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Synthesis and Characterization of Nanoparticles by Using Mulberry (*Morus alba L.*), Figs (*Ficus carica L.*) and Eucalyptus (*Camaldulensis L.*) Leaves' Extracts in Aqueous Medium

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Plants are rich in phytoconstituent biomolecules, which served as a good source of medicine and for many applications. More recently, they have been employed in synthesizing metal/metal oxide nanoparticles (NPs) due to their capping and reducing properties. The most commonly used metals are silver (Ag) and copper (Cu). This green-synthesis approach is environmentally friendly and allows the production of the desired NPs in different sizes and shapes by manipulating parameters, during the synthesis process. In the present work, we report the green synthesis of copper nanoparticles (Cu-NPs) and silver nanoparticles (Ag-NPs), using by mulberry (*Morus alba L.*), figs (*Ficus carica L.*) and eucalyptus (*Camaldulensis L.*) leaves' extracts without using any toxic chemicals, where the extracts of the studied plants' leaves contributed as factors reducing agent as well as a stabilizing agent in green-nanotechnology process. The synthesized nanoparticles are characterized by scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) spectroscopy techniques. The SEM depicts the morphology and size of the synthesized nanoparticles, while the elemental analysis and the relative abundance of each element in the nanoparticles are revealed through the EDX spectra, since EDX spectra confirm the formation of Ag-NPs and Cu-NPs. The results show that the size of Cu-NPs is smaller than that of Ag-NPs for all studied samples. This research gives a clear efficacy and reality of the use of low-cost and environmentally benign plant extracts as a main precursor in the synthesis of NPs.

Рослини багаті на фітокомпонентні біомолекули, які слугують гарним джерелом ліків і для багатьох застосувань. Зовсім недавно їх почали використовувати для синтези наночастинок (НЧ) металів/оксидів металів завдяки їхнім властивостям кеппування та відновлення. Найпоширенішими металами є срібло (Ag) і мідь (Cu). Цей підхід «зеленої» синтези є екологічно чистим і дає змогу виробляти бажані НЧ різних розмірів і форм шляхом маніпулювання параметрами під час процесу синтези. У цій роботі ми повідомляємо про «зелену» синтезу наночастинок міді (Cu-НЧ) та наночастинок срібла (Ag-НЧ) з використанням екстрактів листя шовковиці (*Morus alba L.*), інжиру (*Ficus carica L.*) та евкаліпта (*Camaldulensis L.*) без використання будь-яких токсичних хемікатів. Водночас екстракти листя досліджуваних рослин виконують роль відновлювального аґента, а також стабілізувального аґента в процесі «зеленої» нанотехнології. Синтезовані наночастинки було охарактеризовано за допомогою методів сканувальної електронної мікроскопії (СЕМ) та енергодисперсійної рентґенівської (EDX) спектроскопії. СЕМ відображає морфологію та розмір синтезованих наночастинок, тоді як елементну аналізу та відносну кількість кожного елемента в наночастинках було виявлено за допомогою EDX-спектрів, оскільки EDX-спектри підтверджують утворення Ag-НЧ і Cu-НЧ. Результати показують, що розмір Cu-НЧ є меншим, аніж Ag-НЧ для всіх досліджуваних зразків. Це дослідження чітко підтверджує ефективність і реальність використання недорогих та екологічно безпечних рослинних екстрактів як основного попередника в синтезі НЧ.

Key words: Cu nanoparticles, Ag nanoparticles, plant extracts, scanning electron microscopy, EDX spectra.

Ключові слова: Cu-наночастинки, Ag-наночастинки, рослинні екстракти, сканувальна електронна мікроскопія, EDX-спектри.

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1. INTRODUCTION

Nanotechnology is one of the fastest growing areas of science and technology. The synthesis of metal nanoparticles (NPs) is an active area of research in the field of nanotechnology with an exponential progress in biomedical applications [1]. So far, NPs have been produced through various approaches involving a variety of physical, chemical and biological processes. Generally, most of the physical, chemical and hybrid approaches are expensive and hazardous in nature due to the involvement of sophisticated instruments and toxic materials including solvents, reducing agents and precursors, *etc.* [2].

In recent years, the development of an efficient 'green' chemistry method for synthesizing metal NPs has become a major focus of researchers [3]. Plant extracts are widely used in different NPs' synthesis processes [4], since plant extract-based approaches are inexpensive, efficient, and does not involve a cumbersome process of the isolation of the biomolecules and storing the microbial cultures, *etc.* Moreover, plants'-based synthesis of nanomaterials does not require expensive reagents, high temperatures and pressure, *etc.* [2]. NPs synthesized *via* plant extracts may be used for various biological purposes. Plant extracts can serve as reductive as well as stabilizing agents in synthesis of NPs. Several plant-leaves' extracts have been successfully used for NPs preparation due to their excellent properties for biomedical applications like mulberry, figs and eucalyptus.

Mulberry (*Morus alba L.*), an ecologically and economically important plant species of the Moraceae family, is widely cultivated across many countries of the globe [5]. Mulberry plant is one of the conventional herbs used in medicine since time immemorial due to its chemical composition and pharmacological function [6] and is a rich source of phenolic compounds with antioxidant properties, such as flavonoids, anthocyanins, macronutrients, vitamins, minerals, and volatile aromatic compounds [7, 8]. That aside, *M. alba* as a whole also has a high nutraceutical value due to its low lipid value and high levels of protein, carbohydrates, fibre, organic acids, vitamins, and minerals, which are comparable to other berries [7].

Figs (*Ficus carica L.*) are a sort of deciduous tree, which belongs to the Moraceae family. Its fruit is generally referred as figs, which have been used as food and in medicine for several centuries. Its fruit, root and leaves are used in the native system of medicine in different disorders, such as colic, indigestion, diarrhoea, sore throats, coughs, bronchial problems, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers. *F. carica* has been reported to include antioxidant, antiviral, antibacterial, antiinflammation, haemostatic, hypoglycaemic, hypocholesterolaemic, cancer suppressive and anthelmintic effects [9].

Eucalyptus (*Camaldulensis L.*) is the conventional name of eucalyptus of the Myrtaceae family genus [10]; it is one of the most popular medicinal plants, which is used traditionally for the treatment of wounds, boils and other ailments, with which the test organisms are routinely associated [11, 12]. This plant is a rich source of terpenoids, tannins, sterol, saponins, flavonoids, and phenolic compounds [11, 13].

In recent years, the development of metals' and metal-oxides' NPs has greatly enhanced the biomedical field in terms of biosensing, imaging diagnosis and therapy. The most commonly used metals and their oxides are gold (Au), silver (Ag), and copper (Cu).

Among these, Cu is a relatively low-cost metal that is more cost-effective than Au and Ag. Additionally, Cu-NPs are efficient catalysts, with high yields and easy product separation; and they can be reused repeatedly. Cu-NPs are used extensively as anticancer, antimicrobial, and antioxidant agents. This is mainly because NPs are able to interact with the biological system at cellular levels for various reactions and functions [3]. Cu-NPs also have been used for textiles photocatalysis, electrical conductors, biochemical sensors, oxidative capacity. The Cu-NPs possesses potentially appropriated in cooling fluids for electronic systems and conductive inks due to the surface-plasmon resonance properties [14].

In addition, silver nanoparticles are more interesting due to their distinctive properties, such as high electrical conductivity, chemical stability, catalytic and antibacterial activity. The existing literature confirms that Ag-NPs synthesized by plant extracts hold promising medical applications [15], since Ag-NPs are used in a wide range of applications, including pharmaceuticals, cosmetics, medical devices, foodware, clothing and water purification, agriculture and in wastewater treatment, *etc.* due to their antimicrobial properties [16].

Considering all mentioned, the present research is aimed at application of green chemistry for the synthesis of Cu-NPs and Ag-NPs as one of the most important and highly applied nanoparticles. To do so, the extract of mulberry, figs and eucalyptus leaves existing in Damascus, Syria were used as the reductive agent.

2. MATERIALS AND METHODS

Fresh leaves of mulberry, figs plants were collected from Tishreen park and eucalyptus plant were collected from Dummar suburb area, Damascus city, Syria in June 2022. They were thoroughly washed with tap water to remove the adhering salts and other pollutants and finally with double distilled water. The cleaned fresh leaves were shade-dried at ambient temperature for 2 weeks. Dried leaves were powdered by a mixer grinder.

2.1. Preparation of Mulberry, Figs and Eucalyptus Leaves' Extracts

20 g of each dried and powder leaves were placed into a 400 mL of distilled water for one hour at 25°C, and then heated to 60°C for one hour with continuous stirring by a magnetic stirrer. The mixture was cooled and filtered with Whatman filter paper using a vacuum filter. The extracted solution was stored in a fridge for further use [6].

2.2. Preparation of Copper Acetate and Silver Nitrate Solutions

To prepare copper acetate and silver nitrate solutions, each of silver nitrate and copper acetate powder from Sigma Aldrich was mixed with distilled water to get 0.3 M aqueous solution of $AgNO_3$ and $Cu(CH_3COO)_2$.

2.3. Preparation of Cu-NPs and Ag-NPs

The synthesis of nanoparticles (Cu-NPs and Ag-NPs) was carried out by adding 10 mL from the stock solution of each extract to 90 mL of 0.3 M for each of $AgNO_3$ and $Cu(CH_3COO)_2$ aqueous solutions separately in a 250 mL flask. The addition was conduct slowly at 25°C for 4 hours with continuous stirring by a magnetic stirrer. While stirring, the reaction colour transformed to light brown, then dark brown colour, and afterward, no further colour transformation is perceived until the end of the reaction. After that, the pH of solution adjusted to value of 7. The solutions were left to stand for 24 h, then filtered with Whattman No. 1 filter paper, and then the obtained product was dried at 70°C for 12 h in an oven. Finally, a black powder (of nanoparticles) is achieved. The nanoparticles were removed after weighed and transferred to a closed container and kept for further analysis [2, 17].

2.4. Characterization of Cu-NPs and Ag-NPs

The characterization of synthesized nanoparticles was carried out as follows according to the methods described earlier by Baran (2018). The surface structure was visualized by SEM (TESCAN, Czech Republic) at an accelerating voltage of 30 kV, and elemental analysis measurement was done using EDX (EDAX, USA) at an accelerating voltage of 20 kV [18].

3. STATISTICAL ANALYSES

The data was subjected to one way-ANOVA IBM SPSS software package for Windows (Version 20, SPSS Inc., Chicago, IL); the statistical significance was evaluated at $P \leq 0.05$. The results were presented as mean ± standard deviation based on three replications.

4. RESULTS AND DISCUSSION

Plants are autotrophic organisms, biochemically additional to the primary metabolites necessary for the organisms' life. They synthesize secondary metabolites, also known as natural products, which are not essential for plant growth, but are compounds, which help with adaptive processes, defence against predators or pathogens, ecological interactions, symbiosis, metal transport, and competition, among others. Secondary or phytochemical metabolites like including phenols, flavonoids, alkaloids, and terpenoids can act as reducing and stabilizing agents responsible for the synthesis of nanoparticles. In general, nanoparticles' synthesis involves mixing plant extracts with metal precursor salts like copper acetate and silver nitrate. They can interact under different reaction conditions (pH, temperature, concentrations, *etc.*). Here, the formation of nanoparticles takes place at some stages. It begins with the set of species equilibrium; once dissolved, the extracts and the precursor salt coordination complexes are formed between the metal ion and the phytochemicals of the extract. The sites, where the nanoparticles will grow, are created at the second nucleation stage. At the third stage of growth and adsorption, small adjacent nanoparticles come together to form particles of a larger size. Finally, during termination, the final shape of the nanoparticles occurs [19]. The general synthesis mechanism is shown in Fig. 1.

In the present study, water is used as a medium of extraction, considering that it is safe, inexpensive, and numerous components (carbohydrates, proteins, glycosides, phenolic compounds) can be soluble in water [5]. Copper nanoparticles and silver nanoparticles were synthesized using mulberry, figs and eucalyptus leaves' extract. The first sign of the synthesis of nanoparticles was the colour change of the reaction solution. The reason behind this change is the reduction of each silver and copper ions to silver and copper nanoparticles, since the addition of plants extracts to copper acetate and silver nitrate leads to the colour change of the resulting solution to light brown in a short time, and by the end of the reaction time, the colour gradually grows darker [15]. The observed colour change is not only due to the reduction of Ag^{+} to Ag^{0} or Cu^{+2} to Cu^{0}

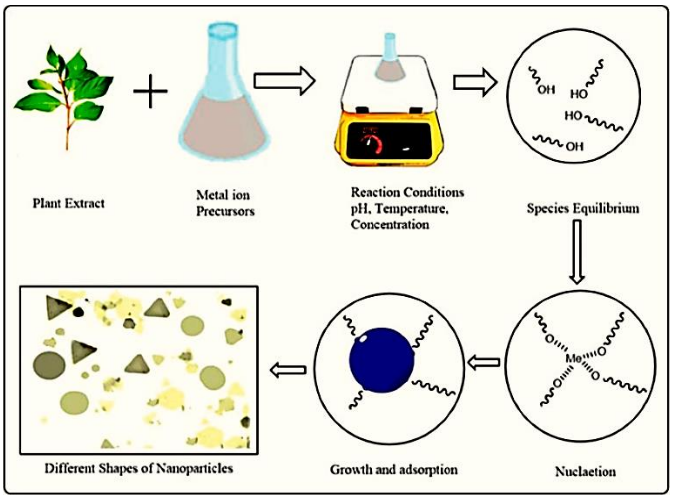

Fig. 1. General mechanism for nanoparticles' synthesis utilizing plant extracts [19].

by the biomolecules present in the mentioned extract, but is also due to the stimulation of surface plasmon resonance (SPR); since the reduction of particle size is associated with increased stimulation of the external surface electrons, a phenomenon known as SPR, this colour occurs due to the observable effect of surface plasmon excitations in metal nanoparticles [6, 15].

After synthesizing of nanoparticles, it is important to know their morphological and physicochemical properties, since, depending on their size, shape, surface morphology, structure, and homogeneity, their properties can vary. The most used characterization techniques are scanning electron microscopy (SEM) for providing the information about nanoparticle morphology, size, and homogeneity and energy dispersive x-ray (EDX) spectroscopy for estimating the abundance of elements present over nanoparticles' surface [19].

SEM has an ability to contribute morphology and microstructure of bulk as well as nanostructures [14]. Based on the obtained SEM images, the synthesized nanoparticles had various shapes, most of which are irregular, agglomerated and are multiform. Building blocks of various bioactive reducing agents, lower capping ability of leaf extract, H-bonding presented in bioactive molecules could be the reason for the agglomeration of the nanoparticles [20]. The size of the Cu-NPs was determined between 8–16 nm for mulberry extract, 13.3–21 nm for figs extract, and 6.8–30.9 nm for eucalyptus extract, while the size of the Ag-NPs was determined between 12.6–23.9 nm for mulberry extract, between 22.6–39.5 nm for figs extract, and between 12–23.9 nm for eucalyptus extract. Based on our literature search, the size of NPs synthesized using a plant source resulted in sizes ranging between 2–577 nm for Cu-NPs, and between 15–57 nm for Ag-NPs with spherical, oval-shaped, plate, rectangular, cuboidal, and cubical shapes [3, 16, 21]. It is noted from the results of the scanning electron microscopy that the size of the manufactured copper nanoparticles is smaller than that of the silver nanoparticles. Figures 2–7 below show the results obtained from the SEM analysis of the green-synthesized silver and copper nanoparticles, which described their respective morphology.

To use nanoparticles as drug delivery systems, they should be in the range of 10–100 nm. Larger particles are captured by macrophages, whereas smaller particles undergo rapid leakage into blood capillaries and renal elimination; even though NPs smaller than 10 nm can pass through the nuclear pore and interact with chromosomes and DNA that is advantageous for gene therapy and diagnostics, but probably not suitable for drug delivery. For nanomedical applications, the preferred size of nanoparticles is less than 200 nm [8]. In this study, the NPs had a size of less than 100 nm, suggesting the proper size for drug delivery.

EDX is an analytical method used to find the chemical compositions of various elements and determines the relative abundance of particular chemical elements on a solid surface.

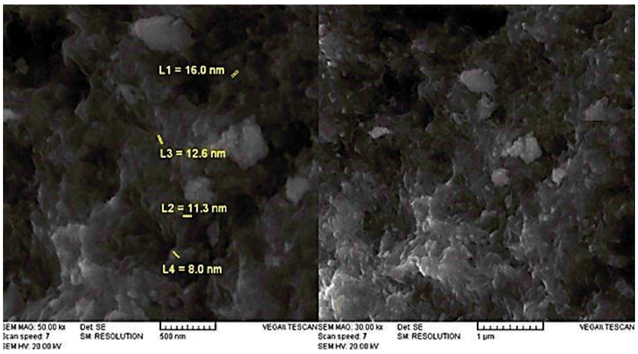

Fig. 2. SEM micrograph of the Cu-NPs using mulberry extract.

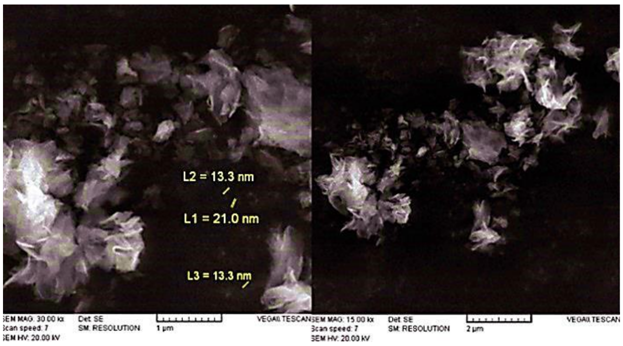

Fig. 3. SEM micrograph of the Cu-NPs using figs extract.

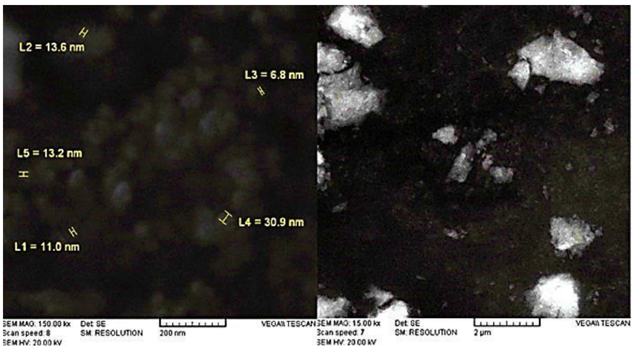

Fig. 4. SEM micrograph of the Cu-NPs using eucalyptus extract.

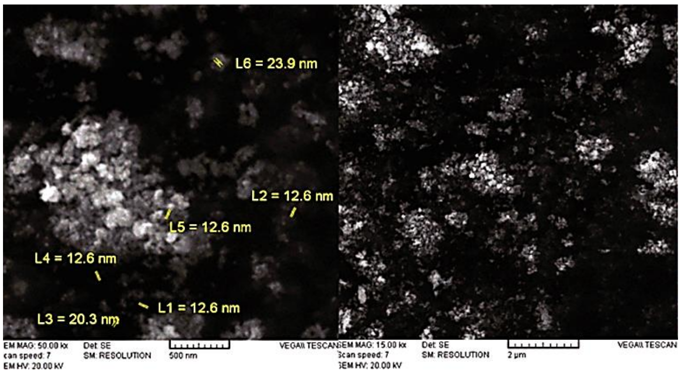

Fig. 5. SEM micrograph of the Ag-NPs using mulberry extract.

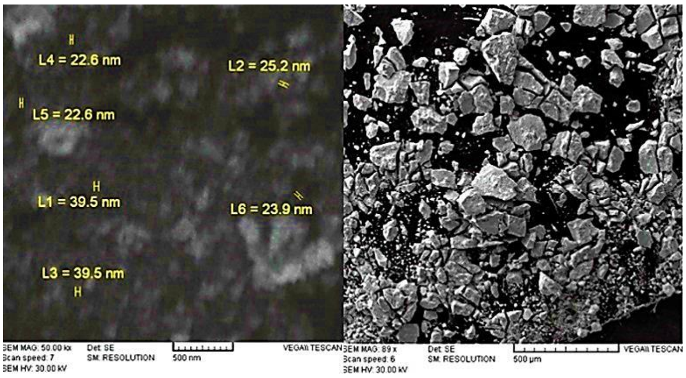

Fig. 6. SEM micrograph of the Ag-NPs using figs extract.

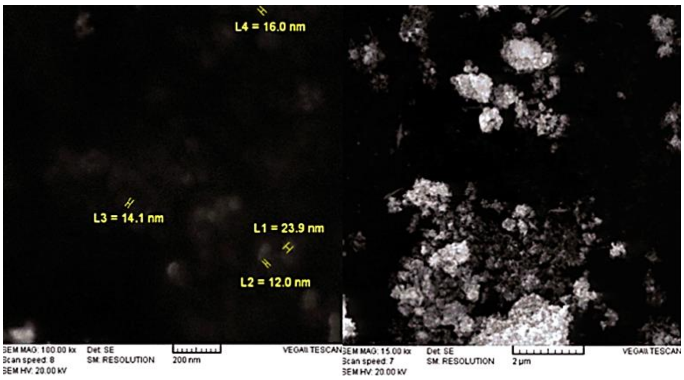

Fig. 7. SEM micrograph of the Ag-NPs using eucalyptus extract.

EDX works on the principle of an interaction of some source of x-ray excitation and a sample. Due to a variance in their atomic structures, every single element proffers the individual assortment of peaks on its electromagnetic emission spectrum. Bombardment of high-energy particles on an atom, which contains ground state electrons, results in the formation of electron hole by releasing an excited electron from the inner shell. An electron from the outer shell and higher energy shell try to fill the empty shell and this energy disparity between the high-energy shell and the lower energy shell may be dispensed under the form of an x-ray. This could be covered by an energy-dispersive spectrometer. Emitting x-rays helps to identify specific elements and its proportion [14]. The elemental composition of Cu-NPs and Ag-NPs were assessed by EDX analysis, confirming the occurrence of copper and silver as a major metallic element, as shown in Figs. 8–13. The EDX spectra of Cu-NPs mainly contained a specific and intense peak at $\cong 3$ keV for Cu (48.13%, 54.56% and 54.55%) for mulberry, figs and eucalyptus extracts, respectively, while the EDX spectra of Ag-NPs contained a specific and intense peak at $\cong 3$ keV for Ag (83.25%, 95.69% and 74.81%) for mulberry, figs and eucalyptus extracts, respectively. This result obtained is incompatible with the literature reported by Dada *et al.* (2017) & Sila *et al.* (2019) [22, 23].

The EDX spectrum determined the presence of O and C in Cu-NPs and Ag-NPs. EDX measured the weight percentage of O (39.61%, 35.56% and 33.67%) and C (12.26%, 9.88% and 11.78%) in Cu-NPs for mulberry, figs and eucalyptus extracts, respectively. In addition, the synthesized silver nanoparticles had 12.15%, 2.84% and 17.91% of O, and 4.59%, 1.74% and 7.28% of C for mulberry, figs and eucalyptus extracts, respectively. The presence of other elements might be related to the breakdown of capping agents from the surface of nanoparticles; also, most likely due to phytochemicals capping NPs, this is supported by the findings of other researchers [8, 17, 22]. The presence of carbon indicates the presence of stabilizers. The studied particles did not notice impurities from the heteroelements of silver, oxygen and carbon in Ag-NPs and copper, oxygen and carbon in Cu-NPs; so, these results indicated that the reaction product was composed of high-purity nanoparticles [17].

5. CONCLUSION

In this modern era, NPs have been widely employed in cosmetics, textiles, photonics, agriculture, and heavy industries as catalysts. Additionally, the application of NPs has been extended to medicine.

In this paper, we report green synthesis of copper nanoparticles and silver nanoparticles from leaves' extract of mulberry, figs and

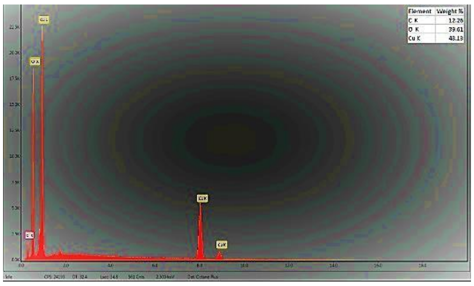

Fig. 8. Analysis of the elemental composition by the EDX analysis of Cu-NPs using mulberry extract.

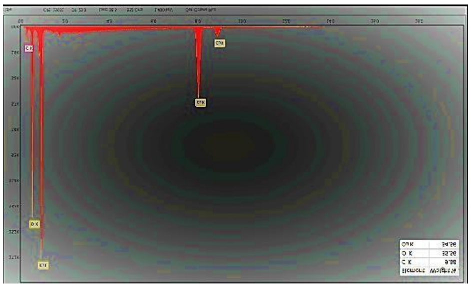

Fig. 9. Analysis of the elemental composition by the EDX analysis of Cu-NPs using figs extract.

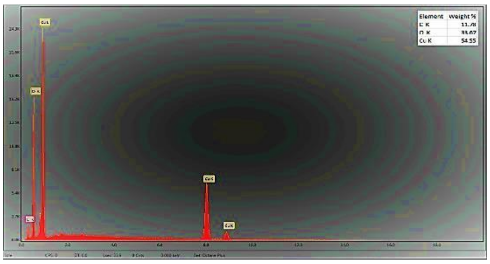

Fig. 10. Analysis of the elemental composition by the EDX analysis of Cu-NPs using eucalyptus extract.

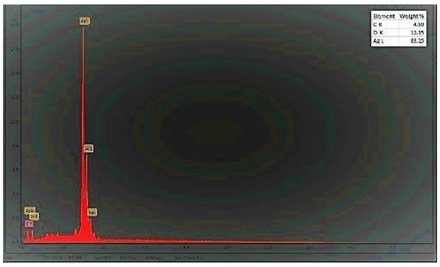

Fig. 11. Analysis of the elemental composition by the EDX analysis of Ag-NPs using mulberry extract.

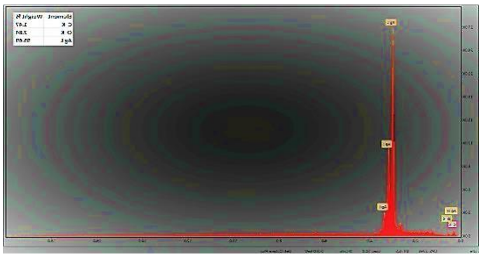

Fig. 12. Analysis of the elemental composition by the EDX analysis of Ag-NPs using figs extract.

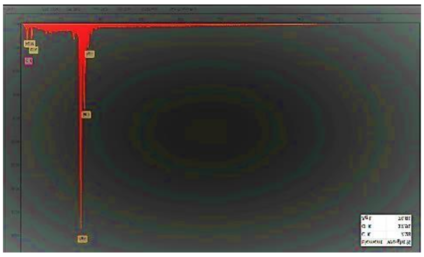

Fig. 13. Analysis of the elemental composition by the EDX analysis of Ag-NPs using eucalyptus extract.

eucalyptus leaves. This is eco-friendly, cost effective, easy and proficient way for synthesis of nanoparticles. The functional group present in the leaf extract were mainly responsible for the reduction of silver and copper metal ions into silver and copper nanoparticles.

The synthesized copper nanoparticles were analysed using SEM and EDX.

The mulberry, figs and eucalyptus plants may be effectively utilized for the production of silver and copper nanoparticles with economically for many applications.

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