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Biosynthesis of Cupper Nanoparticles Using *Coriandrum sativum* L. Ethanolic Extract

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ABSTRACT

Bio-nanotechnology comprises the synthesis of nanoparticles through a reliable and eco-friendly process suitable for a necessary application such as a Green synthesis process, which is executed by the biological molecules in plant, extracts that. A versatile technique was implemented for the synthesis of Copper nanoparticle using an ethanol extract of Coriandrum sativum L. seeds. The reduction of cupper in aqueous solution of $CuSO_4•5H_2O$ (2 mM) was confirmed by UV Vis spectrophotometer showing a typical resonance SPR (Surface Plasmon Resonance) at about 400-500 nm which is specific for the synthesis of nanoparticles (CuNPs). The formations of Copper nanoparticles from the Coriandrum sativum L. seed extract was identified first by observing the color changes, the color was changed from greenish brown to blue-brown color. The optimization of the various conditions, including, pH, time, the concentration of cupper sulfate pentagonal water $CuSO_4•5H_2O$, temperature and the mixing ratios of reactants to prepare the cupper nanoparticles (CuNPs) were studied also. Temperature 70ºC, and pH was 9.8, incubation time 30 min and the ratio of ethanolic extract 70% and cupper sulfate pentagonal water 25:75. X-ray Diffraction & Scanning Electron Microscope (SEM) analysis of cupper nanoparticles indicated that they exist in amorphous nature and the size of the particle lies in the range of 30-89 nm.

Keywords: Cupper nanoparticles, *Coriandrum sativum* L., Ethanolic extract, UV-Visible, X-ray Diffraction, Scanning Electron Microscope (SEM)

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science, nanotechnology deals with structures ranging from 1 to 100 nm nearly in one-dimensions [1] and has perfectly new or improved characteristics which are based on specific characteristics like size, shape, morphology and crystalline structure [2]. The field is spread rapidly, that making an impact in all spheres of human life [3]. In the last years, Green Nanotechnology attracted many researchers from different field like chemistry, physics, material science, medicine, engineering and bio-technology [4,5]. There are modern methods for the synthesis of nanoparticles, which should be required minimum cost reagent, eco-friendly and less drastic reaction condition [6]. The cupper nanoparticle was acquired much importance because of less cost of synthesis, has excellent chemical and physical properties, and very reactive because of their surface- to volume ratio and easily interact with other particle [7]. Cupper nanoparticle is working as water-gas shift catalysts and gas detoxification catalysts [8]. A mechanism of cupper nanoparticle synthesis using plant extracts shown in Figure 1.

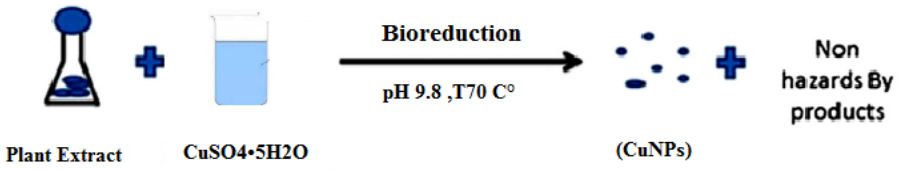

Figure 1: Mechanism of Cupper Nanoparticles

Among the different bio-reductants, *Coriandrum sativum* L. seed extract was selected for the present study since it has metals and vitamin contents, including calcium, phosphorus, niacin, carotene, thiamine, riboflavin, and iron. It's also has sodium and oxalic acid. Techniques Utilization by reduction the plant extracts are all termed as green synthesis process. Natural product extraction from the plants is a good reducing and capping agent, it helps in the biosynthesis of cupper nanoparticles [9]. The essential oil includes is around 1% and the main component reported in the oil is linalool, in the range of 30-80% of total seed oil of coriander [10]. Coriander, like many spices, contains antioxidants, which can retard or inhibit the spoilage of food seasoned with this spice. The coriander was used in anti-inflammatory agent is evident by a traditional formulation from Sri Lanka, Maharasnadhi Quather (MRQ), Which include coriander seeds as one of its main components. MRQ have been reported to have anti-inflammatory properties both in animal models and human subjects and analgesic [11].

MATERIALS AND METHODS

Materials

Cupper sulfate pentagonal water, and used refining. All other reagents were of analytical grade with extreme purity. All glassware's were properly washed with distilled water and oven dried Prior use. *Coriandrum Sativum* L. has been collected from the market.

Preparation of ethanolic extract (70% v/v) of *Coriandrum sativum* L. seed

Three hundred g of *C. sativum* seeds (*Coriandrum Sativum L.*) were macerated for 1 h in 50 l of ethanol (70% v/v) with using an electric blender to prepare the mixture, then heated at 45ºC. The ethanolic extract was filtered and stored at room temperature for further use.

Preparation of cupper nanoparticles

To prepare the cupper nanoparticles(CuNPs), 25 ml of ethanolic extract of *Coriandrum sativum* L. was added to 75 ml of 2 mM $CuSO_4 \bullet 5H_2O$ and heated with stirrer at 70ºC for 30 min. The formation blue-brown color was indicated to synthesis of Copper nanoparticles.

The optimum factors were studied of Cupper nanoparticles synthesis, the experiments were executed in different conditions are pH (3, 6.4, 7 and 9.8), Cupper ion concentration (0.5, 1, 2 and 2.5 mM), temperature (40ºC, 50ºC, 60ºC, 70ºC and 80ºC), *C. Sativum* concentration (5 g, 10 g, 15 g) in 50 ml deionized water, time of reaction (10 min, 30 min and 60 min), and the *Coriandrum sativum* L. seed extract to cupper sulfate pentagonal water ratio (10:90, 15:85, 20:80. and 25:75). The pH of the reaction was adjusted by using 0.1 N Hydrochloric acid and 0.1 N sodium hydroxide. The effect of these parameters on the synthesis of Cupper nanoparticles was observed by UV-Vis spectrophotometer.

Phytochemical analysis

Chemical experiences were organized on the ethanolic extracts of the plant sample using standard methods.

Qualitative analysis of phytochemical constituents

Test for amino acids or primary and secondary amines

1 mL of ethanolic extract of the *Coriandrum sativum* L. The seed was boiled for a little min in a boiling water bath with 1% of ninhydrin solution that prepared freshly. The color blue-violet was indicated to find of amino acids, primary and secondary amine [12].

Test for anthroquinones

A 1 g of ethanolic extract was shaken with 20 mL of benzene and was filtered. 1 mL of 10 % ammonia solution was added to the filtrate then the mixture was shaken and appear violet color in the lower phase indicated the presence of the anthroquinone [13].

Test for flavonoids

From ethanolic extract 0.1 g was taken and heated with 5 mL of ethyl acetate over a water bath for 3 min. The mixture was filtered and 2 mL of the filtrate was shaken with 0.5 mL of dilute ammonia solution and appear a yellow coloration indicated the existence of the flavonoids [14].

Test for glycosides

A half milliliter of concentrated sulfuric acid was put in a test tube. 2.5 mL of ethanolic extract was mixed with 1 mL of glacial acetic acid consist of 1 drop of ferric chloride. The above mixture was added to 0.5 ml of concentrated sulfuric acid. Appearance in the brown ring indicates a positive result [15].

Test for saponins

1 g of ethanolic extract was shaken with distilled water in a test tube and the test tube was warmed in a water bath and the entity of stable froth indicates the presence of saponins [16].

Test for tannins

1 g of ethanolic extract was boiled with 40 mL of distilled water in a test tube. This was filtered and 0.1% of the ferric chloride ($FeCl_3$) reagent was added to the filtrate. If tannin, is present in the sample a blue-black coloration will appear [17].

Test for terpenoids

From ethanolic extract 2.5 mL was mixed with 1 mL of chloroform in a test tube and 2.5 mL of focused sulfuric acid was added to the mixture to form a layer and appear a reddish brown coloration was indicated the existence of terpenoids [18].

Test for phlobatannins

Test for Phlobatannins Deposition of a red precipitate when an ethanolic extract of the *Coriandrum sativum* L. seed was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins [16].

Quantitative analysis of phytochemical constituents

Flavonoids component

10 g of ethanolic extract was extracted constantly with 100 mL of 80% aqueous methanol at room temperature. The mixture was filtered completely by Whitman filter paper no. 42 (125 m). The filtrate was transferred into a crucible and vaporized into dryness and weighed to a constant weight.

20 g of ethanolic extract of *Coriandrum sativum* L. seed was put into a conical flask and add 100 mL of 20% aqueous ethanol. The conical flask was heated on a hot water bath for 4 h and with stirring at about 55ºC. The solution was filtered and the precipitate was again extracted with 200 ml 20% ethanol. The combined extract was decreased to 40 ml on a hot water bath at about 90ºC. Conveyed the concentrate into a 250 ml separatory funnel, 20 ml diethyl ether was added in it followed by strong shaking. The ether layer was neglected while the aqueous layer was restored. The purification process was refined and 60 ml of n-butanol was added. All n-butanol extracts were washed double with 10 ml 5% aqueous sodium chloride (NaCl). The remaining l solution was heated in a water bath. After vaporization, it was dried in the oven to a constant weight. The saponins content was calculated in percentage.

Characterization of green synthesis cupper nanoparticles

By color change

The color various in the reaction mixture of cupper nanoparticles (CuNPs) was recorded through visual observation. The color change from greenish brown to blue-green in the ethanolic extract indicated that the Cupper nanoparticles were synthesized.

UV-visible spectral analysis

The reduction of Cupper ions (Cu^{++}) to Copper nanoparticles (Cu^{0}) was identified spectrometerically by double beam UV-Vis spectrophotometer (PD-303 UV) at different wavelengths (400-500 nm). The graph of absorbance on Y-axis and wavelength on X-axis.

X-ray diffraction studies (XRD)

The synthesized Cupper nanoparticles were cold centrifuged at 15,000 rpm for 30 min and collect the was collected residue. The residue was washed with distilled water to remove any purity to get the powder. The X-ray diffraction screening was performed for the detection of the crystalline nature of the copper nanoparticles was work by X-ray diffractometer Shimadzu XRD-6000 AS (3K. NOPC).

Scanning Electron Microscope (SEM)

Moreover, characterization was done by Scanning Electron Microscope (SEM). It is a type of electron microscope that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that have information about the samples surface topography and composition, SEM measurement was performed on INSPECT S50 FEI Company.

Atomic Absorption Spectrophotometer (AAS)

The supernatant solution was analyzed by AAS to reveal the quantity of Cu^{2+} ions. The rate of less in the concentration of the Cu^{2+} ions indicates the conversion of Cu^{2+} to the Cu°. The concentration of cupper ions was analyzed using atomic absorption spectrophotometer (AA-6300 SHIMADZU). Through the path of the reaction at constant time, a part of the sample was taken, settled for 30 min and then centrifuged.

RESULTS AND DISCUSSION

Characterization of cupper nanoparticles

Phytochemical analysis

Phytochemical qualitative analysis is useful to estimation the active biological components of plants. *Coriandrum sativum* L. seed extracts showed positive results for constituents analyzed, except for anthraquinone, phlobatanins, Terpenoids and tannins as shown in Table 1 and Figure 2. The medical importance of these plants lies in close to chemical substances that create a definite physiological action on the human [19]. *Coriandrum sativum* L. is also well used for its antioxidant, anti-diabetic, anti-mutagenic, antianxiety and antimicrobial activity along with analgesic and hormone balancing effect that promotes its use in foods because a lot of health benefits and its protective effect to preserve the food for longer periods. Saponins are a special type of glycosides which have soapy advantages; they have active antifungal properties and medicinal value [20].

The flavonoids are a type of secondary plant metabolites with antioxidant and chelating properties. The antioxidant activity of flavonoids depends on the substitution kind of hydroxyl groups and frame [21].

Nitrogen is one of the important nutrients needful to helpmate perfect plant up growth plants spend nitrogen to form amino acids needed in the forming of protein [22]. The glycosides are work to down the blood pressure, according to many reports and it play a role in the therapy of failing heart disorders and are known to offer helpful effects on cardiac arrhythmias [23].

Table 1: The qualitative analysis of *Coriandrum sativum* L. extracts

Phytochemical Constituents	Ethanolic Extract	Color
Amino acids or primary and secondary amine	+++	Blue - violet
Alkaloids	--	--
Anthroquinones	--	--
Flavonoids	++	Yellow
Glycosides	+++	Brown ring
Phlobatanins	--	--
Saponins	++	Foam
Tannins	--	--
Terpenoids	--	--

Whereas :- (+): Present, (++): Mildly present, (+++): Strongly present, (-): Absent

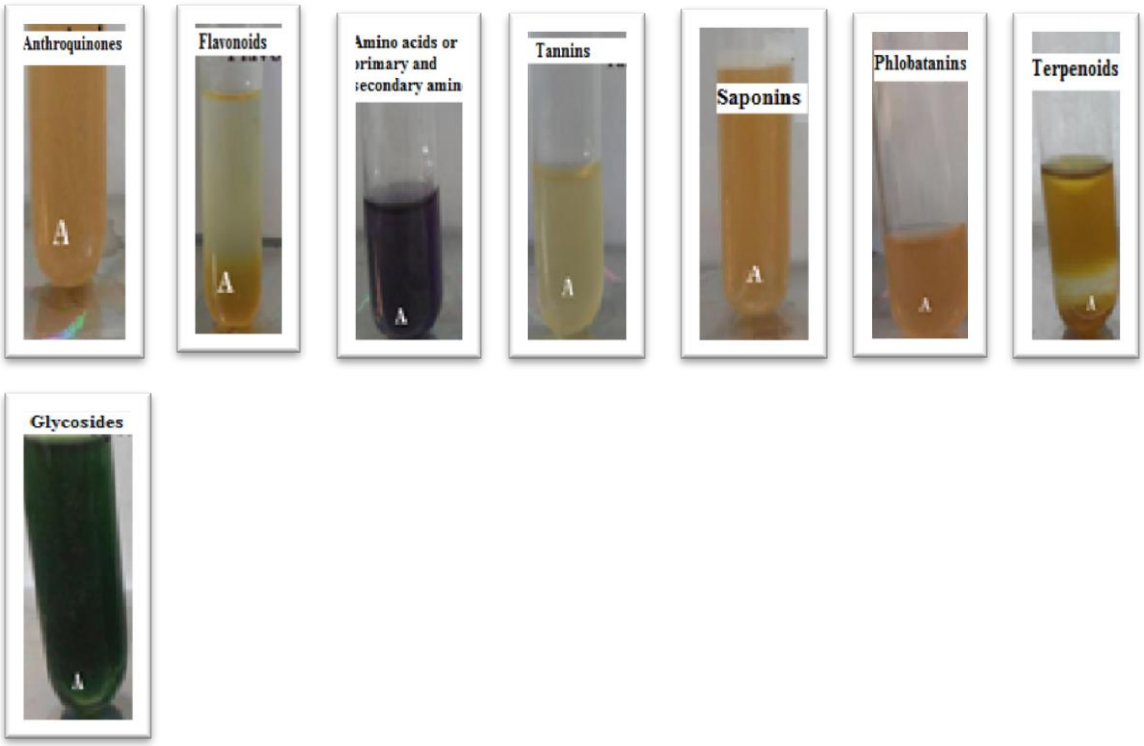

Figure 2: The qualitative analysis of *Coriandrum sativum* L. ethanolic extract

The quantitative analysis results of *Coriandrum sativum* L. Ethanolic extracts are shown in Table 2 indicated the presence of saponins and flavonoids in different quantities in ethanolic extracts, these results related to the efficiency of extraction depends on the nature of the solvent.

Table 2: The quantitative analysis of phytochemical screening of *Coriandrum sativum* L.

The percentage products %		
Saponins	**Flavonoids**	**Phytochemical constituents**
3.07%	18.95%	Ethanolic extract

By color change

The sequential color change indicates the formation of CuNPs by our seed *Coriandrum sativum L.* ethanolic extract.

Plant extract was added into cupper sulfate pentagonal water $CuSO_4.5H_2O$ (2 mM) solution within a few minutes and the appearance of the color was changed from greenish brown to blue-green (Figure 3). This is the primary test for the checking of formation of CuNPs [24]. The reduction was indicated by the increased intensity of surface Plasmon absorption peak observed within 400 nm to 500 nm [25].

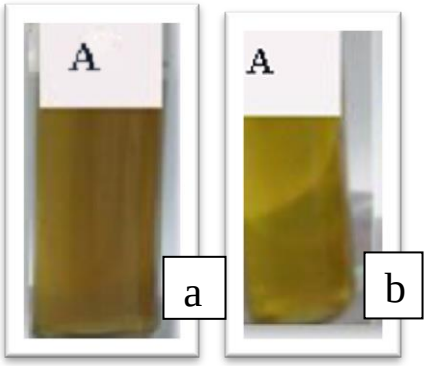

Figure 3: Color Change (a) *Coriandrum sativum* L. Ethanolic extract, (b) the ethanolic extract indicates the formation of copper nanoparticles

Effect of Cupper ion Concentration

The blue-green coloured cupper nanoparticles synthesized from 2 mM cupper sulfate pentagonal water. At the 2 mM concentration shows a narrow band with increased absorbance whereas other concentrations shows a broad peak at 452 nm. The absorption was increased with increasing the concentration of cupper ions from 1 mM to 2.5 mM. In 2 mM concentration the nanoparticles synthesis and size reduction was started quickly due to the more availability of functional groups in the *Coriandrum Sativum L.* seed ethanolic extract. While increasing the substrate concentration the large size and aggregation of nanoparticles was occurring due to the occurrence of compete between cupper ions and functional groups of seed ethanolic extract [26] explained the concentration of cupper sulfate pentagonal water was 1 Mm. Figure 4 shows the effect of $CuSO_4 \cdot 5H_2O$ concentration in the CuNPs.

0.5 mM *1* mM 2 mM 2.5 mM

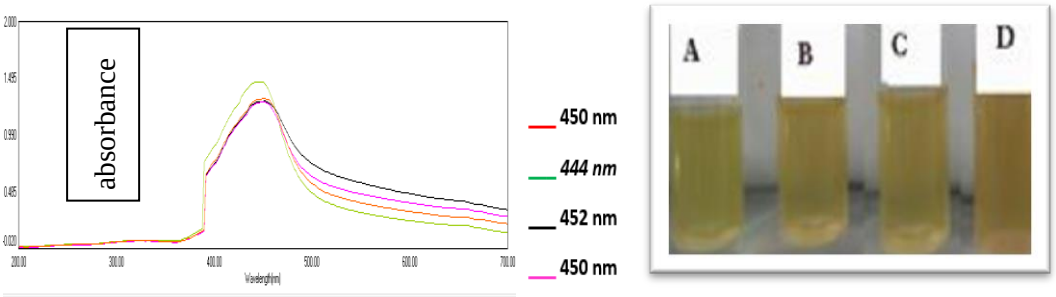

Figure 4: UV Spectrum of Synthesis of Cupper Nanoparticles (ethanolic extract) at Different Concentration of $CuSO_4 \cdot 5H_2O$, In the Image (A=0.5, B=1, C=2 and D=2.5 Mm)

Effect of temperature

The effect of temperature on the rate of formation of CuNps was show in Figure 5. The CuNps were formed within 30 min at 70ºC. However, at room temperature and above 90ºC under boiling condition the solution becomes charred and no particle formation is seen. Temperature increase commensurate with the increase in the proportional composition of nanoparticles, but certain limits. Hence, the reaction at 70ºC favors the biosynthesis of CuNp using aqueous *Coriandrum sativum* L. extract. The literature [27] was observed that the best formation of camps was observed at 80ºC.

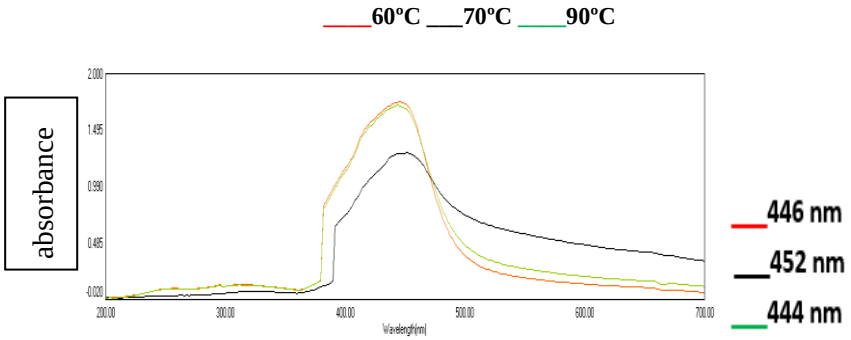

Figure 5: UV-Visible spectra of CuNPs ethanolic extract showing effect of different reaction temperature

Effect of pH

The present work shows that the pH of the solution has an effect on the progress of bio-reduction of copper sulfate pentagonal water solution. The pH of the *Coriandrum sativum* L. extract at 5.4 is mixed with $CuSO_4.5H_2O$ solution to formation cupper nanoparticles at pH 9.8. The surface plasmon absorbance of copper colloids was obtained for all pH except at PH 3 and 6.4. This probably indicates very small particles at such low pH. The Plasmon resonance is clearly visible for pH 9.8, At pH 12, the peak is still detectable but much weaker when compared with other pH, Figure 6 shows UV-Visible absorption spectra for the pH ranging from (3-12).

The literature [28] explained the impact of pH on the shape and size of metal nanoparticles and found that a large number of particle formation in basic medium pH while in acidic pH increase aggregation of nuclei instead of the formation.

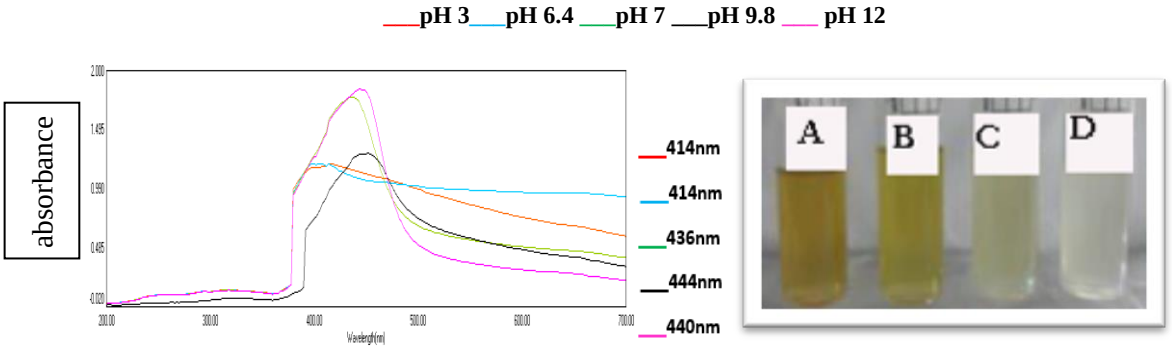

Figure 6: UV Spectra of the pH Effect of in Nanoparticles Using Ethanolic Extract, (A=3, B=6.4, C=7 and D=9.8)

Effect of time

Cupper nanoparticle synthesis was evaluated at different reaction time using UV–vis spectroscopy. Figure 7 shows the UV–vis spectra of cupper nanoparticles at different reaction time of 0, 15, 30, 45 and 60 min and shows colour changes of cupper nanoparticles in different reaction time, the characteristic Surface plasmon resonance (SPR) band for cupper nanoparticles, centred at 452 nm. The variation of absorbance with respect to time. Formation of cupper nanoparticles started within 30 min, after that only slight variation can be observed. The intensity of the SPR peak increased as the reaction time increased, which indicated the increased concentration of the cupper nanoparticles. This result implies that the cupper nanoparticle prepared by this green synthesis method is very stable without aggregation. The stability results from a potential barrier that develops as a result of the competition between electrostatic repulsion and weak Vander Waals forces of attraction. The absorbance of cupper colloid solution increased with span of time and preferable absorption was observed after 15-20 min of reaction [29]. Another search the optimum duration necessary for the end of reaction was 25 min [30,31] was explained The formation of CuNPs within 30 min incubation.

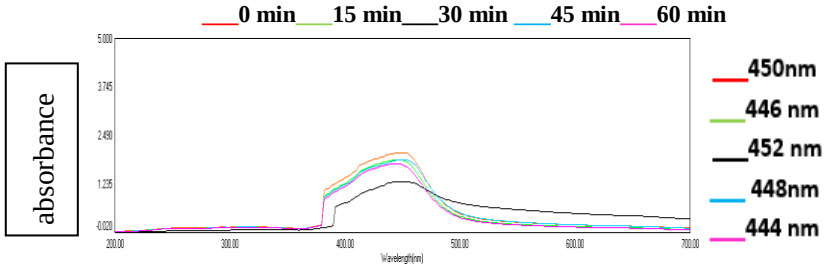

Figure 7: UV-Visible Spectra for the Effect of Time Reaction for Ethanolic Extract CuNPs

Concentration of *Coriandrum Sativum* L. extract

Ethanolic extract increase the *Coriandrum sativum* L. compounds responsible for the formation of cupper nanoparticles was increaseing were observed by increasing the absorbability in Figure 8.

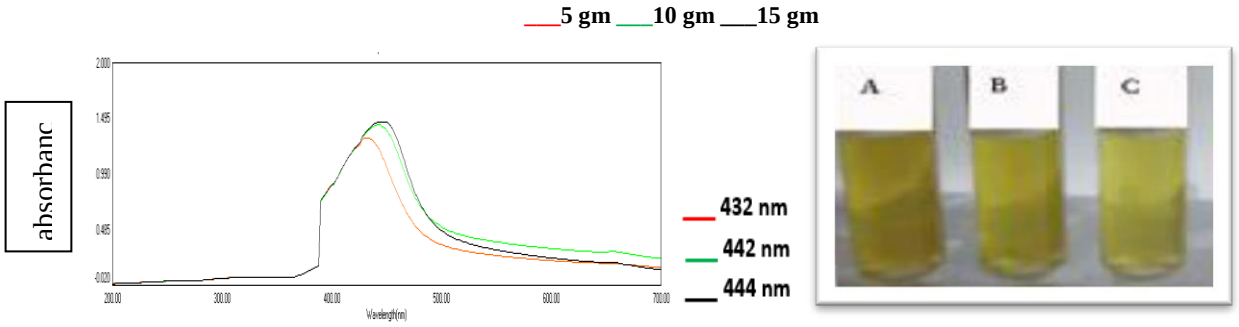

Figure 8: UV-Visible spectra of concentration *Coriandrum sativum* L. ethanolic extract, (A=5 g, B=10 g and C=15 g)

the effect of the closing tightly

Mistakenly been observed leaving open the tubes leads to a decrease in absorbability when leaving other tubes are closed leads to an increase in absorbability was due this volatile aromatic tubes are enclosed in affect formation nanoscale cupper (Figure 9).

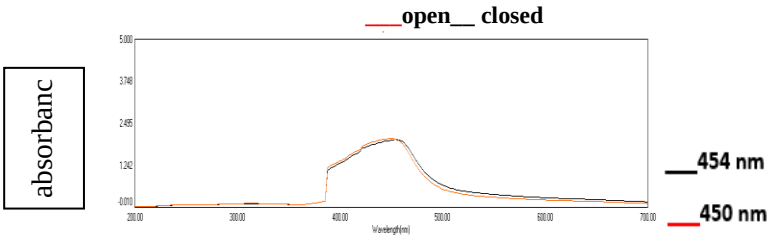

Figure 9: UV-Visible spectra of CuNPs ethanolic extract showing the effect of the closing tightly

effect of the time of storage

The formation of copper nanoparticles effect that time, as shown in Figure 10, it means the study of nanoparticles of copper this green synthesis prepared stable and noted by mistake when the storage tube should be closed because it is pointed out that the volatile material is involved in the synthesis of nanoscale copper particles dramatically.

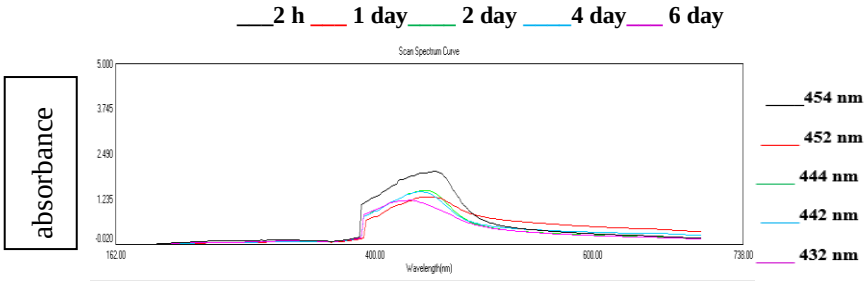

Figure 10: UV-Vis absorption spectrum of cupper nanoparticles at different times for ethanolic extract concentration ratio of copper sulfate pentagonal water and *Coriandrum Sativum L.* extract

The effect of *Coriandrum sativum* L. seed extract concentration in the mixed solution on the biosynthesis of cupper nanoparticles was investigated. The difference concentration of extract in the mixed solution was obtained by changing the volume of the added extract solution different volumes (10-25 ml) of extract were added to (75-90) ml of 2 mM Copper Sulfate Pentagonal Water. Optimizing has been observed for the concentration of cupper and extraction in the ratio (25:75), and low absorption as shown in Figure 11 [32] was observed the optimal ratio 10: 40 ml of 1 mM Copper Sulfate [33], show the ratio 10: 90 of seed ethanolic extract in the mixture of reaction was efficient for the synthesis of copper nanoparticles.

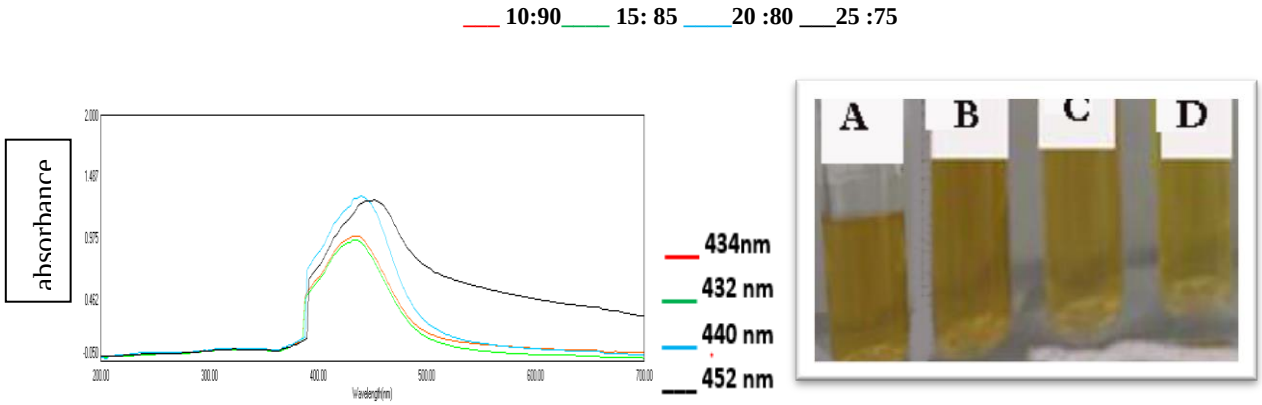

Figure 11: UV-Visible spectra of CuNPs ethanolic extract showing the effect of *Coriandrum sativum* L. extract and $CuSO_4.5H_2O$, (A=25:75, B=20:80, C=15:75 and D=10:90)

X- Ray diffraction studies (XRD)

Figure 12 shows the X-ray diffraction (XRD) pattern of the CuNPs powder synthesized from Cupper Sulfate Pentagonal Water 2 Mm and *Coriandrum sativum* L. extract at 80ºC. The average crystallite size of the synthesized cupper nanoparticles is calculated in the range 30-90 nm by using Debye Scherrer's equation [34]: $D=0.89\ \lambda/\beta\cos\theta$. Where λ represents wavelength of x-ray source 0.1541 nm) used in XRD, D is the crystallite size of CuNPs, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with value from 0.9 to 1 and θ is the Bragg angle. The observed peak noise may be back to the effect of nonuse of particles and the presence of different crystalline biological macromolecules in ethanoic extract. The green synthesis of CuNPs was further supported by X-ray Diffraction (XRD). Thus XRD is commonly applied for determine the chemical composition and crystal structure of a material [35,36].

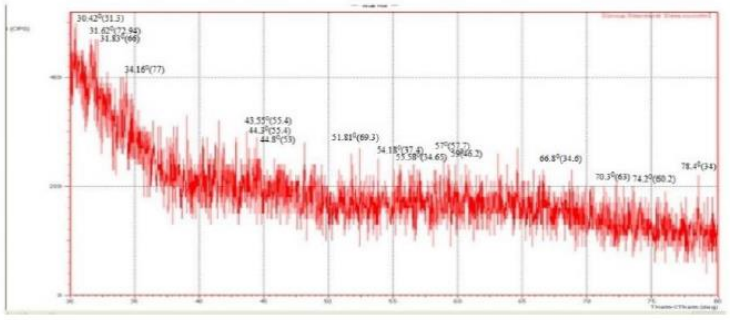

Figure 12: X-Ray pattern of CuNPs ethanoic extract

Scanning electron microscope (SEM) analysis

The surface morphology and size of cupper nanoparticles were acquired by Scanning Electron Microscopy (SEM) analysis. The Figure 13a and 13b shows the Cu NPs synthesized by the seed extract. The hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are In charge of the synthesis cupper nanoparticles using plant extract. Whereas the ethanol extract is appearing shows that spherical and relatively uniform shape of the copper nanoparticles was confirmed in the range of 89-30 nm [37,38]. This may due to availability of different quantity and nature of capping agents present in the extract.

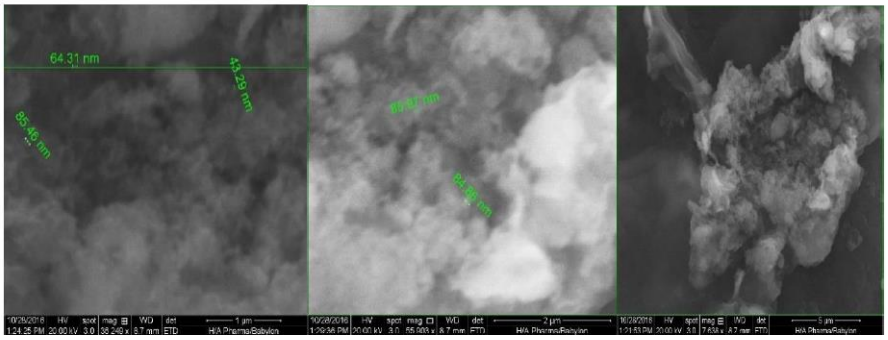

Figure 13: SEM micrograph of CuNPs ethanolic extract

Energy dispersive X-ray crystallography (EDX)

The EDX pattern clearly shows that cupper nanoparticle formed by the reduction of copper ions using fresh ethanolic *Coriandrum sativum* L. extract are crystalline in nature (Figure 13). The quantitative and qualitative analysis of metals may be concerned in the formation of copper nanoparticles. They were identified by EDX analysis and were recorded in the spot-profile mode. The optical absorption peak is observed at 1 Kev, which is typical for the absorption of metallic copper nanoparticles. Strong signals from the cupper atoms are observed, while weaker signals for C, O, Si, Mg, Al, Ca, K, Fe, S and Na atoms were also recorded. From the EDS signals, it is clear that cupper nanoparticles reduced by ethanolic extract of *Coriandrum sativum* L. seed have the weight percentage of elemental copper as 15% (Figure 14).

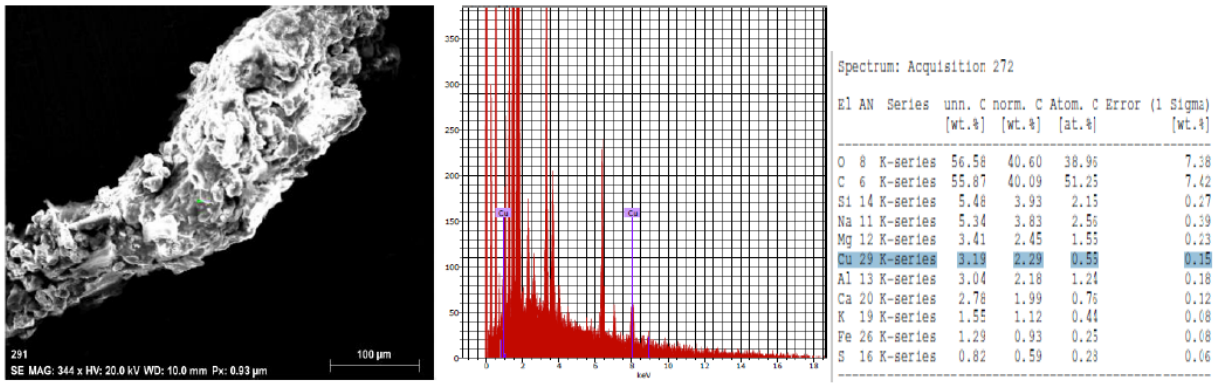

Figure 14: EDX analysis of CuNPs ethanolic extract

Map of cupper nano particulars

Pictures map in Figure 15 reflects the particular copper has a yellow color points.

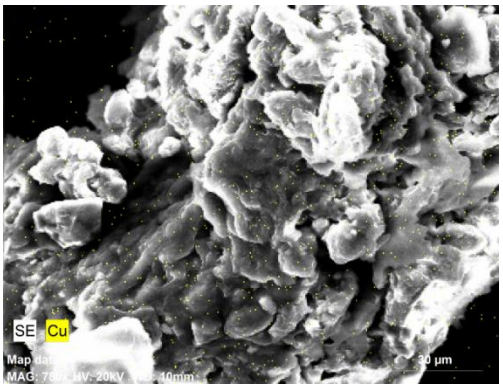

Figure 15: Map image of CuNPs ethanolic extract

Flame atomic absorption spectrophotometer

The conversion of cupper ion to the copper particle is possible to measure by using Flame Atomic Absorption technique which shows the concentration of cupper ion lower. The standard solution of 0.2, 0.4, 1 and 5 ppm Copper Sulfate Pentagonal Water was prepared and analyzed by AAS at zero time. The cupper ion concentration was monitored at different time, after adding *Coriandrum sativum* L. seed ethanolic extract Figure 16 shows the rate of lower in the concentration of copper ions (0.4 ppm) at 0, 15, 30 and 45, min respectively) which describe the transformation of cupper ion to cupper particle. When a plus copper ion concentration increases the time to be adjusted a nan particulate, this is what we observe in Figure 15, where the copper ion required to be reduced to more components when the highest concentration. Our result in Consistent with other studies metal like Ag.

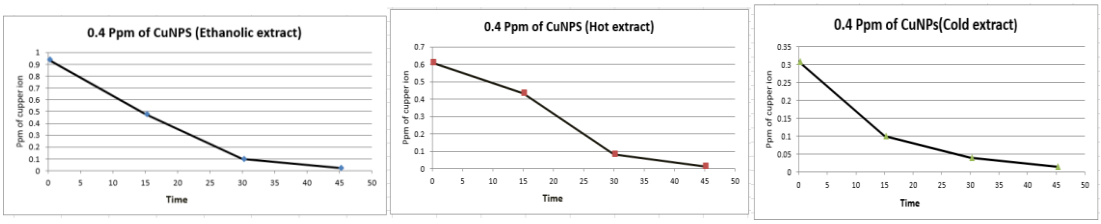

Figure 16: Atomic absorption analysis of copper sulfate pentagonal water (ppm) with reaction time (min) using coriandrum sativum ethanolict extract to the synthesis of cupper nanoparticles

REFERENCES

[1] M. Rai, A. Yadav, A. Gade, *Cr. Rev. Biotechnol*., **2008**, 28(4), 277-284.
[2] N. Khandelwal, G. Kaur, N. Kumar, A. Tiwari, *Dig. J. Nanomater. Biostruct*., **2014**, 9(1), 175.
[3] M. Rai, P. Ingle, A.R. Gupta, I.S. Birla, S.P. Yadav, A.A Abd-Elsalam, *Current Nanosci*., **2013**, 9(5), 576-587.
[4] J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, J. Hong, *Nanotechnol*., **2007**, 18(10), 105104.
[5] P. Heera, S. Shanmugam, J. Ramachandran, *Int. J. Curr. Res. Acad. Rev*., **2015**, 3(10), 268-275.
[6] V.D. Kulkarni, P.S. Kulkarni, *Int. J. Chem. Stud*., **2013**, 1(3), 1-4.
[7] U. Kathad, H.P. Gajera, *Int. J. Pharm. Bio. Sci*., **2014**, 5(3), 533-540.
[8] N.N. Hoover, B.J. Auten, B.D. Chandler, *J. Physical. Chem. B*, **2006**, 110(17), 8606-8612.
[9] R. Sathyavathi, M.B. Krishna, S.V. Rao, R. Saritha, D.N. Rao, *Adv. Sci. lett*., **2010**, 3(2), 138-143.
[10] N.G. Sahib, F. Anwar, A.H. Gilani, A.A. Hamid, N. Saari, K.M. Alkharfy, *Phytotherapy. Res*., **2013**, 27(10), 1439-1456.
[11] M.I. Thabrew, M.G. Dharmasiri, L. Senaratne, *J. ethnopharmacol*., **2003**, 85(2), 261-267
[12] S.N. Chinedu, A.Y. Oluwadamisi, S.T. Popoola, B.J. David, T. Epelle *Pak. J. Biol. Sci*., **2014**, 17(6): 849-854.
[13] P.B. Hawk, B.L. Oser, H.W. Sumerson, *Pract. Physiol. Chem.*, **1954**, Mcgraw-Hill, Book Company Inc., NY, USA, 63-172
[14] D.E. Okwu, *Int. J. Mol. Med. Adv Sci*., **2005**, 1(4): 375-381.
[15] S.F. Zohra, B. Meriem, S. Samira, M.S.A. Muneer, *J. Nat. Prod. Plant Resour*., **2012**, 2(4): 512-516.
[16] H.O. Edeoga, D.E. Okwu, B.O. Mbaebie, *African. J. Biotechnol*., **2005**, 4(7), 685-688
[17] J.A. Olajide, N.B. Danladi, A.S. Maria, *Cent. Euro. J. Exp. Bio*., **2012**, 1(2): 53-61.
[18] O.V. Njoku, C. Obi, *Afr. J. Pure. Appl. Chem*., **2009**, 3(11): 228-233.
[19] O.O. Aiyelaagbe, P.M. Osamudiamen, *Plant. Sci. Res*., **2009**, 2(1):11-13.
[20] H.S. Al-Kawaz, L.A.M. AL-Mashhady, *Res. J. Pharmaceut., Biol. Chem. Sci*., **2016**, 7(2): 2212-2223.
[21] K. Rukmini, P.S. Devi, C.M.K. Chitturi, *Int. J. Pharm. Bio. Sci*., **2015**, 6(1): 1360-1369.
[22] S. Majaw, J. Moirangthem, Ethnobotanical Leaflets, **2009**, 13:578-589.
[23]D. Krishnaiah, T. Devi, A. Bono, R. Sarbatly, *J. Med. Plants Res*., **2009**, 3(2): 067-072.
[24] S.A. Anuj, K.B. Ishnava, *Int. J. Pharma and Bio Sci*., **2013**, 4(4), 849-863.
[25] H.S. Al-Kawaz, L.A.M. AL-Mashhedy, *Int. J. Pharmacy & Therapeutics*., **2016**, 7(1): 31-41
[26] J. Mekala, M.R. Rajan, R. Ramesh, *Indian J. Res.*, **2016**, 5(2).
[27] S. Shankar, X. Teng, J.W. Rhim, *Carbohydrate Polymers*., **2014**, 114, 484-492.
[28] V. Arya, **2010**, *Digest J. Nanomaterials and Biostructures*., 5(1), 9-21.
[29] P. Kaur, R. Thakur, A. Chaudhury, *Green Chem. Lett. Rev*., **2016**, 9(1), 33-38.
[30] Jayalakshmi, A. Yogamoorthi, *Int. J. Nanomaterials and Biostructures*., **2016**, 4(4): 66-71.
[31] S. Hariprasad, G.S. Bai, J. Santhoshkumar, C.H. Madhu, D. Sravani, *Int. J. Chem. Tech. Res*., **2016**, 9(2), 98-105.
[32] J.K.V.M. Angrasan, R. Subbaiya, *Int. J. Curr. Microbiol. Appl. Sci*., **2014**, 3(9), 768-774.
[33] S. Thakur, R. Rai, S. Sharma, *Int. J. Bio-Technol. Res*., **2014**, 1(4), 21-34.
[34] H.P. Klug, L.E. Alexander, *Int. J. Industrial Chem*., **1954**, 4 (29):1-6.
[36] J. Singh, G. Kaur, M. Rawat, *J. Bioelectron. Nanotechnol*., **2016**, 1(1), 1-9.
[37] M.S. Usman, N.A. Ibrahim, K. Shameli, N. Zainuddin, W.M.Z.W. Yunus, *Molecules*, **2012**, 17(12), 14928-14936.
[38] I. Subhankari, P.L. Nayak, *World. J. Nano. Sci. Technol*., **2013**, 2(1), 14-17.