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Characterisation of copper oxide nano particles using *Annona reticulata* extract and their antioxidant and photocatalytic activities

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ABSTRACT

Metal nanoparticles using extracts of plant components as a green approach is considered to be economic and ecofriendly. During the green synthesis of metallic nanoparticles, the polyol components present in the plant extracts are responsible for the bioreduction of metal ions whereas water soluble heterocyclic components stabilize the nanoparticles formed. The present study deals with the synthesis of pure copper oxide nanoparticles (CuO) with appropriate reactants at room temperature. Characterizition of the synthesized product was performed by uv vis spectroscopy, followed by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analysis (XRD), XRD pattern confirmed the crystalline nature and monoclinic structure of synthesized composition. The functional groups present in the sample were identified by FTIR spectroscopy. These analytical techniques clearly confirm the formation of copper oxide with monoclinic structure.

Keywords: Green Synthesis, CuO NPs, XRD, FTIR.

1. INTRODUCTION

Copper oxide nanoparticles have attracted significant attention because of their wide range of applications such as high-Tc superconductors[1-3], sensors[4],catalytic[5-7], optical[8], electrical[9], giant magnet resistance material, solar energy transformation and preparation of organic–inorganic nanostructure composites[10-11]. Further it can be used as anti-bacterial agent when incorporated in coatings, plastics textiles, etc [12]. Copper and copper-based compounds are efficient biocides, which are generally used in pesticide formulations [13] and several health related applications. Green synthesis of nanoparticles has several advantages over chemical and physical synthesis, method using leaves extract. In addition, the plant-mediated synthesis is a rapid, flexible, and suitable process for large-scale production of nanoparticles. Different chemical methods are available for the synthesis of copper oxide nanoparticles namely wet chemical method [14], direct thermal decomposition method[15], microwave irradiation method[16], sol-gel method[17] Although different methods exist for the synthesis of copper oxide nanoparticles, most of the methods are inefficient, costly and generate toxic wastes to the environment. Therefore there arises an urgent need to develop an eco friendly technique. Recently green synthesis of nanoparticles using plant such as *Cassia auriculata* [18], *Hibiscus rosasinenss* [19], *Calotropis gigantea* [20] *Ocimum sanctum* [21] and tea leaf [22] have been reported. The presence of bioactive functional elements act as reducing groups in green chemistry [23]. In recent study, green synthesis of Copper oxide nanoparticles was achieved by using microorganisms [24], plant extract [25], seed [26] and bark [27]. In the group of medicinal plants, the *Piper nigrum* possess excellent medicinal properties due to the presence of enormous phytochemicals. The piperine is an alkaloid, majorly found in *Piper nigrum*, which belongs to the Piperaceae family. Due to the presence of enormous quantity of phytochemicals, the powder of *Piper nigrum*(black pepper) are taken under consideration for the synthesis of copper oxide nanoparticles. *Piper nigrum* is native to south India and is extensively cultivated there and elsewhere in tropical regions [28]. *Ocimum tenuiflorum* leaf extract for the synthesis of copper oxide nanoparticle have been used and the leaf extract contains eugenol, eugenic acid, caryophyllene, urosolic acid, luteolin, rosmarinic acid, aesculin, limatrol, linalool, apigenin, isothymusin, carotene and ascorbic acid [29-30].

Annona reticulata Linn. is one of the traditionally important plant used for the treatment of various ailments.[31] It belongs to family Annonaceae.[32] The synonyms of plant are Sitaphal, Bullock's heart and Custard apple [33] Near about 119 different species of the Annona genus (Annonaceae) are identified among which most of them are shrubs and trees. Traditionally the plant extract is used for the treatment of diarrhoea [34] and pediculosis [35]. Geographical distribution of *A. reticulata* is widely distributed in tropical and subtropical regions. In India it is widely cultivated and naturalized as a fruit consuming plant and deciduous tree. Fruits are edible, somewhat heart shaped, rough and yellow in colour which change to yellowish red on ripening. Fruits are sweet, astringent and useful in blood complaints. [36] Seeds are smooth and blackish in colour. Traditionally the plant has been employed for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark is a powerful astringent and used as a tonic whereas leaves used for helminthiasis treatment.

So the present study aims at exploring the different medicinal properties of the *Annona reticulata* like antipyretic, analgesic, anti-inflammatory, wound healing, etc and to synthesise nanoparticles as a potent substitute for the chemical drugs.

2. EXPERIMENTAL SECTION

2.1 Plant material and its preparation

Leaves of *Annona reticulata* were collected from the Botanical garden of ITM University during July, 2018 from the Gwalior region (Madhya Pradesh). The plant leaves were dried in shade, finely powdered with mortar and pestle and stored in glass vials until extraction.

For extract preparation 5 gm of fine powdered leaves of *Annona reticulata* were macerated with 100 ml of distilled water at room temperature. The mixture was kept for boiling for 60 minutes at 100 °C. The obtained solution was cooled, filtered and then was stored in refrigerator for further use.

2.2 Synthesis of nanoparticles:

The prepared 50ml leaf extract was boiled at 60-70 °C and then the 5gms of $Cu(NO_3)_2.5H_2O$ was added to the boiling leaf extract and was left for boiling until it was converted to a fine paste. The paste thus formed was dried in oven and finally the obtained powder was stored in sealed bottle for further testings.

2.3 Evaluation of secondary metabolites

Qualitative analysis for tannin:

Crude extract was mixed with 2 ml of 2% solution of Ferric Chloride. A blue-greenblack colouration indicated the presence of phenols & tannins. (1ml sample + 500µl $FeCl_3$)

Qualitative analysis for saponins:

Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 seconds. 1cm layer of further stable for 1 min indicates the presence of saponin. (1ml sample + 5ml distilled water).

Qualitative analysis for flavnoids:

Extract were treated with few drops of sodium hydroxide solution. Formation of yellow colour which become colourless on addition of dilute acid, indicate the presence of flavnoid. 10% NaOH solution (2ml) + 100 µl sample gives yellow colour.

2.4 UV – Visible Spectrophotometry

The aqueous extract of the synthesized CuO nanoparticles was used as a reaction mixture for measuring the UV-Vis spectroscopy. The confirmation of the synthesized nanoparticles was obtained by observing the optical property of the reaction mixture using UV-Vis absorption spectroscopy analysis between 200-800nm. [37]

2.5 Determination of Catalytic Properties of CuO Nanoparticles

The catalytic activity of synthesized CuO nanoparticles using the extract of Annona reticulata herb was evaluated using UV–Vis spectrophotometer, Cary E 500, to monitor the absorbance peaks.. The absorbance was measured in the range 350–800 nm at room temperature. In the first reaction mixture, the aqueous solution of methyl orange (1×10^{-4}M) was monitored by measuring the intensity of absorbance. In the second reaction mixture, 4 ml of methyl orange (MO), 0.5 ml of *Annona reticulate* aqueous extract and 3 ml of distilled water were analyzed. The study of decomposition of methyl orange (MO) used the same amounts of methyl orange and *Annona reticulata* aqueous extract, i.e. 4 ml of MO and 0.5 ml of *Annona reticulata* water extract. For the variant MO I, 0.5 ml of the prepared solution of CuO NPs and 2.5 ml of distilled water were used. For other variants, i.e. from variant MO II to variant MO V, the amounts of the prepared solution of CuO nanoparticles were successively increased by 0.5 ml, and the amounts of distilled water were successively decreased by 0.5 ml. All variants were exposed to sunlight for 120 min.[38]

2.6 Reducing power estimation

The electron donating capacity of the bioactive compounds is defined as the reducing power or the antioxidant activity. The antioxidant property of a compound or extract could be described as a redox reaction in which a reactant species (antioxidant) is reduced by the exposure of the oxidant. Different fraction of aqueous plant extract at various concentration (200 - 1000µg/ml) were prepared. 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferrocyanide was mixed in 1 ml of different extract prepared. The test tubes were incubated in water bath for 10 minutes at 50℃ followed by addition of 2.5 ml of 10% TCA and was centrifuged at 3000rpm for 10 minutes. 2.5 ml of upper layer obtained was collected and mixed with 2.5 ml of distilled water followed by addition of freshly prepared 0.5 ml of 0.1% $FeCl_3$. Absorbance was noted at 700nm against a suitable blank.[39]

2.7 Nitric oxide scavenging activity

Extract of different dilutions (0.1 to 1 mg/ml) dissolved in PBS (25mM, pH7.4) were prepared. 200µl sodium nitroprusside (5mM) was added to 800µl of the prepared dilutions. The mixture was then incubated at 37 °C for 2.5 hours under normal light followed by incubation of 20 minutes in dark. 600µl Griess reagent was added followed by incubation for 40 minutes at room temperature and absorbance was noted at 540 nm against a suitable blank. Control solution was prepared (1.6ml H_2O, 400µl SNP and 600µl Griess reagent) and percent of inhibition was calculated by using this equation:

% inhibition = (OD of control- OD of extract/OD of control)*100. [40]

2.8 Metal chelating activity

Metal chelating activity was measured by adding 0.1 mM FeS04 (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of plant extract. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. [41]

2.9 Total Antioxidant Activity

Different dilutions of 0.5 mg/ml extract were prepared and and to it 4 ml of 28 mM sodium phosphate, 4mM Ammonium molybdate and 0.6 M Sulphuric acid was added and were put in capped test tube and were left for incubation in a water bath for 90 min at 95℃. The incubated sample was thereafter cooled to room temperature and the absorbance was measured at 695 nm against blank. Antioxidant activity was expressed relative to that of BHT which was used as standard.[42]

2.10 FTIR

The functional groups attached to the surface of nanoparticles and the other surface chemical residues were detected using FTIR. The characterization involved Fourier transform infrared spectroscopy (FTIR) analysis of the synthesized CuO nanoparticles by Elmer Spectrum 1000 spectrum in attenuated total reflection mode, and using the spectral range 4000-400 cm^{-1} with the resolution of 4 cm^{-1}.[43]

2.11 XRD

X- ray diffraction patterns were observed by using X-ray diffractometer (MiniFlex 600) with Cu having detector (D/teX Ultra). The instrument was operated at voltage 40kW and current 15mA. Diffraction patterns were run at 5-10 ℃ /min in terms of 2θ; crystal and physical state of nanoparticle characterized.[44]

3. RESULTS AND DISCUSSION

3.1 Qualitative estimation of Secondary metabolites

Flavanoids and phenolics are effective scavengers of free radicals due to the presence of OH group. Their presence indicates high analgesic and anti-inflammatory effects and there is high concentration of these metabolites in aqueous extract of *Annona reticulata* leaves. Tannins are present in higher concentration in aqueous extract of *Annona reticulata* leaves and it possess antiviral, antibacterial and antiparasitic effect. Saponins are present in very low quantity in the aqueous extract of *Annona reticulata*.[45] The proportion of various secondary metabolites present in the leaves of Annona reticulata is depicted in table 1.

3.2 UV Vis Spectrophotometry

The absorbance vs wavelength pattern reveals maximum absorbance at 270 nm as depicted in figure 1 which coincided with the expected value of CuO nanoparticles. Thus the λ_{max} of the CuO nanoparticles formulated from *Annona reticulata* can be 270 nm.[46]

3.3 Reducing power estimation

The reducing power of nanoparticles methanolic extracts increases with the increased in concentration. The reducing power was best observed at 1mg/ml concentration. The presence of reducers (i.e antioxidants) causes the reduction of Fe^{3+} or ferricyanide complex to the ferrous form. Therefore, measuring the formation of Prussian blue at 700 nm gives an indication of Fe^{2+} concentration and the reducing capability.[47]

3.4 Total Antioxidant Assay

The absorbance value obtained in the TAA assay were 0.16,0.17,0.319, 0.24, 0.028 for 500, 250, 125, 75 and 30 µg of CuO nanoparticles, respectively. The corresponding absorbance value

for the standard BHT ranged from 0.68±0.04 to 0.1±0.01(Fig 2). Thus the TAA of nanoparticles were in close conformity with that of BHT results.[48] Maximum activity was observed at a concentration of 125 µg.

3.5 NO Scavenging activity

Endothelial cells, neurons, macrophages. etc generate an important chemical mediator i.e NO and is involved in the regulation of various physiological purposes. Numerous plants having rich quantity of phenolic compounds have been reported as potential inhibitors of NO production in various inflammatory reactions. Chronic inflammatory diseases are treated with the compound but excess concentration of these compounds may also cause several diseases. Decrease in the absorbance at 540 nm determines the NO scavenging property. 0.8 ml concentration revealed least absorbance which exhibits that the leaves extract have good NO scavenging property.[49]

3.6 Metal chelating activity

The ferrous state of iron can stimulate lipid peroxidation by reaction and is most powerful pro-oxidant among various species of metal ions [Kuate etal, 2010]. In this assay, percent inhibition of formation of ferrous complex by extracts of *Annona reticulata* was estimated. The results revealed that Fe^{2+} ion chelating activity of extracts were increasing with increasing order of concentration. The metal chelating activity pattern revealed maximum activity ranging from 0.8-1 mg, thus thereby revealing maximum metal chelating activity.[50]

3.7 Catalytic activity

CuO nanoparticles were used as a catalyst for the disintegration of Methyl Orange. All variants were exposed to sunlight for 120 min for the action of nanoparticles on Methyl Orange. Variants were used from MO1 to MO5 as shown in table. The maximum absorbance wavelength of MO was recorded between 400-420 nm. The figure 3 depicts that the degradation of MO occurs due to the increasing amount of prepared solution of CuO nanoparticles. This confirms the catalytic properties of green synthesized CuO nanoparticles.[51]

3.8 FTIR analysis

A characteristic absorption band were exhibited at 3994 cm^{-1} for OH stretch, 2502 cm^{-1} for CH stretch, 1938 cm^{-1} for CO stretch, 1330 cm^{-1} for C=C stretch,929 cm^{-1} for =CH bending and 1496 cm^{-1} for CO stretch.(Fig 4)

3.9 XRD

The major peaks obtained at 2θ values of 32.15(3), 35.46(8), 46.40(2), 51.089(19) and 66.582(5) in the high angle XRD of CuO nanoparticles indicate the existence of crystalline nature of nanoparticles(fig 5). The major peak positions closely matches with the Joint committee for powdered X-ray diffraction standard (JCPDS NO 02-1225) and also matches with values of monoclinic phase copper oxide nanoparticles[52]. The size of particle may be 76.8nm.

Table - 1: Secondary metabolite analysis

Tests	Fairly present	Moderately present	Highly present
Tannin			+++
Flavnoids			+++
Saponins	+		
Phenolic			+++

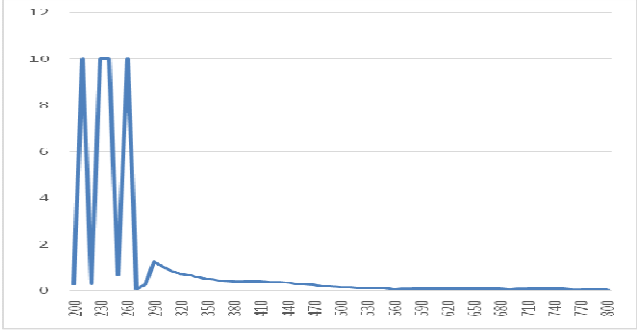

Figure - 1: UV – Vis absorption spectrum of Cu NPs; X axis- wavelength (nm), Y axis- Absorbance.

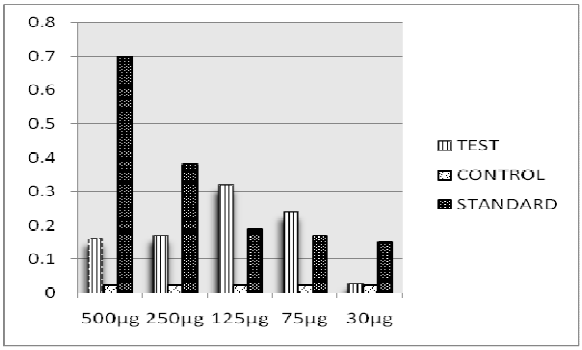

Figure - 2: TAA assay of CuO nanoparticles; X axis – concentration, Y axis- Absorbance.

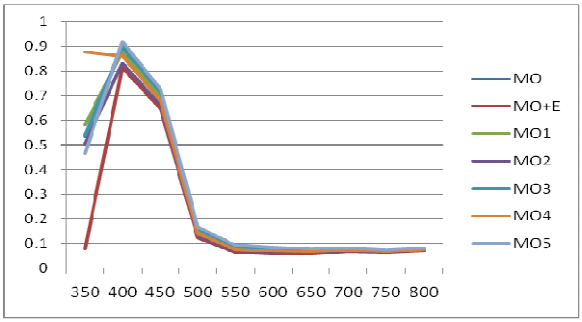

Figure – 3: X axis-wavelength(nm), Y axis-absorbance; Absorption Spectra of MO by CONPs synthesised using Annona reticulata.

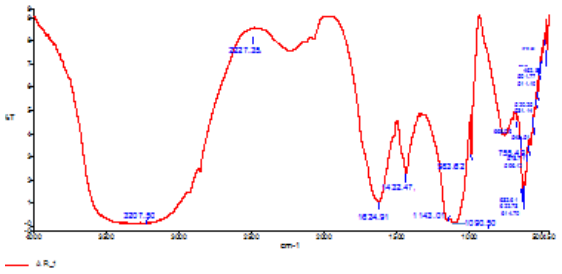

Figure - 4: FTIR Analysis.

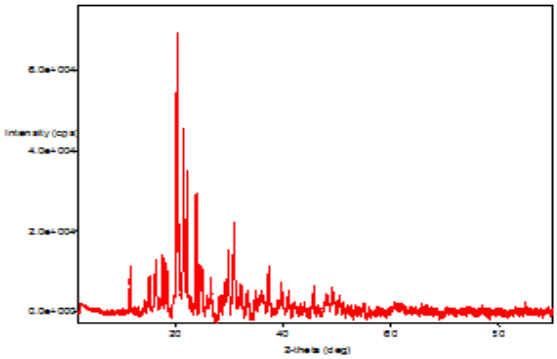

Figure - 5: XRD analysis.

4. CONCLUSION

It is hereby concluded from the present study that the flavonoids and phenolics of leaf extract of *Annona reticulata* had surface active stabilizing molecule for the synthesis of Copper Oxide nanoparticles.

This study also examined the role of aqueous extract of *Annona reticulata* in the formation and stabilization of Copper Oxide nanoparticles. The synthesized nanostructures have been characterized by UV Vis, FTIR & XRD profile. The potential particles had good catalytic & antioxidant activity. To conclude, this study presents the ecological method for preparing nanoparticles without any harmful effects.

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