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RESEARCH ARTICLE

Green synthesis of copper nanoparticles using bracts of *Musa paradisiaca* (Monthan) and study of its antimicrobial and antioxidant activity

Gayathri.V[1], Nivedha.S[1], Pujita.V[1], Ivo Romauld.S[2]
[1]Department of Biotechnology, Rajalakshmi Engineering College, Chennai-602105, Tamil Nadu, India
[2]Department of Bio-Engineering, VISTAS, Chennai-600 117, Tamil Nadu, India
*Corresponding Author E-mail: **pujitavinod@gmail.com**

ABSTRACT:

Nanotechnology is becoming an emerging field of science as it produces nanoparticles that are nanosized with high pore size and surface to volume ratio along with good chemical and physical properties when compared to bulk. Green synthesis of nanoparticles is becoming a most researched topic since it is environmental friendly, cost effective and less toxic. *Musa paradisiaca*, has high antimicrobial and antioxidant activity and hence used as an antimicrobial agent and antioxidant extensively. Our project mainly focuses on the synthesis of copper nanoparticles in the range of nanoscale using bracts of *Musa paradisiaca* (Monthan) and characterization of the formed nanoparticle by using SEM-EDX and FTIR Spectroscopy analysis followed by phytochemical screening of flavonoids and phenols and antimicrobial assay using *E.coli* and *S.aureus* and antioxidant assay. The present study initialized with the preparation of bract extract by heating the bract sample along with distilled water in the ratio of 1:3. The extract was then filtered with Whatman filter paper 42 and then it was mixed with 0.05M copper sulphate in the ratio of 1:1 and heated till the color changed to brownish yellow. The solution was centrifuged and the collected pellet was dried. SEM-EDX analysis was carried out and the surface topology and size of nanoparticles was observed and recorded. Phytochemical screening and FTIR Spectroscopy studies were performed to detect and confirm the presence of functional groups in the bract extract and synthesized nanoparticles. The antimicrobial assay was carried out for the bract extract and the synthesized nanoparticles and effective Zone of inhibition was observed in the synthesized nanoparticle. The antioxidant activity was estimated by DPPH radical scavenging assay and efficient activity was recognized in the synthesized nanoparticle. The synthesized nanoparticle can be effectively used as an antimicrobial and antioxidant agent.

KEYWORDS: *Musa paradisiaca*, Green synthesis, SEM-EDX, FTIR Spectroscopy, Antimicrobial assay, Antioxidant assay.

INTRODUCTION:

India, a richest country in terms of its flowering plants contains more than 50 different species of Musa[1]. Musa belongs to one of the genera of the family Musaceae which includes bananas and plantains. The three major species of *Musa* genus are *Musa acuminate*, *Musa balbisiana* and *Musa paradisiaca*. *Musa paradisiaca* is a hybrid between *Musa acuminate* and *Musa balbisiana*[2].

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The plant is stout and tall, can withstand drought making it suitable for rainfed cultivation. The bracts are large, spatheceous and of various shades of purple, ovate, closely imbricate, spirally arranged and often deciduous. The bracts are overlapped each other closely so that the young inflorescence is compact and conical[3]. Musa bracts are traditionally used as food plants by the larvae of some lepidoptera species, various livestock and humans. *Musa paradisiaca*, has rich antibacterial and antibacterial activity due to presence of various phytochemicals. *Musa paradisiaca* can be used as antidote for snake bite, asthma, burns, diabetes, dysentery, excessive menstrual flow and fever[4]. Nanotechnology is becoming an important field of

science as it produces nanoparticles that are nanosized (1-100nm) with high pore size and surface to volume ratio along with good chemical and physical properties when compared to bulk[5]. Nanoparticles are gaining importance in areas such as mechanics, optics, biomedical sciences, chemical industry, electronics, space industries, drug-gene delivery, energy science, catalysis, optoelectronic devices and photoelectrochemical applications[6]. There is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Hence, green synthesis of nanoparticles using plant extract has taken up its emergence due to various advantages such as cost effectiveness, less toxicity and simple procedures[7]. Copper nanoparticles synthesis is gaining importance since it acts as a promising alternative material to gold and silver in terms of its high conductivity and lower cost[8]. Copper nanoparticles are extensively used in batteries, optical devices, polymers, multilayer metal ceramics, drug delivery and as antimicrobial and antioxidant[9]. This study thus focuses on green synthesis of copper nanoparticles which can be used as an antioxidant and antimicrobial agent to treat various infections and diseases.

MATERIALS AND METHODS:

PREPARATION OF BRACT EXTRACT:

All the chemical reagents used in this experiment were of analytical grade. The *Musa paradisiaca* bracts were collected from Nanganallur market, Chennai, India. The collected *Musa paradisiaca* bracts were thoroughly washed and cut into small pieces. 50g of bract sample was boiled with 150ml of distilled water at 80^{o}C for 30minutes in heating mantel. The bract extract was filtered and used for further experiments[10].

SYNTHESIS OF COPPER NANOPARTICLES USING *Musa paradisiaca* BRACT EXTRACT:

For the copper nanoparticles synthesis, 50ml of *Musa paradisiaca* bract extract was added to 50ml of 0.05M aqueous $CuSO_4.5H_2O$ solution in a 250ml conical flaskand heated at 80^{o}C till color changed to brownish-yellow[11]. The solution was purified by centrifugation at 4500rpm for 20minutes and the pellet was collected and dried to obtain Copper nanoparticles.

PHYTOCHEMICAL SCREENING:

The bract extract and synthesized copper particle were tested for the presence of flavonoids and polyphenols. The analysis was carried out using the standard procedure[12].

FLAVONOID TEST:

1ml of bract extract and synthesized particle were mixed with 1ml of 1%NH_3 solution and observed for yellow coloration.

POLYPHENOL TEST:

1ml of bract sample and synthesized particle were mixed with 1ml Na_2Co_3 solution and 1ml of Folin's reagent and examined for blue/green coloration.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS:

Scanning Electron Microscopy was performed to examine the size and surface morphological behaviour of the synthesized copper particle. The sample preparation was carried out by sputtering the synthesized copper particle with gold layer using Ion Sputter Coater with Gold Target instrument. The thin film formed was air-dried under ambient conditions and the images were studied using Hitachi S-3400N instrument with electron beam accelerated at 300V to 30kV and signals are detected by Secondary Electron (SE) /Back Scatter Electron (BSE) detectorsand magnification up to 300000.

ENERGY DISPERSIVE X-RAY (EDX) STUDIES:

EDX was performed to detect the presence of copper in the synthesized particles. The synthesized particles elemental composition was done in spot profile mode using instrument coupled with SEM analysis. The presence of copper was found using the detection graph and the elemental composition was estimated as net counts, atom percentage and weight percentage.

FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY:

FTIR Spectroscopy analysis was carried out to detect the presence of functional groups that are present in the bract extract and on the surface of the synthesized Copper particle. The dried pellet was analysed using Perkin Elmer Fourier Transform Infra-Red (FTIR) Spectroscopy C100599 Instrument which has a resolution of 0.4cm^{-1} at the working range of 350-7800cm^{-1}.

ANTI-MICROBIAL ACTIVITY:

Musa paradisiac bract extract and synthesized copper nanoparticles were used for antibacterial study through the disc diffusion method[13]. Spread plate was prepared with nutrient agar and antimicrobial activities were tested against two different pathogenic bacterial strains namely *Staphylococcus aureus* and *Escherichia coli*.

QUALITATIVE ASSAY OF ANTI-OXIDANT ACTIVITY:

Antioxidant activity of bract extract and synthesized copper nanoparticles was estimated through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay[10]. In order to evaluate the antioxidant potential through free radical scavenging, the change in optical density of DPPH radicals was monitored. The various

concentrations of synthesized copper fine nanoparticles and extract were mixed with 2mL of DPPH solution (4mM) and left in dark at room temperature. After 30min, the absorbance was measured at 517nm. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

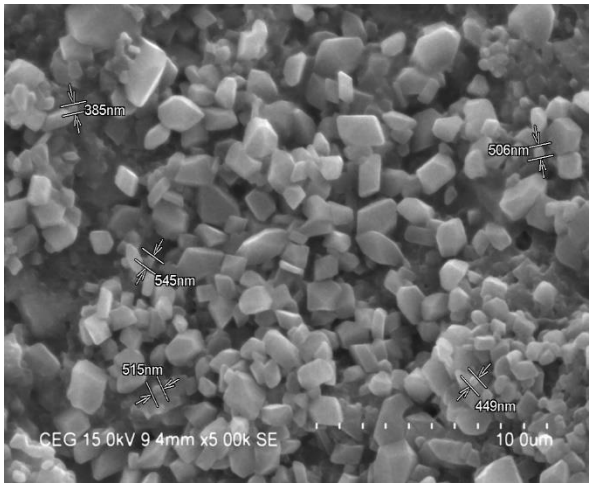
$$\text{Inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of sample}) \times 100}{(\text{Absorbance of control})}$$

RESULTS AND DISCUSSION:

The bract sample was extracted from *Musa paradisiaca* using aqueous solvent. Copper nanoparticle was synthesized from the bract extract using copper sulphate pentahydrate ($CuSO_4.5H_2O$). The synthesized nanoparticle was characterized by SEM-EDX analysis, FTIR spectroscopy. The antimicrobial and antioxidant activity was tested for the bract extract and the synthesized copper nanoparticles.

SEM ANALYSIS:

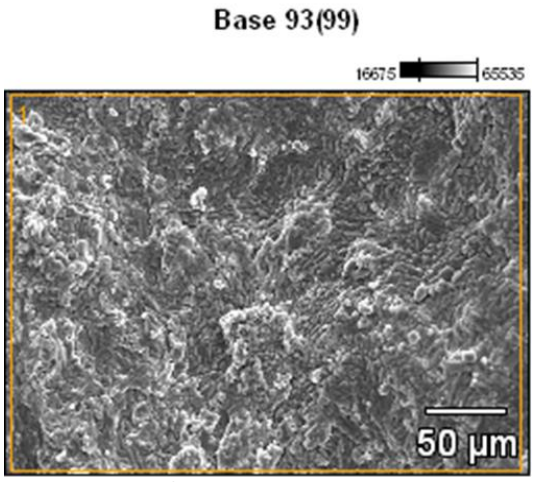
SEM Analysis was carried out to determine the shape of the synthesized nanoparticles. The size of the nanoparticles was found to be in the nanoscale range (1-1000 nm) with an average nanoparticles size of 480nm.



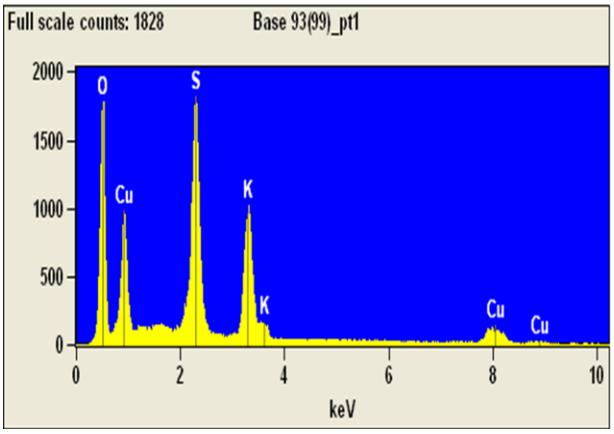
Img.1: SEM Result of synthesized copper nanoparticles

EDX ANALYSIS:

EDX analysis was performed for elemental identification and quantitative compositional information. The EDX confirmed the presence of copper along with sulphur, oxides and potassium. 11.87% (Weight percentage) of copper was present in the synthesized nanoparticles.



Img.2: EDX analysis of synthesized copper nanoparticles



Img.3: Elemental composition of the synthesized nanoparticles

Net Counts

	O	S	K	Cu
Base 93(99)_pt1	19961	25350	16171	1569

Weight %

	O	S	K	Cu
Base 93(99)_pt1	49.75	20.04	18.34	11.87

Atom %

	O	S	K	Cu
Base 93(99)_pt1	70.82	14.24	10.69	4.25

Img.4: EDX of synthesized copper nanoparticles

PHYTOCHEMICAL SCREENING:

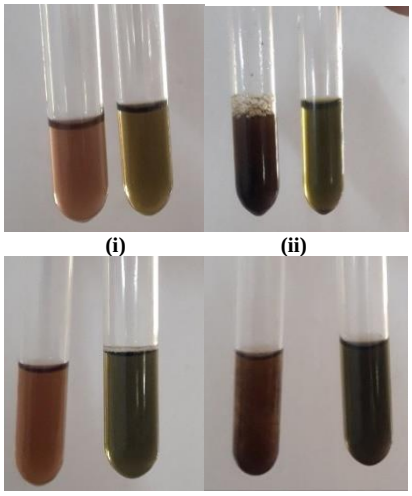
TEST FOR FLAVONOID:

The colour of the extract and copper nanoparticle changed from brownish red to yellow after the addition of ammonia. Yellow colouration of the extract and the synthesized copper particle showed positive result for

the flavonoid test and the presence of flavonoid was detected.

TEST FOR POLYPHENOL:

The colour of the extract and copper nanoparticle changed from brownish red to Blue green after the addition of Na_2Co_3 solution and Folin's reagent. Blue green colouration of the extract and the synthesized copper particle showed positive result for the polyphenol test. Thus the presence of polyphenol was identified.



(i) (ii) (iii) (iv)

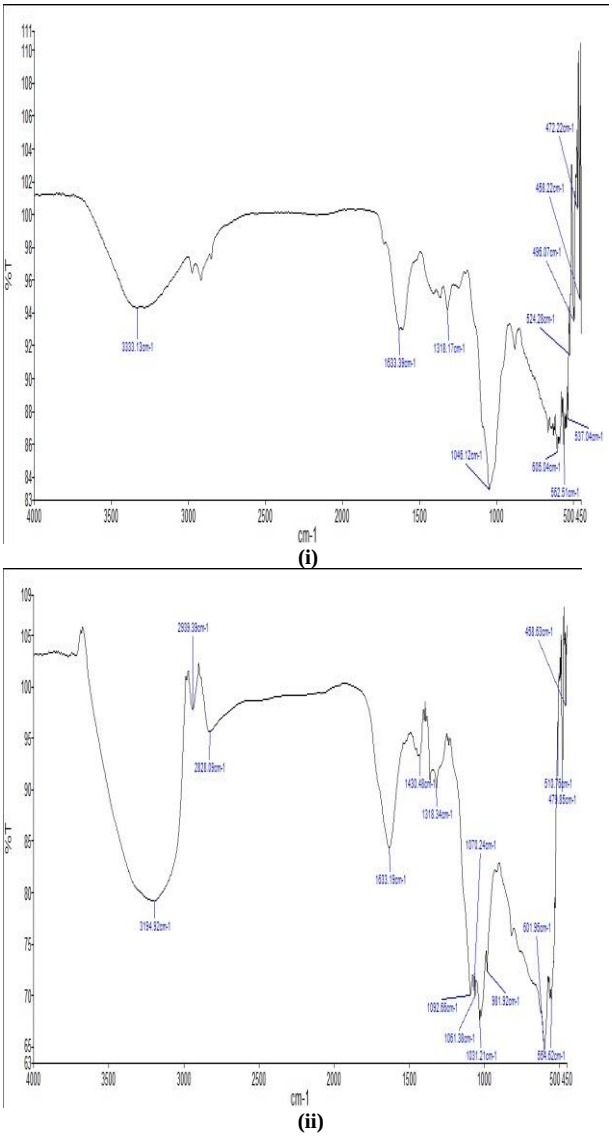
Fig.1:Phytochemical screening of bract extract and synthesized nanoparticles
(i)Presence of flavonoid of bract extract (ii)Presence of flavonoid of synthesized nanoparticle (iii)Presence of polyphenol of bract extract (iv)Presence of polyphenol of synthesized nanoparticle

FTIR SPECTROSCOPY STUDIES:

The FTIR reported the peaks at different wavenumbers and indicated the presence of different functional groups in extract. The C=O stretching of flavonoid was found at 1633.39 cm^{-1} and the O-H stretching of phenol was found at 1318.17 cm^{-1} in both the bract extract and the synthesized nanoparticles. Other functional groups like alkane, anhydride, alcohol, secondary amine and sulfoxide were also present.

Table 1: Presence of functional groups in bract extract

Wavenumber (cm^{-1})	Functional group	Compound
3333.13	N-H stretching	Secondary amine
1633.39	C=O stretching	Carbonyl group of flavonoids
1318.17	O-H stretching	Phenol
1046.12	CO-O-CO stretching	Anhydride
605.04	C-Br stretching	Halo compound
562.51	C-Br stretching	Halo compound



(i)

(ii)

Fig.2: (i)FTIR peaks for synthesized copper nanoparticles (ii)FTIR peaks for bract extract

Table 2: Presence of functional groups in synthesized copper nanoparticle

Wavenumber (cm^{-1})	Functional group	Compound
3194.92	O-H stretching	Alcohol
2939.39	C-H stretching	Alkane
2828.09	C-H stretching	Aldehyde
1633.19	C=O stretching	Carbonyl group of flavonoids
1430.48	C-H bending	Alkane
1318.34	O-H stretching	Phenol
1070.24	S=O	Sulfoxide

ANTIMICROBIAL ACTIVITY ANALYSIS:

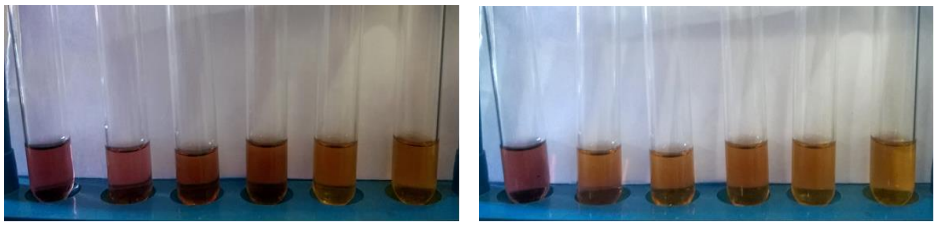
The antimicrobial activity was tested for the bract extract and the synthesized Cu nanoparticles using *E. coli* and *S. aureus* and the Zone of inhibition was determined using Well Diffusion method.

Table 3: Antimicrobial assay using bract extract and synthesized nanoparticles

Zone of inhibition (cm)	Bract extract	Synthesized nanoparticles	Water (negative control)	Gentamycin (positive control)
E. Coli	1.35 ± 0.25	2.23 ± 0.33	0	2.3 ± 0.1
S. Aureus	0.95 ± 0.25	1.43 ± 0.28	0	2.13 ± 0.047

(i) (ii) (iii) (iv)

Fig.3: (i) Zone of inhibition in *E. coli* for bract extract (ii) Zone of inhibition in *E. coli* for synthesized nanoparticles (iii) Zone of inhibition in S. aureus for bract extract (iv)) Zone of inhibition in S. aureus for synthesized nanoparticles



(i) (ii)

Fig.4: (i)Antioxidant assay of bract extract (ii) Antioxidant assay of synthesized nanoparticle

Table 4: Results of antioxidant assay for synthesized copper nanoparticle

Concentration of copper nanoparticle (µg/mL)	OD values at 517nm				Inhibition (%)
	Trial 1	Trial 2	Trial 3	Mean	
Control				0.943	0
100	0.778	0.784	0.781	0.781	17.18
200	0.636	0.631	0.632	0.633	32.82
300	0.423	0.429	0.426	0.426	54.82
400	0.369	0.375	0.372	0.372	60.55
500	0.240	0.247	0.245	0.243	**74.17**

Table 5: Results of antioxidant assay for bract extract

Concentration of bract extract (µg/mL)	OD values at 517nm				Inhibition (%)
	Trial 1	Trial 2	Trial 3	Mean	
Control				0.943	0
100	0.864	0.869	0.868	0.867	8.16
200	0.773	0.778	0.777	0.776	17.76
300	0.699	0.705	0.702	0.702	25.55
400	0.629	0.624	0.628	0.627	33.56
500	0.552	0.546	0.549	0.549	**41.78**

The Zone of inhibition of the extract for *E. coli* and *S. aureus* was found to be 1.35±0.25 cm and 0.95±0.25 cm respectively. The Zone of Inhibition of the synthesized nanoparticles for *E. coli* and *S. aureus* were found to be 2.23±0.33 cm and 1.43±0.28 cm respectively. Gentamicin was used as a positive control and showed Zone of inhibition for *E. coli* and *S. aureus* as 2.3±0.1cm and 2.13±0.04 cm respectively. Water was used as negative control. The study revealed that the synthesized copper nanoparticles showed effective antimicrobial activity against *E. coli* and *S. aureus* when compared to the bract extract.

ANTIOXIDANT ACTIVITY ANALYSIS:

The antioxidant activity analysis was done to evaluate the free radical scavenging ability of the extract and the synthesized nanoparticles. The activity was found by DPPH Free Radical Scavenging Assay. The assay revealed that there is an increase in antioxidant activity of bract extract and the synthesized Copper nanoparticles with increase in concentration. The maximum activity was found to be 41.78% inhibition for the bract sample and 74.17% inhibition for synthesized Copper nanoparticles at 500µg/mL. Thus the study revealed the presence of effective antioxidant activity for the synthesized nanoparticles than the extract.

CONCLUSION:

Copper nanoparticles were synthesized from the bract extract of *Musa paradisiaca* using $CuSO_4.5H_2O$. The synthesized nanoparticles showed effective antimicrobial activity against *E. coli* and *S. aureus* and antioxidant activity. Thus the bract of *Musa paradisiaca* can be used as a potential therapeutic agent to treat various infections and diseases.

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