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Green Synthesis of Silver Nanoparticles Using Mixture of Gnetum Africanum and Heinsia Crinita Leaf Extracts

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Abstract: *Silver nanoparticles are known for their high sensitivity, antimicrobial activities, catalysis and microelectronics applications. These applications have made silver nanoparticles synthesis very important research area. Silver nanoparticles can be synthesized through chemical, physical and biological methods. The biological technique also referred to as the green method, is the most economical, easy, ecofriendly and less time consuming approach in synthesizing the nanoparticles. This work looks at the green synthesis of silver nanoparticles using mixture of Gnetum Africanum (okazi) and Heinsia Crinita (atama) leaves extract as reducing agent for silver nitrate solution. The synthesized silver nanoparticles were characterized using Scanning Electron Microscopy (SEM) and X-ray Diffractometry (XRD). The experimental result showed that volume ratio of 2:1 of Gnetum Africanum to Heinsia Crinita leave extracts reacting with 0.01M silver nitrate ($AgNO_3$) formed less aggregated nanocrystalline silver nanoparticles with standard peaks corresponding to the miller indices of 110, 122, 111, 200, 231, 142, 241, 220 and 311 at 2θ values of 27.98°, 32.42°, 38.16°, 44.44°, 46.40°, 54.82°, 57.52°, 64.38° and 77.08° with face centered cubic structure and average nanoparticle size of 8 nm.. The SEM image shows tiny white silver nanoparticles on the slide, indicating the formation of silver nanoparticles. Silver nanoparticles synthesized using only Gnetum Africanum leaves extract had face centered cubic structure with slight shift in 2θ values for miller indices 200, 142 and 311 from XRD analysis. This might be due to bio-organic or metallo-proteins present in the supernatant that also aid stabilization of the nanoparticles. The average nanoparticles size for silver nanoparticles synthesized using Gnetum Africanum leaves extract is 9 nm. The SEM images showed tiny scattered white particles confirming the formation of silver nanoparticles using Gnetum Africanum with less aggregation. Heinsia Crinita leaf extract on the other hand gave similar peaks with Gnetum Africanum extracts with average nanoparticles size of 6 nm. The SEM image showed that the particles aggregated due to poor stabilizing effect of Heinsia Crinita leaf extract. Similar result was obtained for volume ratio of 2:1 Heinsia Crinita to Gnetum Africanum leaf extracts used in synthesizing silver nanoparticles. The results showed that volume ratio of 2:1 of Gnetum Africanum to Heinsia Crinita leaves extract is best for the synthesis of silver nanoparticles when mixture of Gnetum Africanum and Heinsia Crinita leaves are used. The result also showed that Heinsia Crinita leaves is a poor reducing agent for the formation of silver nanoparticles with silver nitrate salt..*

Keywords— Silver nanoparticles, Green synthesis, Gnetum Africanum, Heinsia Crinita, SEM, XRD

1. Introduction

Metal nanoparticles such as nanocystalline silver particles have wide range of applications in the field of high sensitivity bimolecular detection, diagnostics, therapeutics, catalysts and micro-electronics. Silver nanoparticles are also known as an effective antimicrobial agents and are effective against fungi, bacteria's, viruses and also exhibit low toxicity in in vitro and in vivo applications [1-6]

Silver nanoparticles can be synthesized through different ways and routes. These routes can be divided into three different broad ways which includes physical vapor deposition, ion implantation and wet chemistry. The most common method for nanoparticles synthesis falls under the category of wet chemistry or the nucleation of particles within a solution. This nucleation occurs when a silver ion complex usually silver nitrate ($AgNO_3$) or silver perchlorate ($AgCLO_4$) is reduced to colloidal silver nanoparticles in the presence of a reducing agent.

The use of chemical such as $AgNO_3$ or $AgCLO_4$ which are not economical and environmental friendly has made researches to look for an economically viable and environmentally clean synthesis route to synthesize silver nanoparticles. One method of doing this is through the green synthesis of silver nanoparticles where plant extracts are used as the reducing agent on the silver salts. The plant extract contains phytochemicals which reduce the silver ions in silver salt to silver nanoparticles. Phytochemicals such as flavonoids, terpenoids, polyphenols and alkaloids are known as reducing agent for silver salt and also plants constituents such as carbohydrate, enzymes and fats are known to reduce silver to nanoparticles [7]. Various plant extracts have been used to synthesize silver nanoparticles with varying size and shapes [8-15].

Plants such as Gnetum Africanum also known as Okazi in the Southern part of Nigeria and Heinsia Crinita (atama) leaves extract can be used as reducing Agent. The leaves of these plants contain phytochemicals that can act as reducing agent in reducing silver ions to nanoparticles.

Gnetum Africanum is a vine gymnosperm species (wild spinach) found natively throughout tropical Africa. It is a perennial plant that grows approximately 10 m long, with thick papery-like leaves growing in groups of three. Its leaves and tree and found to be medicinal. Research shows that the phyto-chemicals found in the raw leaves contains 1.37% tannin, 1.86 % alkaloid, 1.04 % flavonoid, 3.82% cyanogenic glycosides, 1.09% saponin and 0.05% sterols and there is no significant difference in the phytochemicals of the raw leaves compared to the cooked leaves [16].

Heinsia Crinita also known as bush apple is a shrub with bisexual flowers and leafy calyx lobes that produces edible fruits. It is often found in the southern part of Nigeria and its leaves are used in cooking delicacy called atama soup. The leaves have also been used in treating diseases such as bacterial infections, diabetes, hypertension and infertility [17-18]. Research has shown that the leaves obtain from aqueous extract of Heinsia Crinita qualitatively contains saponins, tannins and alkaloids in high concentrations while flavonoids are in medium concentration [19].

Green synthesis of silver nanoparticles can also be carried out through bio-reduction processes. The process involves reduction of metal ions to nanoparticles through the combinations of bio-molecules found in the extracts of organisms such as enzymes/proteins, amino acids, polysaccharides, and vitamins. Studies have reported successful synthesis of silver nanoparticle using organisms [20-24]. This research paper looks at the green synthesis of silver nanoparticles using mixture of Gnetum Africanum and Heinsia Crinita leaves extracts abundant in Southern part of Nigeria.

2. MATERIALS AND METHOD

2.1 Materials and Equipment

Silver Nitrate ($AgNO_3$), Fresh Gnetum Africanum and Heinsia Crinita leaf were bought from the local market at Igbogene, Yenagoa Bayelsa State, Magnetic stirrer and hot plate, Beakers (500 ml), volumetric flasks (100 ml). SEM, X-ray diffractometer (PANalytical X'pert Pro MPD).

2.2 Method

2.2.1 Preparation of $AgNO_3$ Solution

A stock solution of 0.1 M silver nitrate was prepared by measuring 16 grams of silver nitrate salt and place in a 100 ml volumetric flask with little amount of de-ionized water and the mixture stirred until the powder was thoroughly dissolved, then the volumetric flask was filled with de-ionized to the 100 ml mark. Then 0.01M silver nitrate solution was prepared by diluting 10 ml of 0.1 M stock solution in 1000 ml of de-ionized water.

The Silver nitrate solution was prepared in a dark room because of the sensitivity of silver nitrate to light and the volumetric flask wrapped with foil paper to avoid colour change in the solution. Figure 1 showed the prepared silver nitrate stock solution wrapped with foil.

Figure 1 Silver Nitrate stock Solution

2.2.2 Preparation of Plant Extract

Gnetum Africanum leaves were washed several times with running water and then with distilled water to remove dirt and dust particles. The leaves were then sliced into pieces and put into a beaker. 20 grams of the leaves was then heated with 100 ml of de-ionized water at a temperature of 60^0C for 15 mins and then filtered with No.1 Whatman filter paper to obtain the extract. The extract was then kept in a cool place at 4^oC for later use. Figure 2 shows (a) Gnetum Africanum leaves, (b) the heating process of the leaves and (c) the leaves extract

Figure 2 The Extraction process of Gnetum Africanum leaves extract.

The preparation of Heinsia Crinita leaves extract followed the same procedure as that of Gnetum Africanum leaves extract. Figure 3 shows the fresh Heinsia Crinita leaves in (a), the heating process (b) and the extract from the leaves (c)

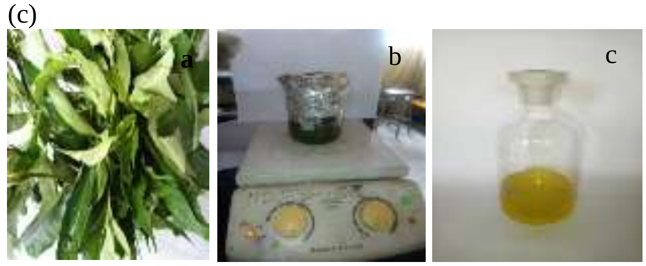

Figure 3 The Extraction process for Heinsia Crinita leaves

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2.2.3 Synthesis of Silver Nanoparticles

Sample A ($AgNO_3$ solution mixed with Gnetum Africanum extract)

10 ml of the Gnetum Africanum extracts was added to 10 ml of 0.01M $AgNO_3$ solution and then stirred with a magnetic stirrer for 5 mins until it forms colloids. The yellowish brown colour change shows the formation of silver nanoparticles.

Sample B ($AgNO_3$ solution mixed with Heinsia Crinita extract)

10 ml of the extract was added to 10 ml of 0.01M $AgNO_3$ solution and then stirred with a magnetic stirrer for 5 mins until it forms colloids. The light yellow colour change observed shows the formation of silver nanoparticles.

Sample C ($AgNO_3$ solution mixed with 2:1 ratio Heinsia Crinita to Gnetum Africanum extracts)

Volume ratio of 2:1 of the leaves extracts were measured and reacted with silver nitrate solution. 10 ml of Heinsia Crinita was mixed with 5 ml of Gnetum Africanum with 10 ml of the 0.01M solution and stirred for 5 mins and the pale yellow show the formation of silver nanoparticles.

Sample D ($AgNO_3$ solution mixed with 2:1 ratio Gnetum Africanum to Heinsia Crinita extracts)

Volume ratio of 2:1 was also measured but in this case, 10 ml of Gnetum Africanum mixed with 5 ml of Heinsia Crinita was mixed with 10 ml of 0.01M $AgNO_3$ solution and stirred for 5 mins and the pale brown colour formed shows the formation of silver nanoparticles.

3. RESULT AND DISCUSSION

Figure 4 shows the colour change in the synthesized silver nanoparticles for sample A. In this figure, (a) is the silver nitrate solution, (b) is the Gnetum Africanum extract and (c) is the silver nanoparticles formed as a result of the Gnetum Africanum extract acting as a reducing agent on the silver nitrate.

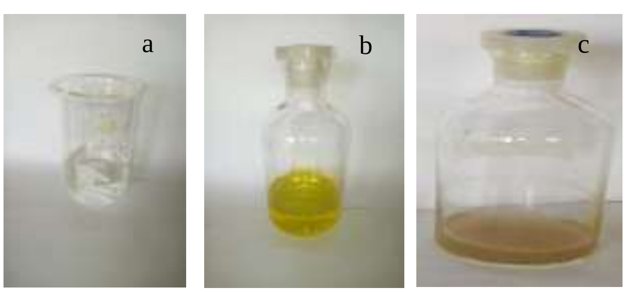

Figure 4 synthesized silver nanoparticles using Gnetum Africanum leaf extract in sample A

Figure 5 shows the synthesized silver nanoparticles using the Heinsia Crinita leave extract as described in sample B. In the figure below, (d) is the silver nitrate solution, (e) is the Heinsia Crinita leave extract solution and (f) is the synthesized silver nanoparticles solution as shown by the colour change.

Figure 5 synthesized silver nanoparticles using Heinsia Crinita leave extracts in sample B

Figure 6 shows the synthesized silver nanoparticles using mixing ratio 2:1 of Heinsia Crinita extract (10 ml) aganst 5 ml of Gnetum Africanum as explained in sample C. In the figure, (g) Gnetum Africanum leave extract, (h) Heinsia Crinita leave extract and (i) synthesized silver nanoparticles indicated by the slight colour change.

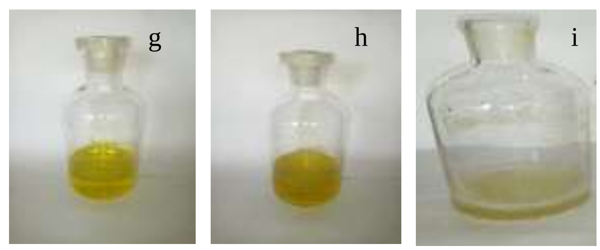

Figure 6 synthesized silver nanoparticles using different volume of the extracts in sample C

Figure 7 shows the synthesized silver nanoparticles by using mixing ratio 2:1 of Gnetum Africanum (10 ml) aganst 5 ml of Heinsia Crinita extract as explained in sample D. In figure 7, (j) Gnetum Africanum leaves extract, (k) Heinsia Crinita leaves extract and (l) synthesized silver nanoparticles.

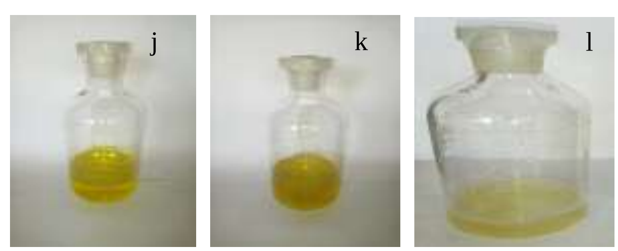

Figure 7 synthesized silver nanoparticles using varying volume of both extract in sample D

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Figure 8 and 9 shows the SEM and diffractometer images of Gnetum Africanum extract synthesized silver nanoparticles as shown by the colour changes in Figure 4 respectively

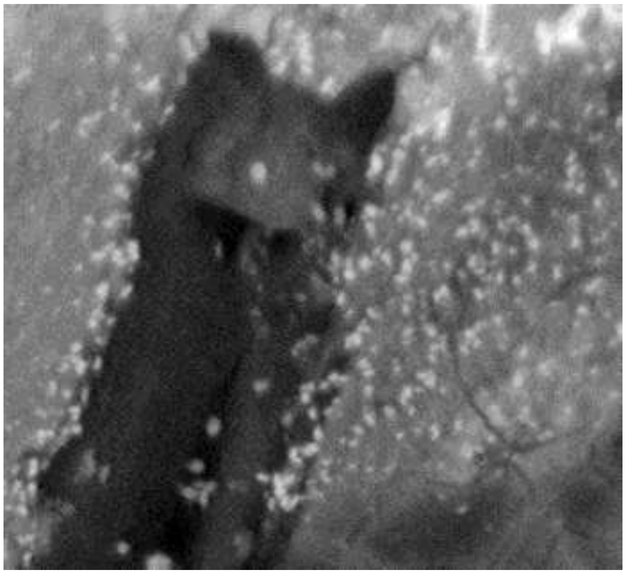

Figure 8 SEM images of silver nanoparticles using Gnetum Africanum.

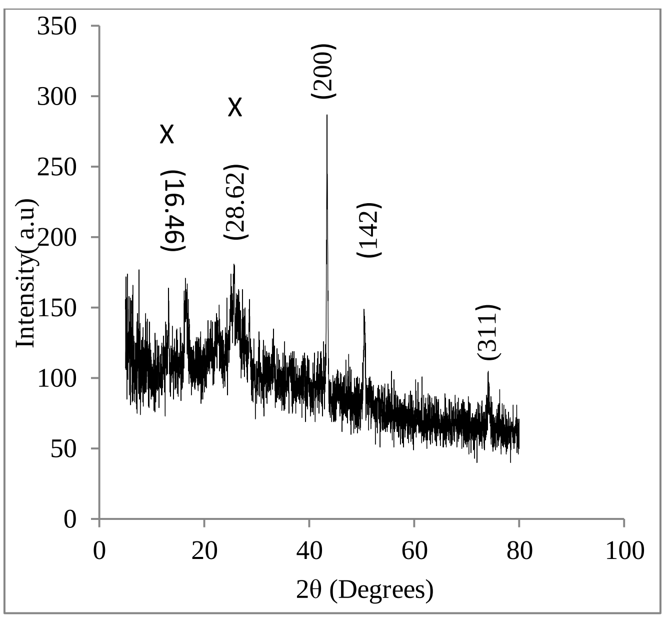

Figure 9 XRD analysis of silver nanoparticles synthesized using Gnetum Africanum extract

The SEM micrograph in Figure 8 shows silver nanoparticles as indicated by the presence of white particles which are seen on the SEM slide indicating the formation of silver nanoparticles. The nanoparticles were seen scattered around the slide indicating the stabilization of the nanoparticles with the leaves extract. This result is corroborated by the XRD result in Figure 9.

Figure 9 shows three distinct diffraction peaks at 2θ values of 43.40^0, 51.17^0 and 74.18^0 which have been identified to be due to silver nanoparticles and corresponding to the Miller indices (hkl) values of (200), (142) and (311) planes of silver. The diffractogram in Figure 9 has been compared with the standard powder diffraction card of the Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04-0783. The XRD study has thus confirmed that the resultant particles in the prepared sample are silver nanoparticles having face centered cubic crystal structure. Although there are slight shifts from the standards, this might be due to the composition of the leaf extract which is acting as a reducing agent in the silver nitrate solution. The two extra peaks in the diffractogram at 16.46^0 and 28.62^0 degrees marked X, might be as a result of the un-reacted silver nitrate solution and hence remained in the sample in minute quantity or it might be due to bioorganic or metallo-proteins present in the supernatant that are responsible for the stabilization of the nanoparticles as reported by Baker syed et al, 2019 [25]. The average crystalline size of the silver nanoparticles was estimated using Debye-Scherer formula as shown in equation 1.

$$D = \frac{0.9\lambda}{\beta cos\theta} \qquad 1$$

Where D is the crystalline size, λ is the wavelength of the X-rays used for diffraction and β is full width at half maximum (FWHM) of a peak. The average size of the silver nanoparticles was calculated to be 9 nm.

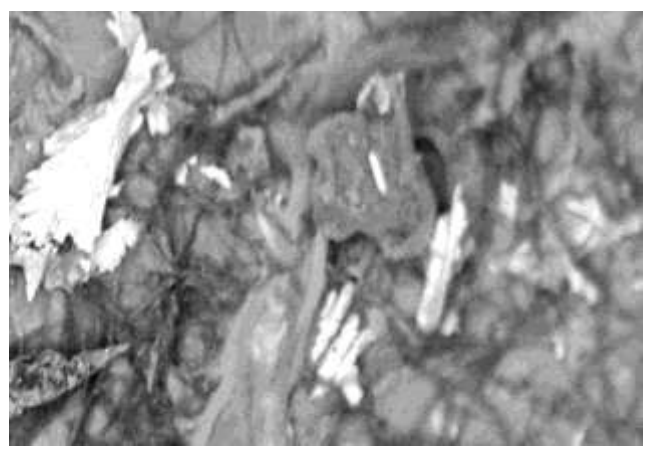

Figure 10 SEM image of synthesized silver nanoparticles using Heinsia Crinita leaves Extract.

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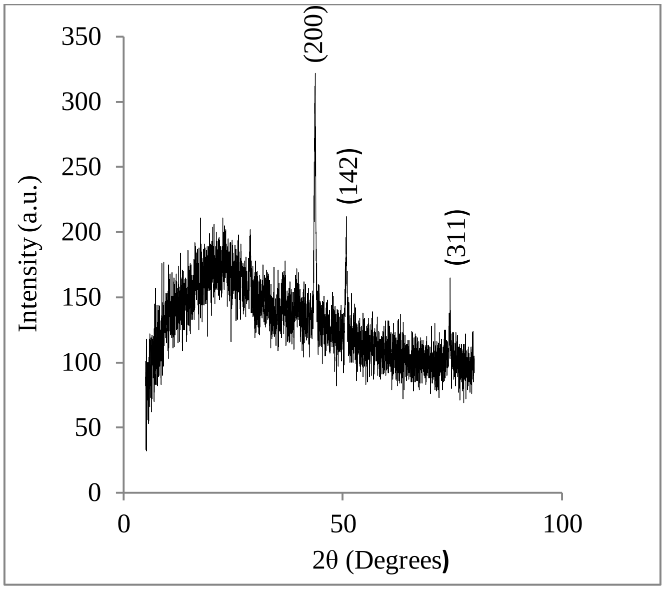

Figure 11 XRD analyses of silver nanoparticles synthesized using Heinsia Crinita leaves Extract.

Figures 10 and 11 above shows the SEM image and XRD analysis of silver nanoparticles synthesized using Heinsia Crinita leaves extract. Figure 10 showed that the nanoparticles synthesized are clustered together. This might be due to the inability of Heinsia Crinita leaves extract to act as a good stabilizing agent after reduction or it might be due to long duration it took to carry out the analysis after synthesis. Figure 11 shows the XRD analysis of three distinct diffraction peaks at 2θ values of 44.01^0, 51.08^0 and 74.42^0 which can be indexed to the (200), (142) and (311) planes of silver. The diffractogram has been compared with the standard powder diffraction card of JCPDS, silver file No. 04-0783. The XRD study thus confirmed that the resultant particles in the prepared sample are silver nanoparticles having face centered cubic crystal structure. The slight shifts from the standards might be due to the composition of the leaf extract which is acting as a reducing agent in the silver nitrate solution. The average crystalline size of the silver nanoparticles after rigorous agitation using was found to be 6 nm.

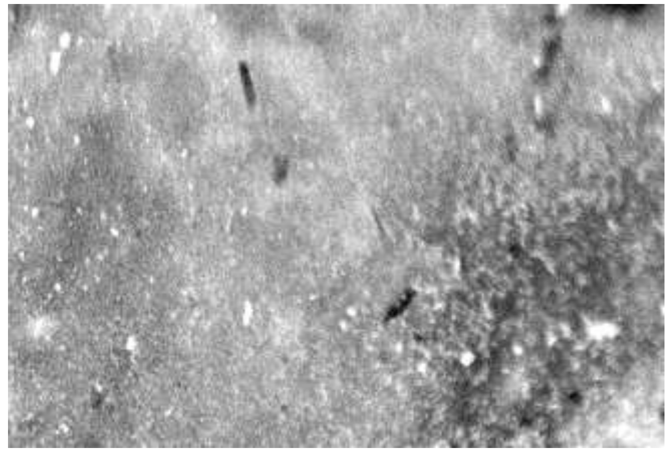

Figure 12 SEM image of synthesized nanoparticles using 10 ml Gnetum Africanum and 5 ml Heinsia Crinita extracts.

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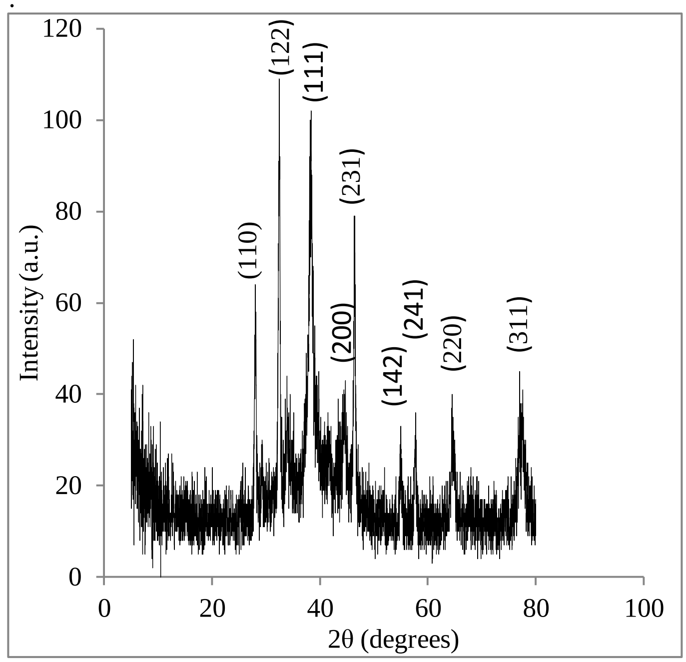

Figure 13 XRD image of synthesized nanoparticles using 10 ml Gnetum Africanum and 5 ml Heinsia Crinita extracts.

Figure 12 shows the SEM image of the synthesized silver nanoparticles using 10 ml Gnetum Africanum and 5ml Heinsia Crinita extracts as prepared in sample D and shown in Figure 7. The particles can be seen scattered around the SEM slide which indicates minimal aggregation of particles. This means that the particles were stable. This result is corroborated with the XRD pattern shown in Figure 13.The XRD pattern showed numbers of Bragg reflections that may be indexed on the basis of the face centered cubic structure of silver. A comparison of the XRD spectrum with the standard from JCPDS File No. 84-0713, confirmed that the silver particles formed were in the form of nano-crystals, as evidenced by the peaks at 2θ values of 27.98°, 32.42°,

38.16°, 44.44°, 46.40°, 54.82°, 57.52°, 64.38° and 77.08°assigned to the (110), (122), (111), (200), (231), (142), (241), (220) and (311) planes of a faced center cubic (FCC) lattice of silver nanoparticles respectively. The average size of the nanoparticles was estimated to be 8nm.
Silver nanoparticles synthesized in sample C (10 ml Heinsia Crinita mixed with 5 ml Gnetum Africanum), followed similar pattern as in sample B (10 ml Heinsia Crinita mixed with silver nitrate). It showed similar aggregation and clustering and similar XRD analysis. Though not included, it indicated that Heinsia Crinita extract are not good reducing agents for the formation of silver nanoparticles compared to Gnetum Africanum extract. It might also indicates that Heinsia Crinita extract is a poor stabilizing agent and contains less of the phytochemicals required to reduce silver salt to silver nanoparticles.

4. CONCLUSION

From the experimental results, it can be confirmed that silver nanoparticles was formed using Gnetum Africanum leaves extract and Heinsia Crinita leaves extract with silver nitrate solution. The formation of silver nanoparticles using 2:1 ratio of Gnetum Africanum (10 ml) mixed with Heinsia Crinita extract (5 ml) gave the best and standard silver nanoparticles peaks from XRD analysis indicating its nano-crystalline form of silver nanoparticles with average particle size of 8 nm.. The silver nanoparticles synthesized using only Heinsia Crinita extracts showed aggregated nanoparticles and non-standard diffractometry peaks similar to nanoparticles synthesized with Heinsia Crinita extracts in ratio of 2:1 with Gnetum Africanum extracts. The average nanoparticles size sythesized using Heinsia Crinita extract is 6 nm. Silver nanoparticles formed using 10 ml Gnetum Africanum extract showed that the nanoparticles were not clustered and have an average size of 9 nm. This also indicates that Gnetum Africanum is a better reducing and stabilizing agent compared to Heinsia Crinita extracts in synthesizing of silver nanoparticles.

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