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REVIEW ARTICLE

Green Synthesis of Copper Nanoparticles by Using Plant Leaf Extract as Reductant

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ABSTRACT
In the present work total reducing strength or phenolic compounds in leaf extract of Conocarpus erectus and Neriumindicum were determined and then Copper nanoparticles (Cu NPs)were synthesized by using only methanol extract of Conocarpus erectus leaves as reducing agent because of its higher values of total phenolic compounds (296 ± 9 µg/g) in comparison to Neriumindicum (185 ± 6 µg/g). Characterization of the green synthesized Cu NPswas performed by SEM (Scanning Electron Microscope) and XRD (X-Ray Diffractometer) techniques. The size of Cu NPs was estimated in the range of 30-70 nm. The usage of plant extract for the preparation of Cu NPs makes the process cost effective, non-toxic and green method.
Keywords:*Green Synthesis, Conocarpus erectus, Neriumindicum, Copper nanoparticle*

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INTRODUCTION

Copper nanoparticles (Cu NPs) play an important role because of their high surface area to volume ratio, excellent catalytic, optical and magnetic properties and high thermal conductivity as compared to other transition metals like gold or silver. But because of high oxidation potential of copper a reducing and stabilizing agent is required to reduce copper salt[1]. There are number of techniques which have beenextensively used to form Cu NPs. It includes, for example,micro-emulsion method [2], arc submerged system[3], flame-based aerosol methods [4], sono-chemical [5], hydrothermal[6]and solid state techniques[7]. Recent years Biological (or green) approach using plants or plant extracts and microorganisms for the syntheses of metal nanoparticles have recently been advised as substitutes to hazardous (chemical) methods [8]. Phenolic compounds like tannins, flavonoids and phenolic acids are considered to be involved in redox activities so they are key performer to the reducing or antioxidant activity of medicinal plants, fruits or vegetables. The phenolic compounds because of their redox activities behave as hydrogen donators, reducing agents, singlet oxygen quenchers and also metal chelating agent [9]. In plant extracts there are numerous types of phenolic compounds. They are highly reactive compounds and get involved in redox reactions. The presence of total phenolic substances within the plant extract could be liable for metal ions reduction and creation of the respective metal's nanoparticles [8].

Conocarpus erectus is the species family *Combretaceae*. It is an evergreen tree and grows on coastal areas of hot regions of the world [10]. *Nerium indicum* is also an evergreen shrub or small tree of the genus *Nerium* and family Apocynaceae. It also grows everywhere in tropical regions [11]. Though extract of both compounds have been reported rich in phenolic compounds but *Conocarpus*leaf extract was reported a large variety of these compounds [11, 12].

The aim of present work is to synthesize cobalt nanoparticles by using leaf extracts of plant (*Conocarpus erectus* or *Nerium indicum*) having higher values of total phenolic compounds because to the best of our knowledge through literature it was the first time to use plant leaf extract (*Conocarpus erectus* or *Nerium indicum*) for the green synthesis of Cobalt nanoparticles.

MATERIAL AND METHODS

All reagents such as Cobalt Nitrate and Methanol which were used throughout the research work were of analytical grade supplied by Merck (Germany) and Sigma-Aldrich (USA). Equipment were Analytical balance (Sartorius, Germany), SEM analyzer (Hitachi S4160, Japan) XRD analyzer (Karaltay, DX-2700 MIN), Magnetic stirrer/Hot plate (MS-H-Pro+), vacuum filtration assembly (Thomas 4595D45), Thermostat/incubator (Seimens), Spectrophotometer (Tomos), grinder (West point).

Preparation of extract of leaves

100g of dried leaves of *Conocarpus erectus* and *Nerium indicum* were first grinded and then powdered leaves was added to 500 mL methanol, ethanol and distilled/deionized water in 1000mL flask and well mixed. This extraction was done by a hot plate/magnetic stirrer at 50^0C for 60min. The obtained extract of plants was filtered by vacuum filtration assembly then filtrate was kept safely to use further.

Determination of total reducing strength or total phenolic compounds

The phenolic concentration in plant extracts was determined by singleton *et al.*[13] using spectrophotometer technique. In this analysis 1 mg/mL of methanol extract of plant was used. The reaction mixture was prepared by dissolving 0.5 mL of methanol extract with 2.5 mL of 10% Folin-Ciocalteu's reagent in 2.5 mL of 7.5% $NaHCO_3$ and water. Blank was prepared by dissolving 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu's reagent in water and 2.5 ml of 7.5 % $NaHCO_3$. The reaction samples were incubated at 45^0C for 45 minutes in a thermostat. The absorbance of reaction mixture was investigated using spectrophotometer at maximum wavelength of 765 nm. The mean value of absorbance was found for the three observations. The calibration line was constructed after repeating the same procedure for the standard solution of gallic acid. Based on the observed absorbance, the concentration of phenolics was noted in mg/mL from the calibration line. Then the total content of phenolic compounds in extract was expressed in terms of milligram of gallic acid per gram of extract (mg of GA/g).Total reducing strength or total phenolic compounds in all plant extracts were determined as described by singleton *et al.*[13] with the help of spectrophotometer. Briefly 0.5mL of extract added with 10%Folin-Ciocalteu's reagent (2.5 mL in equal volume of 7.5% $NaHCO_3$). Blank was prepared by adding methanol (0.5mL), 10% Folin-Ciocalteu's reagent (It was dissolved in water and 7.5 % $NaHCO_3$in equal volumes of 2.5mL).The reaction mixtures were incubated at a temperature of 45° C for 45 minutes in an incubator/thermostat. The absorbance of solution was noted at 765 nm wavelength using spectrophotometer. Standard solution of gallic acid was used as standard.

Green synthesis of copper nanoparticles

The copper nanoparticles were prepared in a 250mL conical flask in which 50mL 0.01 molar solution of copper sulphate was mixed with 10mL of the plant extract (100 g of dried leaves powder of was added to 500 mL methanol, ethanol and deionized/distillated water in 1000mL flask) along with vigorous shaking on a hot plate until during 15 min the colour of mixture became dark.

Characterization

The crystallinity and crystal phases of the grown Copper structures were identified by the powder X-Ray diffraction (XRD). The morphology of Copper nanoparticles was studied using scanning electron microscope (Hitachi S4160).

RESULTS AND DISCUSSION

Total phenolic compounds

Total phenolic compounds in *Conocarpus erectus* and *Nerium indicum* extracts were investigated and results are illustrated in Table 1. It was found that total phenolic compounds in methanol extract of *Conocarpus erectus* and *Nerium indicum* is higher than their extract in water and ethanol. The values in methanol extract are noted as *Conocarpus erectus*, 296±9 µg/g; *Nerium indicum*, 185±6 while in water extract they are noted as *Conocarpus erectus*, 59±8 µg/g; *Nerium indicum*, 27±2 µg/g. The methanol leaf extract of *Conocarpus erectus* composed of higher values of phenolic content so it was used for the green synthesis of Cu NPs in this research. El-Sayed, S. *et al.* [14] have investigated that the total phenolic compound values were higher in ethyl acetate extraction of fruits which are about 303.45 mg/g GAE and for flowers are about 301.15 mg/g GAE, however, they were lower in leaves [186.1 mg/g GAE] and in stem [181.61 mg/g GAE]. On the contrast our results shows the methanol extract of leaves contain higher values of phenolic compounds which might be because of environmental condition and choice of solvent [15].We have selected *Concocarpus erectus* and *Nerium indigum* leaves extract or the green formation of

Cu NPs as a raw material just because of their abundancy and availability in any season. Whereas Vinayagam and Sudha [7] has already reported in scientific literature that the total phenolic compounds values were observed higher in flower extract of *Nerium indicum* [449 mg/100g], as compared to leaves extract [227mg/100g].

Table 1: Total phenolic compounds in leaves extract of *Conocarpus erectus* and *Neriumindicum*

Plant	Total Phenolic Compounds (µg/g)		
	Water	**Ethanol**	**Methanol**
Conocarpus erectus	59 ± 8	158 ± 5	296 ± 9
Neriumindicum	27 ± 2	149 ± 5	185 ± 6

Green synthesis of copper nanoparticles by Conocarpus erectus

In the present investigation Cu NPs are synthesized by reducing metal ions from its salt using phenolic compounds from leaves extract as reducing agent. The reduction mechanism was proposed by Ramnthan *et al*. [16]**.** Fig. 1 shows the elaborated scheme of reduction of copper ions by phenolic compounds which were present in plant leaf extract.

Scanning Electron Microscopy Study

The nature of the produced Copper nanoparticles was analyzed by SEM. Nearly mono dispersed distribution of particle sizes were seen by this method. The SEM image [Fig. 2] disclosed a number of clusters of Cu particles. This image also revealed that size of the Cu nanoparticles were in the range of diameter 30 - 70 nm and a few larger particles of above with the diameter of 100nm. Similar types of images were also reported by various researchers for copper nanoparticles [2-8].

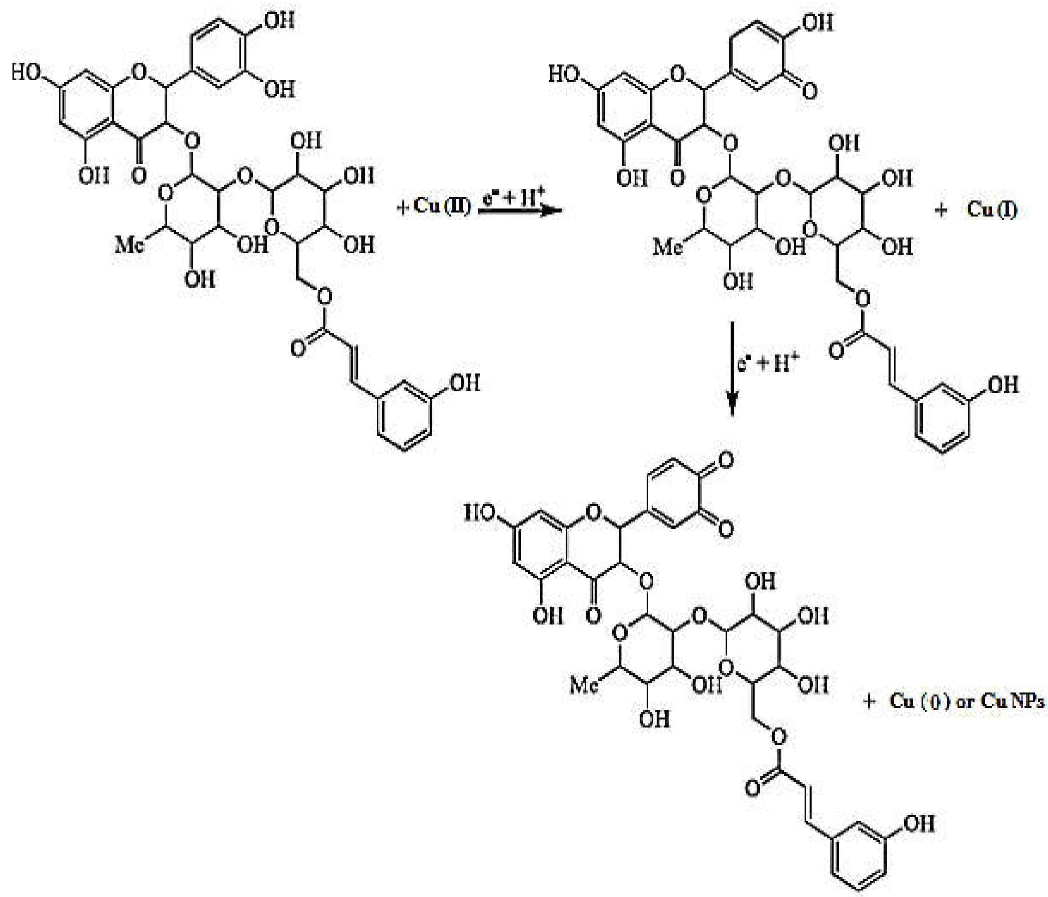

Fig 1: Scheme for the phenolic reduction to form copper nanoparticles

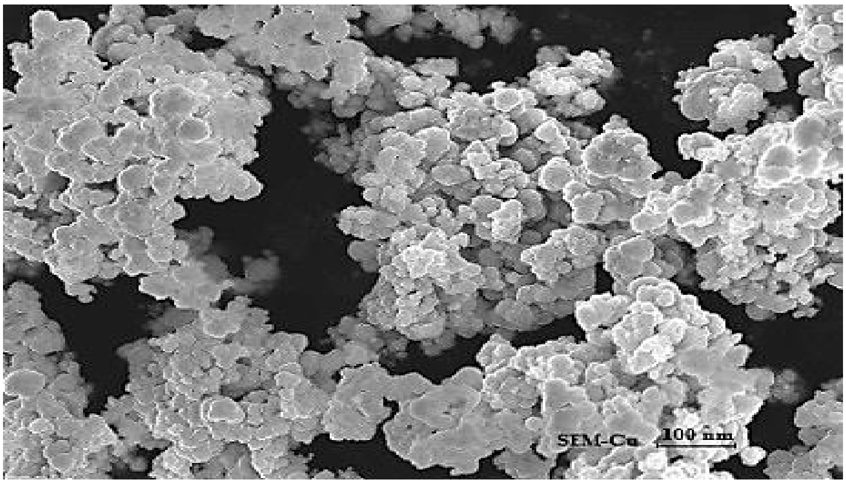

Fig. 2: SEM image of green synthesized Copper nanoparticles by methanol leaf extract of *Conocarpus erectus*

X-Ray Diffraction Study

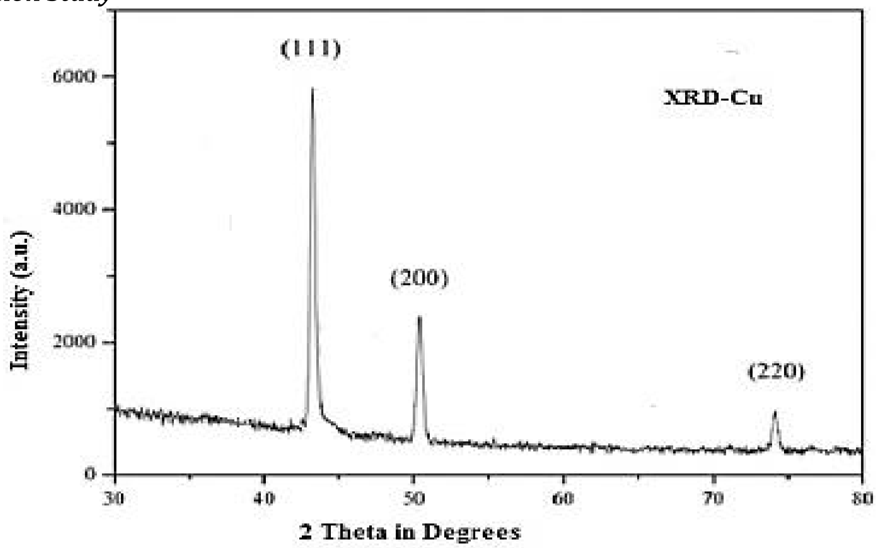

Fig. 3: XRD Pattern of green synthesized Copper Nanoparticles by leaf extract of *Conocarpus erectus*

XRD analysis was used to identify the copper nanoparticles. Fig. 3 illustrated the XRD pattern of copper nanoparticles for the prepared sample in *Conocarpus* extract and agreed with the reported XRD pattern of Cu NPs [17]. Table 2 represents the values at peaks which are almost coinciding with the standard crystalline copper with respect to spherical copper and its corresponding lattice planes [18]. It has also been observed that there are sharp and much intense peaks due to the high crystalline nature of Nanosized copper.

Table 2. Experimental and Standard diffraction angles of Copper nanoparticles

Standard angle JCPDS Copper:04-0836 [16,18]	Experimental diff. angle[2θ in degree]	Diff. Hkl (planes) [2θ in degree]
43.297	42.87	111
50.433	49.79	200
74.130	73.66	220

CONCLUSION

We have prepared Cu nanoparticles by green synthesis technique which is simple and ecofriendly. It was concluded that methanol extract of *Conocarpus erectus* leaves consist of higher phenolic compounds (296±9 μg/g) on contrary to *Nerium indicum* (185±6 μg/g). The

Copper nanoparticles produced were in the range of 30-70 nm. It has also been concluded that extract of *Conocarpus erectus* can be used as a good reducing agent for an ecofriendly and cost effective green synthesis of copper nanoparticles and doesn't involve any hazardous chemicals used previously in chemical reduction methods.

COMPETING INTERESTS

The authors have declared that no competing interest exists

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