

GC-MS Analysis and Green Synthesis of Copper Nano particles Using *Vitex negundo.L* Leaf Extract

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ABSTRACT- Recently, development of reliable experimental protocols for synthesis of metal nanoparticles with desired morphologies and sizes has become a major focus of researchers. Green synthesis of metal nanoparticles using plant extracts emerged as a nontoxic and eco-friendly method for synthesis of metal nanoparticles. In this study biosynthesis of stable copper nanoparticles were done using aqueous leaf extract of *Vitex negundo leaf* from 3mm copper sulphate solution. Synthesized nanoparticles were characterized under UV-Vis spectroscopy at the range of 400 nm to 800 nm at varying intervals of time. The peak at 254nm revealed the presence of CuNPs. It was observed that the *Vitex negundo* leaf extract can reduce copper ions into copper nanoparticles within 10 to 15 min of reaction time. These biosynthesized Cu nanoparticles were characterized with the help of X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and SEM techniques. GC-MS analysis revealed that the leaf extract of *Vitex negundo.L*, contains steroids, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides. These bioactive principles are found to be responsible for bioreduction during the synthesis of spherical copper nanoparticles. The preparation of nano-structured copper particles using *Vitex negundo.L* leaf extract thus provides an environmentally friendly option, as compared to currently available chemical and physical methods.

Keyword: Biosynthesis , Copper nanoparticles, Copper Sulphate , EDX,FT-IR, GC-MS analysis,

1 Introduction :

In recent days nanotechnology has induced great scientific advancement in the field of research and technology. Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. Nanoparticle is the core particle which performs as a whole unit in terms of transport and property [1]. As the name indicates nano means a billionth or 10^{-9} unit. Its size range usually from 1-100 nm due to small size it occupies a position in various fields of nanoscience and nanotechnology. Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material.

So the main aim to study minute size is to trigger chemical activity with distinct crystallography that increases the surface area [2-4]. Thus in recent years much research is going on metallic nanoparticle and its properties like catalyst, sensing to optics, antibacterial activity, data storage capacity [5]. The various nanoparticles, metal nanoparticles assume special importance because they are easier and cheaper to synthesize and are the most promising in applications [6]. Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [7]. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry[8]. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods can not avoid the use of toxic chemicals in the synthesis protocol. Since metal

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nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods for nanoparticle synthesis using microorganism, enzymes and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods[9], [10].

Currently developed synthesis methods for copper nanoparticles include chemical reduction [11–15], Biosynthesized copper nanoparticles should excellent long term stability in aqueous medium [16]. Copper has an excellent electrical conductivity.

They present a wide range of potential applications in nanotechnology including catalysts [17], additives for lubricants [18], heat transfer nanofluids [19], manufacture of electronic and optical devices [20], conductive inks [21], materials for solar energy conversion [22], biosensors [23], antibiofouling agents [24], and cancer cell treatments [25]. Moreover, copper nanoparticles can be a promising candidate to replace expensive noble metal nanoparticles such as silver and gold [26–28]. Due to relatively low costs, this metal plays a significant role in modern electronic circuit. Because of its excellent electrical conductivity, catalytic behaviour, good compatibility and surface enhanced Raman Scattering Activity. Cu nanoparticles have drawn the attention of scientists to be used as essential component in the future nano-device. The leaves of *Vitex Negundo*.L are digitate, with five lanceolate leaflets, sometimes three. The ayurvedic use of *Vitex Negundo* leaves is in treating dysmenorrhea [29]. The characterization study of copper nanoparticle was done by examining size, shape, and quantity of particles. Number of technique is used for this purpose, including UV-visible spectroscopy, Scanning Electron microscopy (SEM), Fourier Transmission Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD).



Fig : Plant *Vitex negundo*.L.

2 Experimental methods:

2.1 Materials and methods:

Vitex Negundo. L leaf samples were collected from the Holy Cross College campus and were cleaned properly in running tap water. Thoroughly washed leaves (100 g) were cut and boiled with 100 ml of deionized water for 15 min under reflux condition at temperature 80° C. The resulting product was filtered and stored in refrigerator for further experiments.

2.2 GAS CHROMATOGRAPHY - MASS SPECTRUM STUDY (GC-MS)

The components of test sample were evaporated in the injection port of the GC equipment and segregated in the column by adsorption and absorption technique with suitable temperature programme of the oven controlled by software. Different components were eluted from the column based on the boiling point of the individual components. The GC column was heated in the oven between 60 to 270°C. The time at which each component eluted from the GC column was termed as Retention Time (RT). MS was conducted–Interpretation of mass spectrum (GC using database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library6. The retention time, molecular weight, molecular formula and composition percentage of the sample material was analysed.

2.3 Synthesis of Copper nanoparticles using leaf extracts:

For the Cu nanoparticle synthesis, 40 ml of *Vitex Negundo.L* leaf extract was added to 60 ml of 3mm aqueous Copper Sulphate solution in a 250 ml Erlenmeyer flask and it is agitated using a magnetic stirrer. According to literature studies copper nanoparticle solution has dark brown or dark reddish in colour. In *Vitex negundo* before addition of CuSO_4 its colour was brown but after its treatment with CuSO_4 its colour changes to dark reddish brown which indicated the formation of CuNPs. It was monitored periodically (time and colour change were recorded along with periodic sampling and scanning by UV-visible spectrophotometry) for maximum 5 hrs. This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles. Then, the colloidal mixture was sealed and stored properly for future use

2.4 Characterization :

The wave length of copper nano particles were confirmed by UV-Visible spectrum of the Perkin Elmer spectrophotometer at a resolution of 1 nm (from 200 to 800 nm) in 2ml quartz cuvette with 1 cm path length. The free dried sample was subjected to morphological characterization using JEOL Jsm-6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis. Fourier transform infrared (FTIR) spectroscopic measurements were done using Shimadzu spectrophotometer and the range of spectra is $4000\text{--}400\text{ cm}^{-1}$ range at resolution of 4 cm^{-1} . The sample were prepared by dispersing the CuNps uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. EDX measurements of the reduced CuNPs were recorded on X- ray diffractometer (x' pertpananalytical) instrument operating at a voltage of 40 kV and current of 30 mA with Cu K (α) radiation to determine the crystalline phase and material identification.

3 Results and Discussion:

UV-Visible spectral analysis

Figure- 1 shows the UV absorption peaks of *Vitex negundo*. UV-Vis spectra were taken at different time period for 3mm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution (30mins to 5hrs). The spectra showed the peak approximately at 254.00nm, clearly indicating the formation of spherical CuNPs in the aqueous extract of *Vitex negundo*. The occurrence of the peak at 254 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the copper nanoparticles which gets excited due to the applied electromagnetic field.

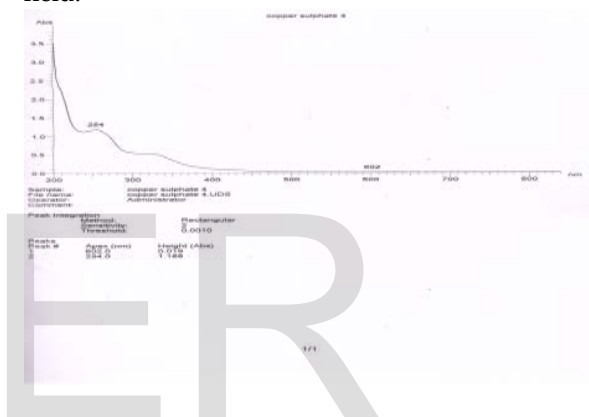


Fig 1: UV- Visible spectra were taken at different time period for 3mM CuSO_4 solution (30mins to 5hrs)

FT-IR Analysis

FTIR gives the information about functional groups present in the synthesized copper nanoparticles for understanding their transformation from simple inorganic CuSO_4 to elemental copper by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of copper nanoparticles mediated by the plant extracts. The FTIR spectrum of Copper Nanoparticles is shown in figure 2. The IR spectrum of copper Nanoparticles shows band at 3304.06 cm^{-1} , 1589 cm^{-1} , 1392 cm^{-1} and 1271.09 cm^{-1} corresponds to O-H Stretching H- bonded alcohols and phenols, N-H bend primary amines, corresponds to C-N stretching of the aromatic

amino group and C-O stretching alcohols, ethers respectively. FTIR spectrum of copper nanoparticles suggested that Cu nanoparticles were surrounded by different organic molecules responsible for the synthesis of CuNPs.

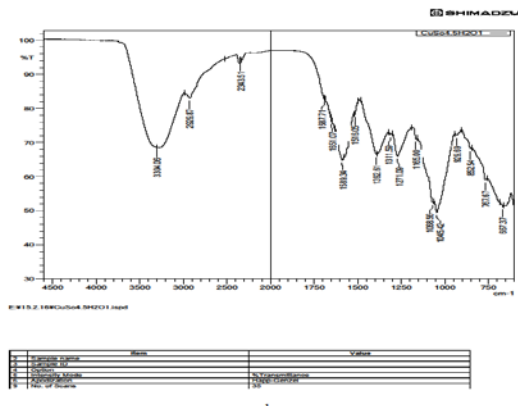
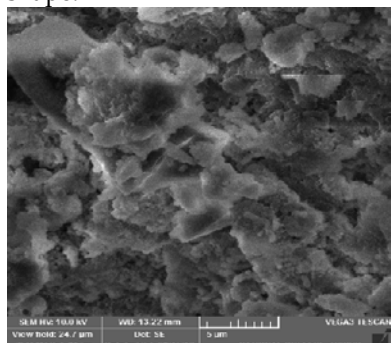


Figure- 2: FTIR graph Synthesised CuNPs

Scanning Electron Microscopy

A scanning electron microscope was employed to analyze the shape of the copper nanoparticles that were synthesised by green method. SEM analysis shows that the plant has tremendous capability to synthesize copper nanoparticles which were roughly spherical in shape.



EDX Analysis

Figure-3: SEM image of CuNPs

Figure 4 shows the EDX spectrum of spherical nanoparticles prepared with this bioreduction method. The peaks around 1.40 keV correspond to the binding energies of Cu. Throughout the scanning range of binding energies, no peak belonging to impurity was detected. The results indicated that the reaction product was composed of high purity Cu nanoparticles.

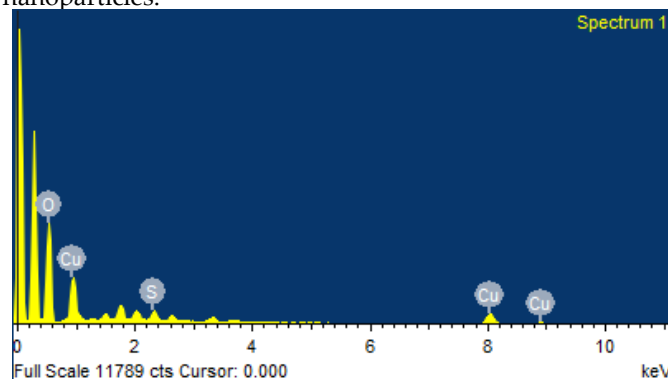


Figure- 4: EDX pattern of CuNPs

Gas Chromatography - Mass Spectrum Study (GC-MS) Structural Isolation and Identification of Phytocompounds

The GC separated compounds are identified from the recorded mass spectra by comparison with the mass spectra from the database of National Institute of Standard Technology (NIST) library.

GC-MS chromatogram of the aqueous extract of *Vitex negundo* showed 14 peaks indicating the presence of 14 chemical constituents (Figure - 5). The 14 active constituents with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the aqueous extract of *Vitex negundo* are presented in Table - 1.

Table 1: Phytocomponents identified in the aqueous extract of the leaves of *Vitex negundo*. by GC-MS

S. No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area%
1	5.21	4-Undecene,10-methyl-,(E)-	C ₁₂ H ₂₄	168	2.27
2	7.34	Butane,2-nitro-	C ₄ H ₉ NO ₂	103	1.52
3	8.90	Hydroperoxide,heptyl	C ₇ H ₁₆ O ₂	132	0.76
4	11.11	Nitric acid, nonyl ester	C ₉ H ₁₉ NO ₃	189	0.76
5	11.59	2-None-1-ol,(E)-	C ₉ H ₁₈ O	142	9.09
6	12.09	3-Octene-1-ol,(z)-	C ₈ H ₁₆ O	128	0.76
7	13.08	n-Decanoic acid	C ₁₀ H ₂₀ O ₂	172	2.27
8	14.92	Phytol	C ₂₀ H ₄₀ O	296	24.24
9	17.29	2-Propenoic acid,2-(dimethylamino)ethyl ester	C ₇ H ₁₃ NO ₂	143	3.03
10	24.68	Squalene	C ₃₀ H ₅₀	410	9.09
11	27.85	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	283	5.20
12	31.08	2-[p-Chlorophenyl]-1H-naphth[2,3-d]imidazole-4,9-dione	C ₁₇ H ₉ ClN ₂ O ₂	308	13.64
13	32.33	6,9,12-Octadecatrienoic acid, phenylmethyl ester,(z,z,z)-	C ₂₅ H ₃₆ O ₂	368	17.42
14	34.50	Bicyclo[3.3.1]nonan-9-one,1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(+)-	C ₁₂ H ₁₉ NO ₃	225	9.85

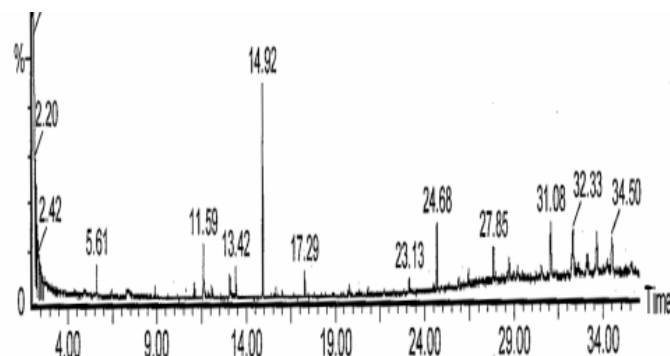


Figure -5 GC-MS of aqueous extract of *Vitex negundo*

4 Conclusion:

Green synthesis of Copper Nanoparticles by the help of green plants is a very cost effective, safe, non-toxic, eco-friendly root of synthesis which can be manufactured at large scale. *Vitex Negundo.L* showed great capability to synthesis Copper Nanoparticles at optimum temperature conditions. The UV absorption peak at 254.00 nm clearly indicates the synthesis of CuNPs. The SEM studies helps at deciphering their morphology and distribution. FTIR studies confirmed the biofabrication of the CuNPs by the different phytochemicals with its different functional groups present in the extract solution. The phytocomponents responsible for biofabrication is revealed from the GC-MS study. The EDX patterns confirmed the purity, phase composition and nature of the synthesized nanoparticles. The GC-MS result of *Vitex Negundo.L* shows that more than 14 phytoconstituents present in the aqueous leaf extract. These bioactive principles are found to be responsible for bioreduction during the synthesis of spherical copper nanoparticles. Thus plant based biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis.

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