and 420 nm for ethanolic, hot, cold extracts and juice respectively indicated silver nanoparticles were formed (Figure 2). Expanding of peak indicated nanoparticles are poly dispersed [17].

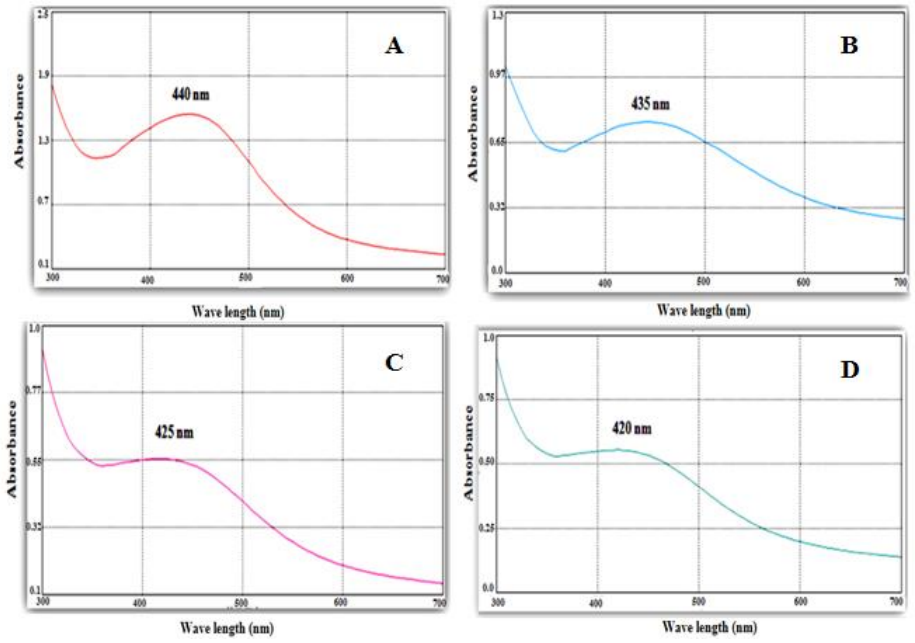

Figure (2): UV-Vis Absorption Spectrum of Silver Nanoparticles for Extracts and Juice
Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

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Effect of pH

pH plays an important role in the nanoparticles synthesis. The effect of pH on synthesis of silver nanoparticles was tested under a different pH (4, 6, 8, 10, 12) as shown in Figure (3).

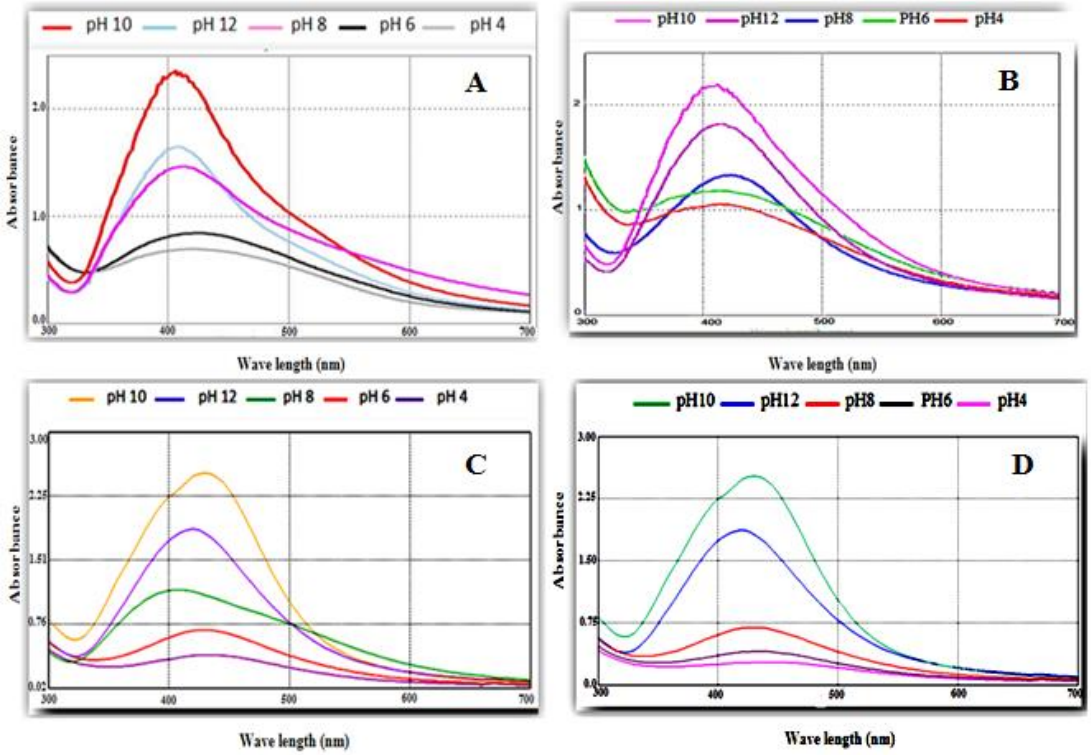

Figure (3): UV-Vis Absorption Spectrum of Silver Nanoparticles at Different pH for Extracts and Juice
Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

When increasing the pH of the reaction mixture an increase in absorbance was observed. It seems that pH affects on the amount of nanoparticles production and stability of them. The reactionmixture turned brown when silver was reduced and the reaction mixture coloring accelerated when increasing Ph [18]. Figure (4) show the change in color with change of pH for extracts and juice.

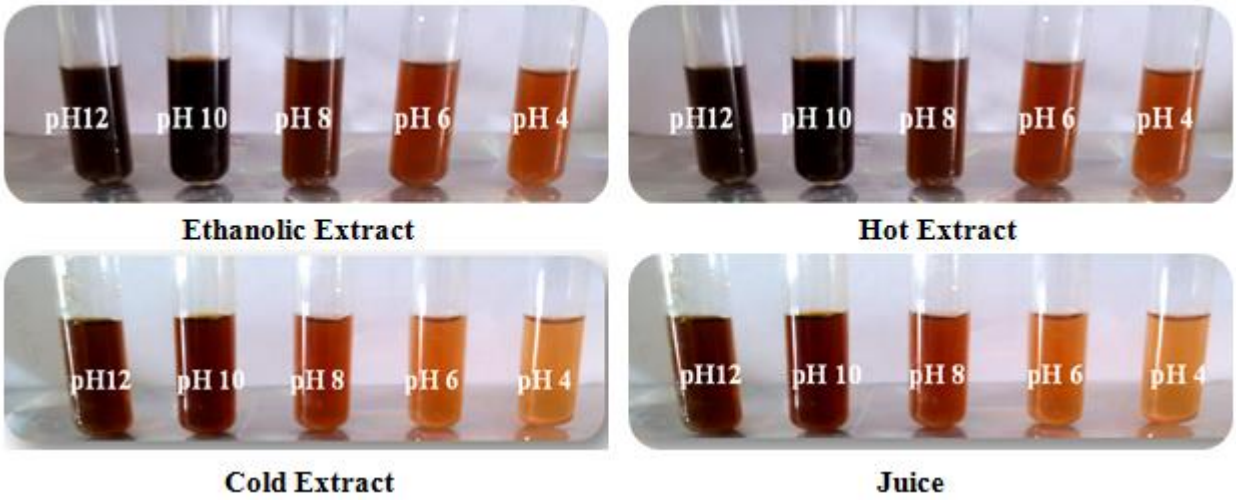

Figure (4): Change the Color with Change pH for Extracts and Juice

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The maximum absorbance was found at pH (10) for all extracts and juice. There is no noticeable difference between all extracts and juice. These results are in a good agreement with the previous study. For instance, Daniel *et al*., show the maximum absorbance was found at pH 10. This may be due to the presence of tannic acid in the leaf extract of the Henna. Tannic acid contains two units, glucose and gallic acid. Under alkaline conditions gallic acid reduces silver nitrate into silver nanoparticles at room temperature, which may be the reason for higher absorbance of silver nanoparticles observed at alkaline pH. While At lower pH nanoparticles shows lower and broader absorbance [19].

Effect of Temperature

Temperature is another physical factor which plays a significant role to monitoring the nucleation process of nanoparticles formation. As shown in Figure (5) the absorbance increase by increasing the temperature and then decreasing at higher temperatures [14]. The maximum absorbance was observed at temperature 80 °C for ethanolic extract, but hot extract at 85 °C. There is a noticeable difference between ethanolic and hot extracts. While the cold extract and juice have maximum absorbance was observed at temperature 55°C. There is no noticeable difference between them.

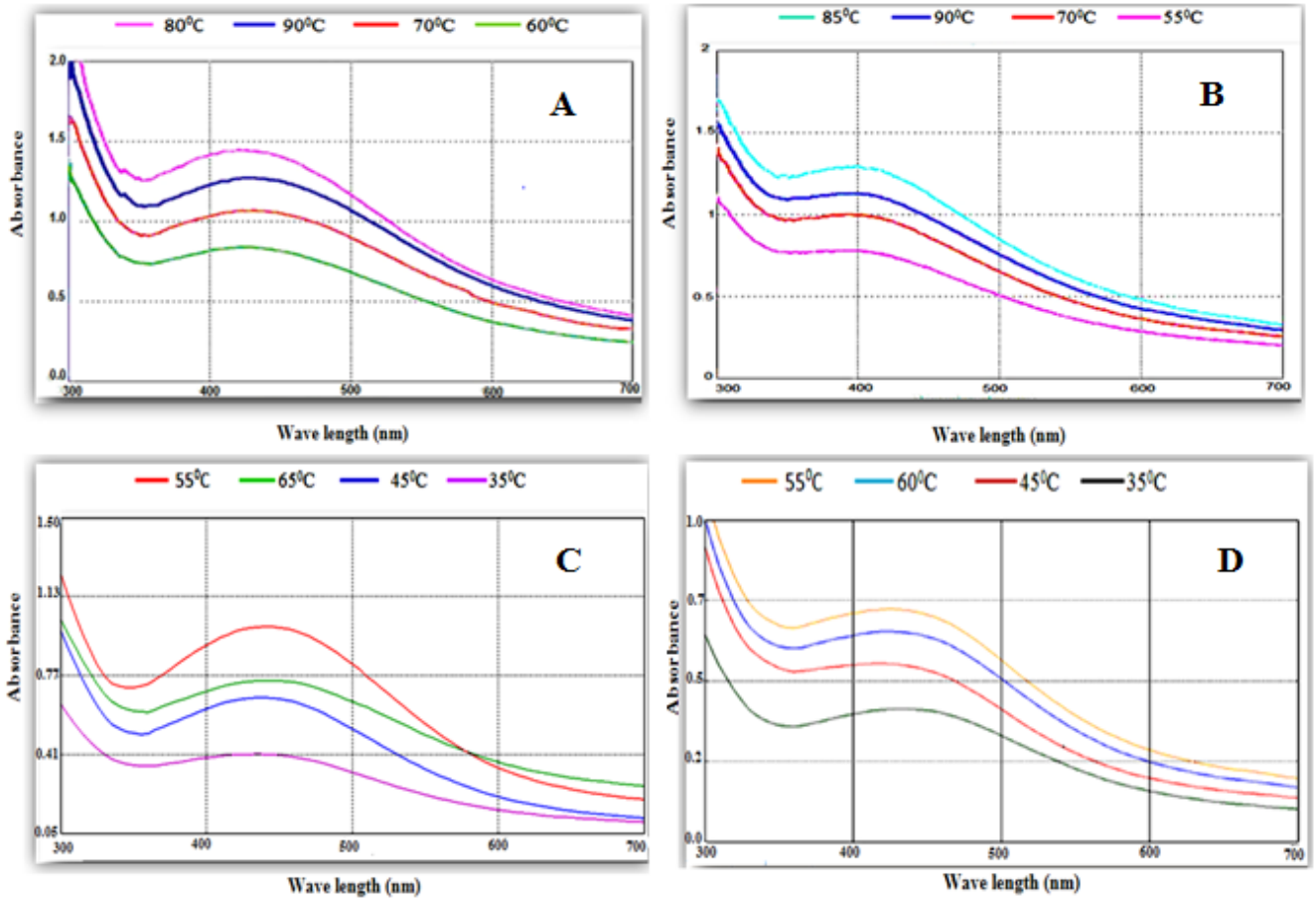

Figure (5): UV-Vis Absorption Spectrum of Silver Nanoparticles at Different Temperature for Extracts and Juice

Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

These results are in a good agreement with the previous studies, like Mittal *et al*., who reported the absorbance increased with increasing of temperature from 25 to 45 °C and thereafter decreased at higher temperatures. Moreover, nanoparticles synthesized at higher temperature offer surface plasmon resonance at narrow absorption range [20].The other study showed that the efficiency of silver nanoparticles synthesis from Oak fruit was highest at 45 °C [21].

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Effect of the Ratio of *Actinidia deliciosa* Extracts and Juice

The effect of extracts and juice concentration in the mixed solution on the biosynthesis of silver nanoparticles was investigated. The difference in concentration of extracts and juice in the mixed solution was obtained by changing the volume of the added extracts and juice solution different volumes (5-15 mL) of extracts and juice were added to 100 mL of 3 mM silver nitrate. The results are shown in Figure (6) and observed the absorbance increased with increase the ratio of extracts and juice.

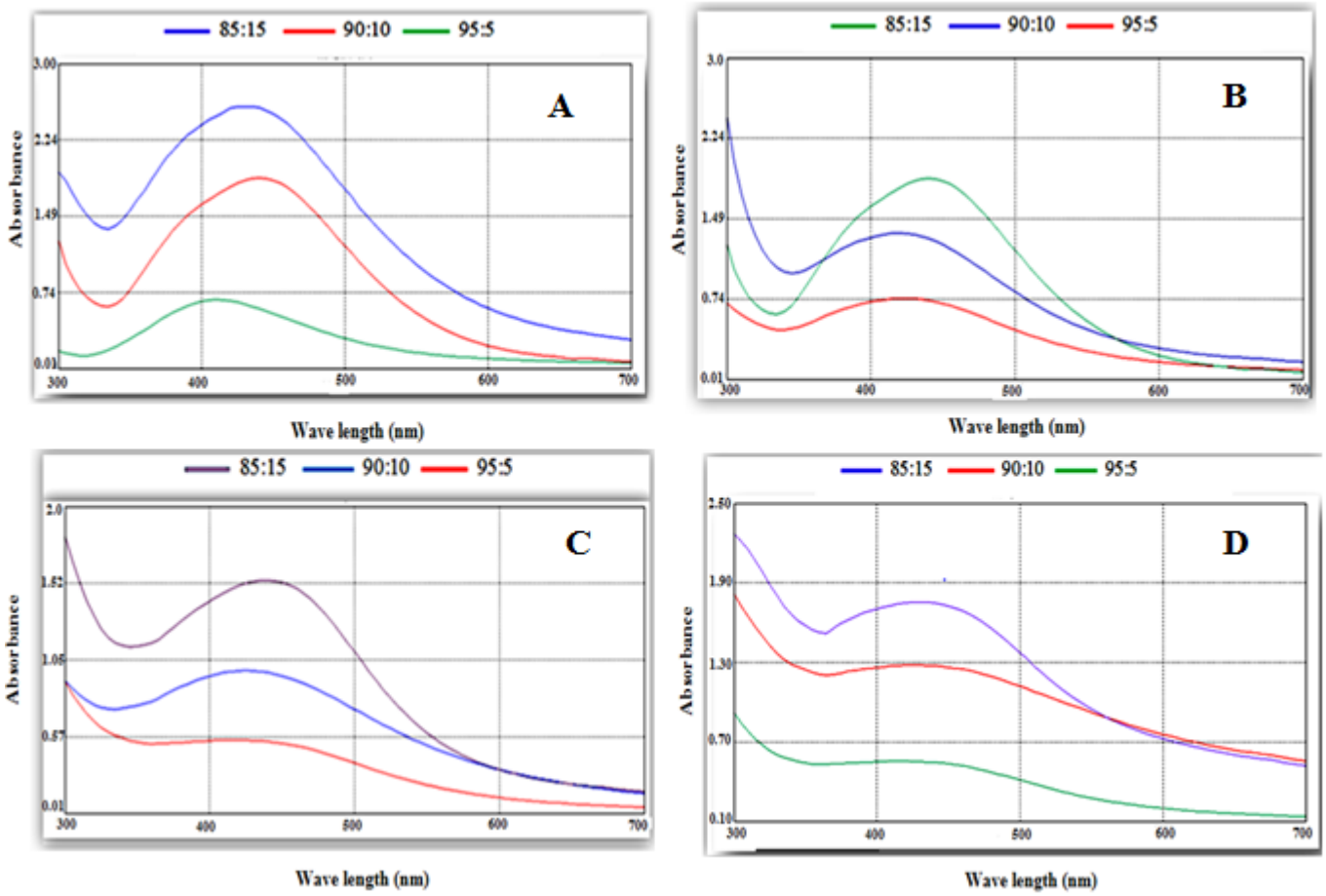

Figure (6): UV-Vis Absorption Spectrum of Silver Nanoparticles at Different Ratios of Extracts and Juice for Extracts and Juice

Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

These results were agreeing with the previous studies such as Saware *et al*., they showed when the increasing ratio of leaf extract of *Ficus benghalensis* increased the intensity of the surface plasmon resonance band. They were observed that with higher volumes of leaf extract the particles were not stable and agglomeration was observed. The peak is broader at lower amounts of leaf extract and sharp peak were observed with increase the ratio of extracts [22].

Another study of Meva *et al*., described the synthesis of silver nanoparticles using seed kernels of *Ricinodendron heudelotii*(Baill) Pierre Pax and show when increase the ratio of extracts increased the absorbance [23].

Effect of Silver Ion Concentration

The UV–Vis spectrum shows the effect of silver nitrate concentration in the silver nanoparticles synthesis by using *Actinidia deliciosa* extracts and juice. Characteristic surface plasmon absorption band was observed for the brown colored silver nanoparticles synthesized from the 3 mM silver nitrate. The absorbance

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was increased while increasing the concentration of silver ions from 1 mM to 3 mM but by increasing the concentration of silver ion there was a fall in absorbance. In the present study 3 mM concentration has the optimum condition for AgNPs as shown in Figure (7).

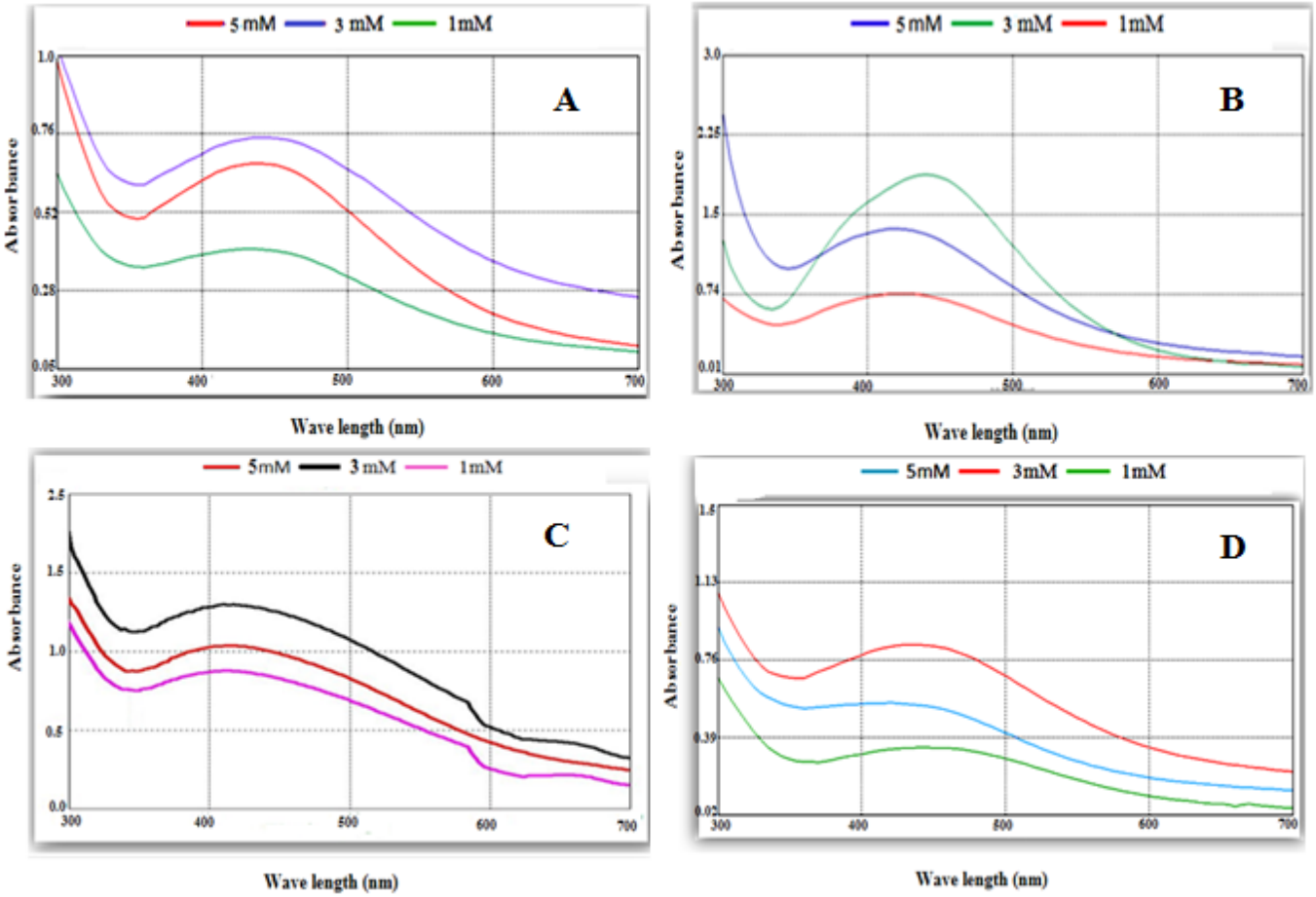

Figure (7): UV-Vis Absorption Spectrum of Silver Nanoparticles at Different Concentration of $AgNO_3$for Extracts and Juice

Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

While Vanaja *et al.,* had been reported that the optimum silver nitrate concentration 1 mM is suitable for nanoparticles synthesis. Similarly, increasing intensity indicates increasing concentration of nanoparticles, higher concentration of silver nitrate suggests the formation of larger nanoparticles [24].

Effect the Time of Storage

Time is another factor which effects on the formation of silver nanoparticles. Figure (8) shows the increasing of absorbance when increased the time of storage and the intensity of color increased also. The intensity of the surface plasmon resonance peak increased as the time increased which indicated increasing formation of the silver nanoparticles. This result means that the silver nanoparticles prepared by this green synthesis method are very stable without aggregation.

In the present study the optimum duration of ethanolic extract was 2 months, then the absorbance decreases, but the optimum duration of hot extract was 1.5 months. While the optimum duration of cold extract was 12 days, but the optimum duration of juice was 7 days.

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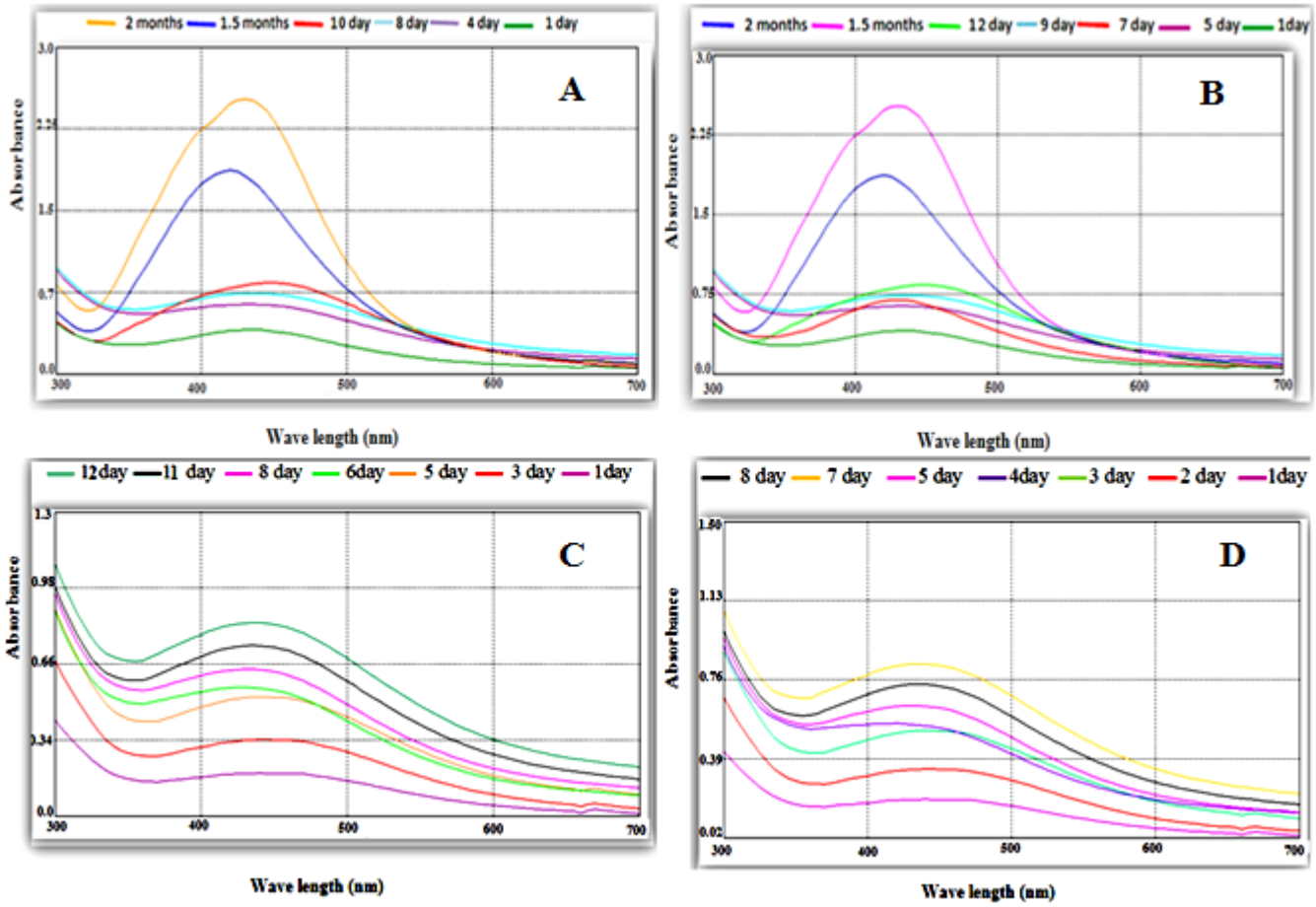

Figure (8): UV-Vis Absorption Spectrum of Silver Nanoparticles at Different Times for Extracts and Juice
Whereas A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

The study of Kumar *et al.,* indicates the reduction of silver ions by using *premna herbacea* Leaf extract and time required for the color change was noted, which remain stable for more 3 months without any changes in the absorption spectrum [25].

Another study of Alzahrani and Welham found that the optimum incubation time for biosynthesis of silver nanoparticles using *watermelon* was 120 min. It was found when the incubation time was less than 15 min there was no formation of AgNPs because the redox potential of the silver nitrate is reduced. Moreover, it was observed that increasing of time lead to increasing of absorption peak and more AgNPs was formed [26].

X- Ray Diffraction Studies (XRD)

The biosynthesized silver nanoparticles were confirmed by the characteristic peaks that were observed in the XRD Figure (9). We observed peaks which indicate the presence of crystalline materials. The XRD pattern was ranging from 20 to 80 at 2θ. The XRD patterns of Ag/extract indicate that the structure of silver nanoparticles is face-centered cubic (fcc) [27].

The XRD peaks were observed at 32.2°, 38.17°, 44.47°, 46.22°, 64.44° and 77.34° were corresponds to the planes 54.36, 111, 200, 100, 220 and 311 for ethanolic extract, but hot extract were observed at 32.21°, 38.71°, 44.47°, 64.44° and 77.34° were corresponds to the planes 54.36 , 111, 200, 220 and 311 which are indexed to the face centered cubic structures of silver nanoparticles.

While the XRD peaks for cold extract were observed at 38.2°, 44.4° and 77.3° were corresponds to the planes 111, 200 and 311 but the XRD peaks for juice were observed at 38.2°, 44.4°, 64.5° and 77.3° were corresponds to the planes 111, 200, 220 and 311 which are indexed to the face centered cubic

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structures of silver nanoparticles. The XRD pattern of these peaks indicates the silver nanoparticles is crystalline in nature and some of the unassigned peaks were observed, it may be due to the fewer bio-molecules of stabilizing agents are enzymes or proteins in the plant extract [28].

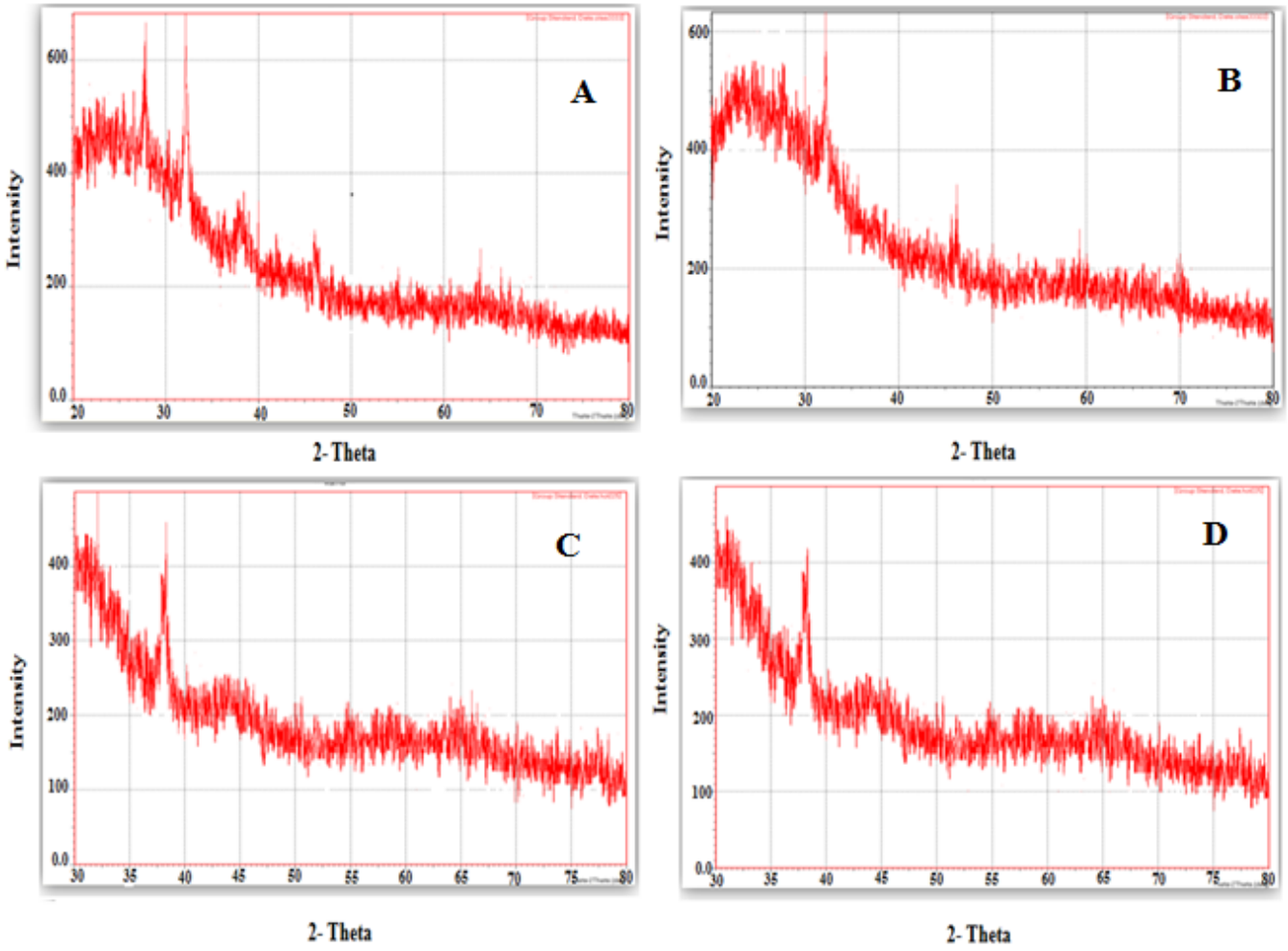

Figure: (9) XRD Patterns of Silver Nanoparticles for Extracts and Juice
Where as:- A: Ethanolic Extract B: Hot Extract C: Cold Extract D: Juice

The present study is compatible with Ahmad and Sharma, who had studied the synthesis of silver nanoparticles (AgNPs) using extracts of *Ananas comosus*. The XRD patterns showed the characteristic Bragg peaks of (111), (200), (220) and (311) sides of the face center cubic (fcc) silver nanoparticles [29].

Scanning Electron Microscopy Analysis (SEM)

The SEM images of the AgNPs are shown in Figure (10) is seen that AgNPs have different shapes were obtained from extracts and juice of *Actinidia deliciosa*being used as reducing and capping agents. The results showed the size of particles for ethanolic extract is 76 nm but the size particles for hot extract is 78 nm while the size particles for cold extract is 83 nm and the size particles for juice is 85 nm.

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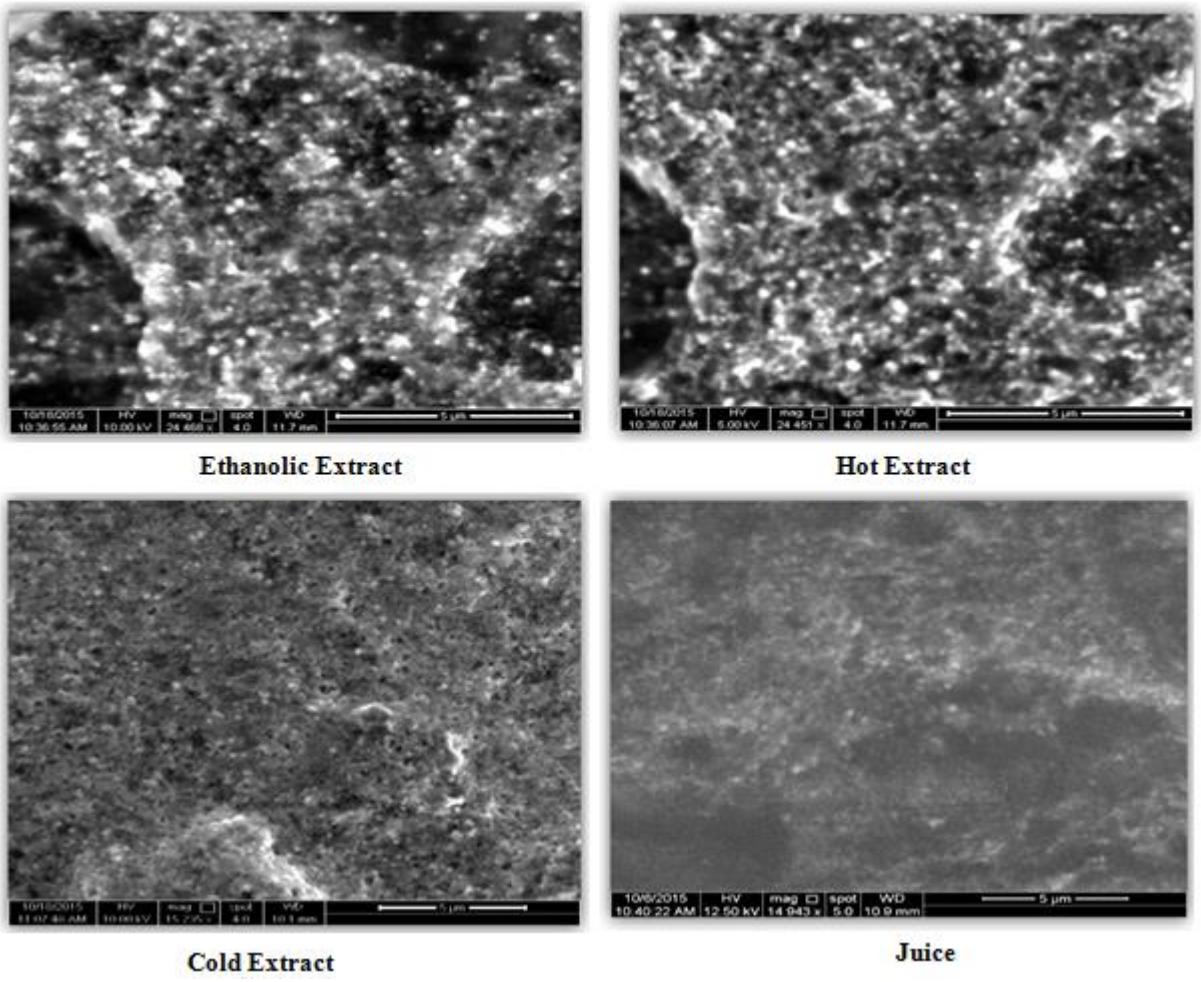

Figure (10): SEM Analysis of Silver Nanoparticles for Extracts and Juice

The study of Banerjee *et al*., showed the SEM image seen that AgNPs different shapes were obtained in case of different leaf extracts being used as reducing and capping agents. Banana, neem and tulsi extracts formed approximately spherical, triangular and cuboidal AgNPs, respectively. This may due to availability of different quantity and nature of capping agents present in the different leaf extracts [30].

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