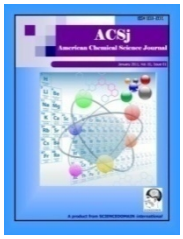

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Synthesis and Characterization of Silver Colloidal Particles of *Ageratum conyzoides L.* Plant Extracts

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Authors' contributions

Author YM performed the statistical analysis and wrote the protocol and first draft. Authors AIO and JTB designed the study and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Colloidal particles with controlled size and composition are of fundamental and technological interest as they provide solution to many technological and environmental challenges. In this study, an environmentally friendly approach was adopted to synthesize silver colloidal particles using aqueous extract of *A. conyzoides* L. plant. The reaction of this plant's extract with a solution of 1mM $AgNO_3$ changed its colour into reddish brown colour due to reduction of silver ions to silver atoms. UV- Visible and FTIR spectroscopy techniques were employed to characterize the colloidal particles. The UV-Visible spectroscopy revealed the formation of silver colloidal particles by exhibiting the typical surface plasmon absorption maximum at 420nm of UV-Visible spectra. The results showed that the size of the silver nanoparticles in the colloidal solution, range from 2-100nm. The FTIR measurements were carried out to identify the possible bio-molecules or functional groups responsible for capping and efficient stabilization of the metal colloidal particles synthesized by the plant extract and the results showed that the synthesized colloidal particles may be surrounded by proteins and metabolites having the functional groups of alcohols, ketones, aldehydes and carboxylic acids. The results signify that *Ageratum conyzoides* is a potential medicinal plant for the synthesis of silver colloidal particles.

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Keywords: Silver; Ageratum conyzoides L; UV-visible spectroscopy; FTIR spectroscopy.

1. INTRODUCTION

Colloidal system is a heterogeneous system in which one substance is dispersed (called dispersed phase) as very fine particles in another substance called dispersion medium. The size of the colloidal particles diameter ranges between 1 to 100nm which is small enough to remain suspended. Therefore, colloid is an intermediate state between suspensions and solutions. Colloids and colloidal systems are essential to life. They function in everybody cell, in the blood, and in all body fluids, especially the intercellular fluids. All life processes take place in a colloidal system, and that is true both of the normal fluids and secretions of the organisms, and of the bacterial toxins, as well as, in large measure, of the reactions, which confer immunity (Available: http://www.xamplified.com/what-is-a-colloid) and also (Available: http://collaidalsilvermaster.com/collaidal%20systems.pdf.)

The most important and distinct property of colloidal particles is that they exhibit larger surface area to volume ratio. The most effectively studied colloidal particles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Metal colloidal particles have tremendous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices. Among the above four, silver colloidal particles play a significant role in the field of biology and medicine [1]. (Available online at http://www.urpjournals.com)

Colloidal particles are increasingly receiving attention as important starting points for the generation of micro and nanostructures [2]. These particles are under active research because they possess interesting physical properties differing considerably from that of the bulk phase. It comes from small sizes and high surface/volume ratio. Manufacturing entire objects from pure silver metal or coating them with silver is prohibitively expensive for consumer items but research has found that impregnating other materials with silver colloidal particles is a practical way to exploit the germ fighting properties of silver [2].

Colloidal particles have a wide range of applications, as in combating microbes [3], biolabelling and in the treatment of cancer [4]. There are various methods for colloidal particles formation such as sol-process, micelle, sol-gel process, chemical precipitation, hydrothermal method, pyrolysis, chemical vapour deposition, bio-based protocols etc [5]. Therefore, there is a growing need to develop environmentally friendly processes for colloidal particle synthesis without using toxic chemicals. Biological methods for metal colloidal particle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods.

Generally, metal colloidal particles can be prepared and stabilized by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used [6]. Studies have shown that the size, morphology, stability and properties (chemical and physical) of the metal colloidal particles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal colloidal particles [6]. Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest [6]. The interaction of Ag in the matrix of the plant extract will satisfy this requirement. This is part of the stimulus for this research work.

Ageratum conyzoides (Asteraceae) commonly called billygoat-weed, chickweed, goatweed, (whiteweed) is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world and also has bioactivity with insecticidal and nematocidal acitivity. This tropical species appears to be a valuable agricultural resource [7]. Hausa people call it 'Gwiwan Jimna', Fali, 'Bavwivwiya' or 'Kureda', Gude 'Kudzen Gwanda' and Fulani, 'Kachkachu'nga' as shown in Fig. 1 (a and b) below.

Fig.1 (a) Leaves and flower of A*. conyzoides L.* **(b) Tropical Whiteweed (*A. conyzoides L.*)**

Ageratum conyzoides is an erect, herbaceous annual, 30 to 80 cm tall; stems are covered with fine white hairs, leaves are opposite, pubescent with long petioles and include glandular trichomes. The inflorescences contain 30 to 50 pink flowers arranged as a corymb and are self-incompatible. The fruit is an achene with an aristate pappus and is easily dispersed by wind. In some countries the species is considered a weed, and control is often difficult [7]. Seeds are positively photoblastic and viability is often lost within 12 months. The optimum germination temperature ranges from 20 to 25ºC [7]. The species has great morphological variation and appears highly adaptable to different ecological conditions.

A. conyzoides is widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. In Central Africa it is used to treat pneumonia, but the most common use is to cure wounds and burns [8]. Traditional Communities in India use this species as a bacteriocide, antidysenteric and antilithic [9], and in Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide [10-11]. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic [12-13]. In Reunion, the whole plant is used as an antidysenteric [14]. The use of this species in traditional medicine is extensive in Brazil. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhoea, rheumatism, spasms, or as a tonic [7]. *A. conyzoides* has quick and effective action in burn wounds and is recommended by Brazilian Drugs Central as an antirheumatic [7].

Ageratum conyzoides has bioactive activity that may have agricultural use, as shown by several research investigations in different countries. Jaccoud [15] reported the use of the leaves as an insect (moth) repellent. The insecticide activity may be the most important biological activity of this species. The terpenic compounds, mainly precocenes, with their

antijuvenile hormonal activity are probably responsible for the insecticide effects. Assays conducted in Colombia by Gonzalez et al. [16] showed activity of this species against *Musca domestica* larvae, using whole plant hexane extract. Vyas and Mulchandani [17] reported the action of cromenes (precocenes I and II), isolated from *Ageratum* plants, which accelerate larval metamorphosis, resulted in juvenile forms or weak and small adults.

In this present study, the reduction of silver ions and formation of silver colloidal particles by the extract of *A. conizoides* plant was investigated via preparation aqueous extract of the plant, synthesis of silver colloidal particles using this extract and characterization of the particles using UV-Visible and FTIR spectroscopic technique.

2. MATERIALS AND METHODS

2.1 Sample Location and Identification

The whole plant material (*Ageratum conyzoides Linn)* was located and collected from the University Staff Quarters, Mubi, of Adamawa State University, Mubi, Nigeria in the month of October, 2011. The plant botanical name was identified and confirmed by Prof. S.S. Sanusi, a plant taxonomist, in the Department of Biological Sciences, University of Maiduguri, Nigeria as reported elsewhere [18].

2.2 Sample Collection and Treatment

The collection of the plant sample was done randomly in the month of October. The sample was deposited in chemistry laboratory, Adamawa State University, Mubi.

The collected sample was freed from twigs and extraneous matter. Soil, grit, sand and dirt were removed by sifting. To remove the remnants of adhering foreign matter, the sample was rapidly and thoroughly washed under tap water and rinsed with distilled water and then shade dried at room temperature for 15 days. The dried plant sample was pulverized to a fine powder using a porcelain pestle and mortar.

3. PREPARATION OF EXTRACT (AQUEOUS EXTRACT)

3.1 Hot Water Extraction

A portion of 5g of the powdered sample (the whole plant) was boiled in 100 cm^3 of distilled water in 250cm^3 conical flask for about 10 minutes and was allowed to stand and cooled. The boiled sample was then filtered using Whatman No.1 filter paper. The extract was stored in refrigerator until use.

4. SYNTHESIS OF SILVER COLLOIDAL PARTICLES

Aqueous solution of silver nitrate (0.001M $AgNO_3$) was prepared by dissolving 0.0425g in 250cm^3 distilled water in 250cm^3 volumetric flask which was used for the synthesis of silver colloidal particles. 10cm^3 of the weed extracts was reacted with 100cm^3 aqueous solution of 0.001M $AgNO_3$ in 250 cm^3 conical flask for the reduction of Ag^+ to Ag and was kept at room temperature for two (2) hours. The time of addition of extract into the aqueous $AgNO_3$ solution was considered as the start of the reaction. The bio reduced silver colloidal particle solution was used for characterisation by UV-Visible spectrophotometer.

5. CHARACTERISATION OF SILVER COLLOIDAL PARTICLES

5.1 UV-Visible Spectrophotometric Analysis

The reduction of Ag^+ to Ag was monitored by measuring the UV-Visible spectrum of the reaction mixture (silver nitrate solution + plant extract) at different time intervals i.e 30, 60 and 90 minutes within the range of 200–800 nm using UV-Vis spectrophotometer (Shimadzu, UV-2550PC Series), because it has already been reported that the absorption spectrum of aqueous $AgNO_3$ solution exhibited λ_{max} at about 220 nm where as silver colloidal particles λ_{max} at about 430 nm [19]. The reduction of silver ions was confirmed by qualitative analysis with NaCl, (precipitate formation) using the procedures of [19].

5.2 Fourier Transform Infrared (FTIR) Spectrophotometric Analysis

The synthesized silver colloidal particles solution was centrifuged (using Cole centrifuge machine Model 0412-1) at 4000 rpm for 30 minutes. The pellet was washed and rinsed with 5 ml of deionised water to get rid of the free proteins or enzymes that are not capping the silver colloidal particles. Finally, the dried form of silver colloidal particles was palletized with KBr and analyzed and recorded from the range 4000-400nm using FTIR spectrophotometer (Shimadzu 8400S) [20].

6. RESULTS AND DISCUSSION

The synthesized silver colloidal particles were first confirmed by visual observation. The colourless silver colloidal solution was changed into reddish brown, pale yellow and yellow in water, ethanol and diethyl ether respectively due to reduction of silver ions to silver atoms as shown in Fig. 2 below.

Fig. 2. A Photograph showing aqueous solution of 1mM $AgNO_3$ with A. *Conyzoides* L. plant extracts before adding the different plant extracts (a) and after addition of Water (b)

The change in colour observed in figure above is due to excitation of Surface Plasmon vibrations [19]. This Surface Plasmon vibrations (SPR) phenomenon and the entire reduction could easily be tracked by the change in colour and confirmed by UV-Vis spectroscopy.

6.1 UV-Visible Spectra Analysis

The synthesized silver colloidal particle using aqueous extract of *Ageratum conyzoides* were detected using UV-Vis spectrophotometer. Absorption spectra of silver colloidal particles formed in the reaction mixture at different time interval are displayed in Fig. 3, while Table 1 shows the absorption maxima (λ_{max}) of the silver colloidal particles prepared from aqueous extract at different time intervals.

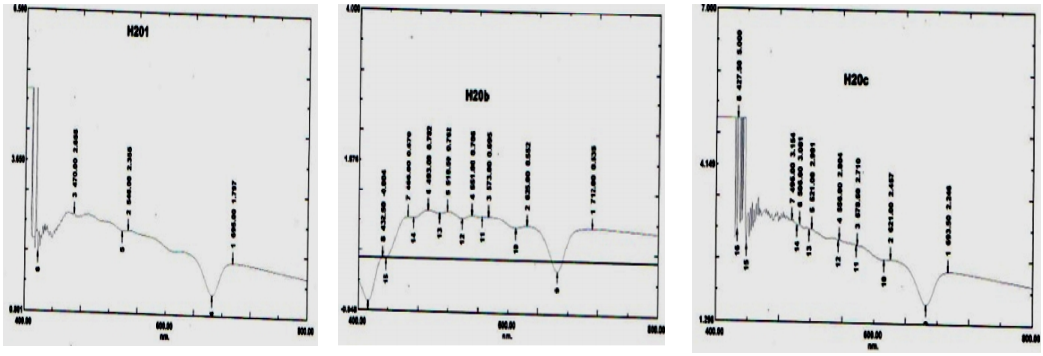

Fig. 3. UV-Vis absorption spectra of silver colloidal particles synthesized from aqueous extract at different time intervals: (a) 30 min, (b) 60 min, (c) 90 min

Table 1. Absorption maxima (λ_{max}) of the silver colloidal particles prepared from aqueous plant extract at different time intervals

Plant extract	λ_{max} (nm)		
	30 min	**60 min**	**90 min**
In water	415	433	427
	433	712	435
	695	-	694

UV-Visible spectroscopy is one of the most widely used techniques for structural elucidation of nanoparticles [1]. Therefore, it could be used to examine the size and shapes of nanoparticles in solution. The UV-Visible spectra shown in Fig. 3 have maximum wavelengths (λ_{max}) at different time intervals as extracted in Table 1 above. These maximum wavelengths occur due to the fact that, the metal nanoparticles have free electrons which give Surface Plasmon Resonance (SPR) absorption bands due to the combined vibrations electrons in resonance with light waves [21]. Hence, sharp bands of silver colloids were observed at 415nm, 433nm, 427nm, 433nm and 435nm for synthesized colloidal silver using aqueous extract. The position of the absorption bands depend strongly upon dielectric constant of the medium and probably surface absorbed species. According to Mie's theory, spherical nanoparticles give rise to a single SPR band where as anisotropic particles confer to two or more SPR bands depending on the shape of the particles [22]. It can then be inferred that, the surface Plasmon band in the silver nanoparticles solution remain around 430nm throughout the reaction period. The Plasmon peaks obtained in this work are close to

assigned peaks for well documented nanoparticles with sizes ranging from 2nm-100nm [23]. This also suggests that, the particles are dispersed in solution with no evidence of aggregation. This is true because, the nanoparticles are extremely stable even after two months of the study. It is possible that these particles are stabilized in solution by bioactive compounds that act as capping agent.

From Table 1 above, the wavelengths at 415-435nm could be assigned to carotenoids or vitamins [24]. The band at 712 nm might be assigned to xanthophylls or oxy-carotenoids [25]. For therapeutic reasons, the extract could be used to provide high concentrations of bioactive molecules from the plant. These bioactive molecules such as proteins, phenols and flavonoids can play two roles in the synthesis of silver nanoparticles. These roles are reducing silver ion (Ag^+) to nano size silver atom (Ag) and capping of nanoparticles [26]. It can therefore be concluded that, *Ageratum Conyzoides* is a potential medicinal plant for the synthesis of silver nanoparticles.

6.2 FTIR Analysis

FTIR measurements were carried out to identify the possible bio-molecules or functional groups responsible for capping and efficient stabilization of the metal colloidal particles synthesized by the plant extracts. The FTIR spectra (4000-400cm^{-1}) of powdered plant sample and the plant aqueous extract was recorded and presented in Fig. 4 below shows the spectra of dried powdered sample and fresh aqueous extract of the sample while Fig. 5 shows the FTIR spectra of synthesized silver colloidal particles using Aqueous Extract of *Ageratum Conyzoides L.* plant. Table 2 and gives the corresponding peak areas for specific regions. The functional groups identification was based on the FTIR peaks attributed to stretching and bending vibrations.

The Peak at 1059.92cm^{-1}which is seen in raw powdered sample but not seen in hot water extract, suggests the presence of -C-O- stretching mode in ether groups of polyols present in flavones, terpenoids and polysaccharides present in the extracts [27]. The band at 1250.88cm^{-1} which is observed in plant's raw sample only, shows -C-O stretching mode or O-H bending vibration due to ethers and carboxylic acid present [24]. Peaks or band around1386.86cm^{-1} can be assigned to geminal methyls [21]. The bands at 1627.01cm^{-1} and 1635.69cm^{-1} can be assigned to stretching vibration of -C=C- or aromatic compounds. The bands or functional groups such as , –C-O- and -C=C- derived from heterocyclic compounds and the amide I band derived from the proteins which are present in the plant extracts could be the capping ligands of the nanoparticles [27].

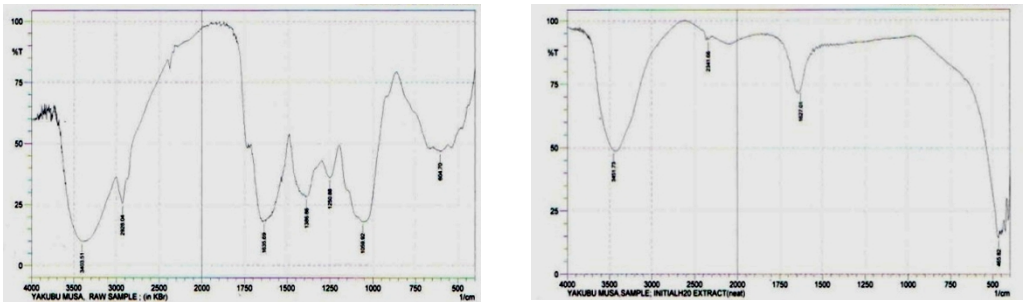

Fig. 4. FTIR spectra of (a) raw powdered dried sample and (b) aqueous extract

The band around 2341.66cm^{-1} as seen in the spectrum of water extract is assigned to C≡N stretching mode due to nitriles. The band or peak at 2928.04cm^{-1} could be assigned to –C-H stretching mode due to (terminal alkenes). The broad intense band at 3403.51cm^{-1} can be assigned to N-H (amide II) stretching frequency arising from the peptide linkages present in the protein of the powdered sample. The bands at 3451.73cm^{-1} is assigned to O-H stretching frequency of H-bonded alcohols and phenols [21].

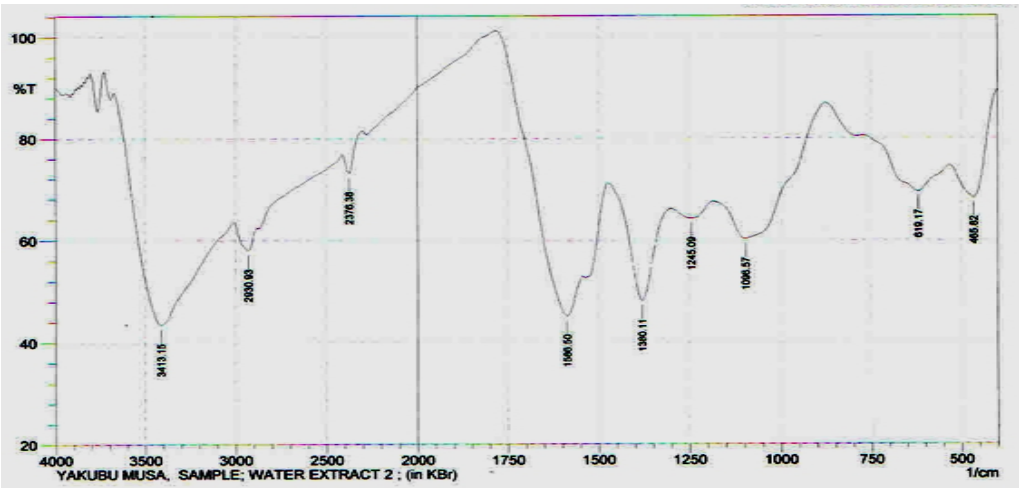

Fig. 5. FTIR spectrum of synthesized silver colloidal particles using aqueous extract of Ageratum Conyzoides L. plant.

Table 2. Assignment of FT-IR spectroscopic bands of raw powdered sample and water extract of *Ageratum conyzoides L*- plant

Bands	Raw sample peaks (cm^{-1})	Hot water extract peaks (cm^{-1})	Assignments
1	------	----------	-CH_2 rocking mode
2	1059.92	----------	-C-O- stretching due to ethers
3	1250.88	----------	C-O stretching due to acid and ethers
4	1386.86	----------	-C-H bending (Alkane)
5	-------	----------	C=C stretching (Aromatic)
6	1635.69	1627.01	Amide band 1, C=C stretching (Alkane or Aromatic group) and C=O stretch (Carbonyls)
7	-------	---------	NH stretching.
8	--------	2341.66	C ≡ N stretching
9	---------	-------	S-H stretching (Mercaptans)
10	---------	--------	CH_3 stretching (Symmetrical)
11	2928.04	--------	-C-H stretching
12	---------	--------	=CH stretching (Terminal alkenes)
13	3403.51	3451.73	O-H stretching (H-bonded)

Relating the FTIR spectra in Fig. 4(b) and 5 above, it can be observed that, there more peaks in Fig. 5 than the peaks in Fig. 4(b). The changes in the –COOH group for –OH i.e. hydroxyl group, the peak appeared at 3451.73cm^{-1} in the water extract before reaction but

after the reacting with silver solution, the peak becomes more intense and shifted to 3413.15cm^{-1} and also for -C≡N of protein, the peak at 2341.66cm^{-1}, the peak intensity increased after encapsulation of silver nanoparticles which appeared at 2376.38cm^{-1}. The band appearing at 1627.01cm^{-1} corresponding to stretching vibration of -C=C or aromatics in the water extract spectrum which appeared more intense and sharp disappeared after encapsulation of silver. The emergence of other spectral bands after reacting the extract with the silver solution such as 2930.93cm^{-1} (-CH_3 stretch in alkanes), 1586.50cm^{-1} (-C-C=C- symmetric stretch), 1380.11cm^{-1} (-CH_3 stretch of geminal methyls), can be attributed to the effect of encapsulation of silver nanoparticles and the mechanism involved in the process.

7. MECHANISTIC PATHWAY OF SILVER PARTICLES FORMATION

The major role of the plant extract was to reduce the silver. Hence, extracts of Ageratum Conyzoides plant are known to contain mono and sesquiterpenes, flavonoides, triterpenes and steroids alkaloids and miscellaneous compounds such as amino acids [18]. Some of the alkaloids of the plant may play a great role in the formation silver nanoparticles as capping agents. The possible mechanisms indicating their roles could be outlined as follows:

7.1 Glycolysis

The probability of reduction of $AgNO_3$ to silver may be illustrated due to the mechanism known as glycolysis (the breakdown of glucose to pyruvate, with the release of usable energy).

Plants fix CO_2 in the presence of sunlight. Carbohydrates are the first cellular constituent formed by photosynthesizing organism on absorption of light.

$$CO_{2(g)} + H_2O_{(l)} \rightleftharpoons H_2CO_{3(aq)} \rightleftharpoons H^+_{(aq)} + HCO^-_{(aq)} \text{ ---------- (1)}$$

This is the metabolic pathway that converts glucose $C_6H_{12}O_6$ into pyruvate and hydrogen ion:

$$H_3C - \overset{O}{\overset{\|}{C}} - \overset{O}{\overset{\|}{C}} - O^-_{(aq)} + H^+_{(aq)} \text{ ---------- (2)}$$

The free energy released in this process is used to form the high-energy compounds, ATP (andenosine triphosohate) and NAHD (Reduced Nicotinamide Adenine dinucleotide). Glycolysis can be represented by the following simple equation:

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD}^+_{(aq)} \longrightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+_{(aq)} \text{ ------ (3)}$$

Nicotinamide adenine dinucleotide, abbreviated NAD^+, is a coenzyme found in all living cells. NAD is a strong reducing agent. NAD^+ is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells. NAD^+ is an oxidizing agent-it accepts electrons from the other molecules and becomes reduced. This reaction forms NADH, which can donate electrons [26]. These electron transfer reactions are the main function of NAD:

$$\left.\begin{aligned} &AgNO_{3(s)} \longrightarrow Ag^{+}_{(aq)} + NO_{3\ (aq)}^{-} \\ &NAD^{+}_{(aq)} + e^{-} \longrightarrow NAD \\ &NAD + H^{+}_{(aq)} \longrightarrow NADH + e^{-} \\ &e^{-} + Ag^{+}_{(aq)} \longrightarrow Ag_{(s)} \end{aligned}\right\} \quad \text{----------} \quad (4)$$

NAD^{+} keeps in getting re-oxidised and gets constantly regenerated due to redox reaction [26]. This might have led to the transformation of Ag ions to Ag.

Therefore, the mechanistic pathway for the formation of silver colloidal particles using glycolysis can be summarized as thus:

$$HOCH_2(CHOH)_4CHO_{(l)} + 2Ag^{+}_{(aq)} \longrightarrow HOCH_2(CHOH)_4COOH_{(aq)} + 2Ag_{(s)} + 2H^{+}_{(aq)} \text{ --- (5)}$$

8. CONCLUSION

The green synthesis of silver colloidal particles using different extracts of *Ageratum Conyzoides L.* plant provides an environmentally friendly, simple and efficient pathway for the synthesis of benign colloidal particles. The size of the silver colloidal particles synthesized may range from 2-100nm. The bioreduced silver colloidal particles were characterized using UV-Visible and FTIR spectroscopic techniques to ascertain the colloidal particles formation and the functional groups that are responsible for the reduction of silver ion to silver. These silver particles were reduced and stabilized by a thin layer of proteins and metabolites that have functional groups of amines, alcohols, ketones, aldehydes and carboxylic acids. From the nanotechnological point of view and literatures available, these obtained silver colloidal particles have potential application in biomedical field. Hence, the simple procedure adopted in this work could have several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

COMPETING INTERESTS

The authors hereby declare that there no competing interests with regards this article.

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