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GREEN SYNTHESIS OF COPPER NANOPARTICLES USING AQUEOUS EXTRACT OF *AEGLES MARMELOS* BARK AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The present study, deals with investigating effect of biosynthesis method. In this method the interaction time on morphology and size of copper sulphate pentahydrate. Nano particles synthesized using aqueous bark extract. The plant extracts plays a vital role in the field of nanotechnology as it is environmentally friendly and does not involved any harmful chemicals. The synthesized nanoparticles were characterized by using UV-vis spectroscopy, X-ray diffraction(XRD),fourier transform infrared spectrometer(FT-IR)analysis, SEM were carried out to determine the nature of the capping agents in *aegles marmelos* bark extract . The antimicrobial activity of the synthesized nanoparticles were also examined. The result confirmed this protocol as simple, rapid, one step eco-friendly, non-toxic and an alternative conventional physico-chemical method.

KEYWORDS: Nanoparticle, antimicrobial, SEM, FT-IR., XRD.

INTRODUCTION

In recent years, nanotechnology is emerging as cutting-edge technology interdisciplinary with physics, chemistry, biology, material science, medicine and engineering. Nanoparticle is a 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 1-100nm [1] due to small size it occupies a position in various fields of nano science and nanotechnology.

Among the nanoparticles Copper Nanoparticle gained much importance because of low cost of preparation, and has excellent physical and chemical properties. Copper Nanoparticle are very reactive due to their surface- to -volume ratio and easily interact with otherparticle.[2] Copper Nanoparticles have wide applications like Anti fouling, biocidal, Catalytic activity, gas sensor, wound dressing and solarcells.[3] The biosynthesis of pure metallic copper nanoparticles in aqueous phase is still an open challengefor bio-nanotechnologists.

The biomolecules like alkaloids, flavones, terpenes, aminoacids and carbohydrates are present in the plant material can acts as reducing agent as well as capping agent can increase the rate of nano synthesis as well as the stability of the product.

Aegle marmelos (L.) Correa is commonly known as bael, belongs to the family Rutaceae, is a moderate sized, slender and aromatic tree. In Tamil, it is called vilvam indigenous to India. It is a deciduous sacred tree associated with Lord Shiva. Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils.[4] A drug balae fructose extracted from the fruits with mucilage and pectin content is very useful for treating chronic diarrhoea, dysentery, hemorrhoids and swellings. The leaf juice mixed with black pepper is used to treat jaundice and asthmatic complaints. The antibiotic potential of the leaf, fruit and root helps in curing diarrhoea,dysentery, asthma and fever. The bark and root soaked in water for a over night in a copper vessel are used to cure blood pressure, diabetes and leprosy.[5-7]

MATERIALS AND METHODS

Preparation of bark extract

Fresh *Aegle marmelos* barks were collected(Fig 1).The collected barks were washed several times under running water and then washed with distilled water 3 to 4 times to remove dust particles. The barks were dried in shade to remove the moisture content and powdered in a blender. About 10g of *Aegle marmelos* bark sample was added to 400ml of distilled water and heated to 60^0c with continous stirring time for 20minutes(Fig 2).The mixture was cooled to room temperature, filtered with whatmann no 1 filter paper to obtain extract(100%).The extract was collected and stored in a brown bottle. The extract functions as both the reducing and stabilizing agent.

Phytochemical screening and qualitative analysis

Aegle marmelos stock solution was used for preliminary qualitative phytochemical screening. The screening was carried out by standard phytochemical methods.

Test for Terpenoids

To the 2ml of extract add 2ml of acetic anhydride and 2-3 drops of concentrated sulphuric acid. Presence of deep red coloration indicates the presence of terpenoids.

Test for Tannins

To the 2ml of extract add 2ml of water and add few drops of ferric chloride. Formation of green precipitate indicates the presence of tannins.

Test for phlobatannins

To the 2ml of extract add 2ml of hydrochloric acid. Formation of red precipitate indicates the presence of phlobataninns.

Test for anthocyanins

To the 2ml of extract added, 2ml of hydrochloric acid and a few drops of ammonia, presence of pinkish red to bluish violet coloration indicates the presence of anthocyanins.

Test for proteins

To the 1ml of extract add 1ml of conc. sulpuricacid. Formation of white precipitate indicates the presence of protein.

Test for saponins

To the 5ml of extract add 5ml of water, the appearance of heat froth indicates the presence of saponins.

Test for steroids

To the 2ml of extract add 2ml of chloroform and 2ml of concentrated sulphuric acid. Appearence of reddish brown ring at the junction indicate the presence of steroids.

Test for glycosides

To the 2ml of extract add 2ml of chloroform and 2ml of acetic acid. The colour changes from violet to blue to green indicates the presence of glycosides.

Test for alkaloids

To the 2ml of extract add a few drops of hager's reagent. The formation of yellow precipitate indicates the presence of alkaloids.

Test for Emodins

To the 2ml of extract add 2ml of ammonium hydroxide and 3ml of benzene red coloration indicates the presence of emodins.

Test for anthraquinones

To the 3ml of extract add 3ml of benzene and 5ml of ammonia. the formation of pink or violet or red coloration in ammoniacal layer, indicates the presence of anthraquinones.

Test for anthocyanins

To the 2ml of extract add 2ml of hydrochloric acid and ammonia. The colour changes from pinkish red to bluish violet, indicate the formation of anthocyanins.

Test for sterols

To the extract add few drops of chloroform and concentrated sulphuric acid,shaken well and tost for some time. The appearance of red colour in upper layer indicates the presence of sterols.

Test for Triterpenoids

To the extract add few drops of chloroform and concentrated sulphuric acid, shaken well and tost for some time. The formation of yellow colour at the lower layer indicates the presence of triterpenoids.

Synthesis of Copper Nanoparticle

Various concentrations of root bark extract solutions (5-10%) and copper sulphate solutions (2Mm-6Mm) have been employed to synthesize copper nanoparticles(CuNP's). The light green colour of the mixture of copper sulphate and bark extract at 0min of reaction time changed very fast at room temperature. After 2min dark green suspended in mixture is obtained. This colour change indicates the biosynthesis of copper nanoparticles(Fig 3a &3b).

Reactant concentration for the synthesis of CuNP'S

$CuSO_4$	RBFR
2mM	5%,7.5% , 10%
4mM	
6mM	

Characterization Techniques

UV-Visible Spectra Analysis

The research results have shown the formation of various nanoparticles from different salt that gives characteristic peaks at 24hrs time interval at different absorptions using UV-visible spectroscopy. Cu Nps show characteristic absorption peaks at the range of 200-800 nm. A progressive increase in the characteristic peak with increase in reaction time and concentration of biological extracts with salt ions is a clear indicator of nanoparticle formation. UV-vis absorption spectrum shows peaks characteristics of the surface plasmon resonance of nanosized particles.

FT-IR Analysis

To determine the biomolecules present in the bark extract, FTIR analysis was carried out which is responsible for the reduction of Copper ions with the spectral range of 400-4000 cm-1. Here the sample was centrifuged at 10,000 rpm for 20 min, dried using hot air oven and ground with KBr to form a pellet. Then the pellet was analyzed using Jusco 5300 model FTIR instrument.

SEM Analysis

Morphology and mean particle size of the Cu were determined by SEM analysis. The samples were prepared for SEM analysis. The SEM analysis was established by using Supra Zeiss with 1nm resolution at 30 kV with 20 mm Oxford EDS detector.

XRD Analysis

XRD measurements of the reduced CuNPs perform 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. The samples were taken in lids and put under instrument for analysis.

Anti-microbial assay

Antibiogram was done by disc diffusion method (NCCLS,1993;Awoyinka et al.,2007)using plant extract. Petri plates were prepared by pouring 30ml of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10minutes.The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of

the nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus* specie of bacteria were spread on nutrient agar plates for bacteria and *Candida Albicans* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filterpapers(6mm diameter)containing the crude extract (50µl,100µland150µ)were laid down on the surface of innoculated agar plate. The plates were incubated at 37^0C for 24hrs for the bacteria and at room temperature (30 ± 1) for 24-48hrs for yeasts strains. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

The qualitative phytochemical screening of *Aeglesmarmelos* bark extract was done and the result are shown in the Table.1.

Table.1 Phytochemical compounds of bark powder of aegle marmelos in aqueous solvent

Phytochemical compounds	Plant name	Water solvent
Terpenoids	*Aeglemarmelos* bark	+
Flavanoids	*Aeglemarmelos* bark	+
Alkaloids	*Aeglemarmelos* bark	+
Steroids	*Aeglemarmelos* bark	-
Glycosides	*Aeglemarmelos* bark	-
Carbohydrates	*Aeglemarmelos* bark	+
Saponins	*Aeglemarmelos* bark	-
Tannins	*Aeglemarmelos* bark	+
Proteins	*Aeglemarmelos* bark	+
Saponins	*Aeglemarmelos* bark	+
Emodins	*Aeglemarmelos* bark	+
Anthraquinones	*Aeglemarmelos* bark	_
Anthocyanins	*Aeglemarmelos* bark	+
Sterols	*Aeglemarmelos* bark	_

UV-Visible Spectral Analysis

The reduction of Cu^+ ions was monitored by UV-Vis Spectrophotometer for the metal ions stability. The characterization of copper Nanoparticles by UV-Spectrophotometer from the range 275nm(Fig 4).

FT-IR

The FTIR spectrum of copper nanoparticles is shown (fig 5).the IR spectrum of copper nanoparticles shows band at 3525cm-1,3317cm-11632cm-1,1105cm-1,619cm-1corresponds to OH stretching H bonded alcohols and phenols, carbonylstretching,NHstretching ,NO_2assymetric stretching,CH stretching, CH bending respectively. FTIR spectrum of Cu

nanoparticle were surrounded by different organic molecules such as alcohols,ketones aldehydes. These functional groups plays an important role in these Cu nano particle synthesis.

Scanning Electron Microscopy [SEM]

The surface morphology and size of the nanoparticles were obtained by Scanning Electron Microscopy analysis. The SEM image of aegle marmelos stabilized copper nanoparticles indicated that the nanoparticles were aggregates roughly around 5µm particle size(Fig 6).

XRD Analysis

The crystallite size of material, packing and morphology tested using XRD spectrometer with Cu source on the basis of powder diffraction method and the size was calculated by using Scherrer equation.

D = $B\lambda/\beta COS\theta$

Where, d is the average crystallite size of the phase under investigation,

B is the Scherrer constant (0.89),

λ is thewavelength of X-ray beam used,

β is the full-width half maximum (FWHM) of diffraction and

θ is the Bragg's angle.

X ray diffraction studies of CNPs were investigated from the angle of 100 to 800. The intensity plotted against angle(2θ in degrees) showed the average crystallite size. The average particle size is calculated to be 45 nm by using Scherrer formula. The crystal structures of copper nanoparticles were shown (Fig 7).

ANTIMICROBIAL ACTIVITY

The antibacterial activity of the copper nanoparticle synthesized from Aeglemarmelosbark extract was examined against *Staphylococcusaureus*bacteria using disc diffusion method.The maximum zone of inhibition by copper nanoparticle is obtained (Fig 8a).

The antifungal activity of the copper nanoparticle synthesized from Aeglemarmelos bark extract was examined against *Candida albicans* fungi using the disc diffusion method.The maximum zone of inhibition by copper nanoparticle is obtained (Fig 8b) (Table 2).

Fig 1: Aegle marmelos bark

Fig 2:preparation of stock solution after heating

Fig 3(a) synthesise of copper nanoparticle before stirring.

Fig 3(b) synthesise of copper nanoparticle after stirring.

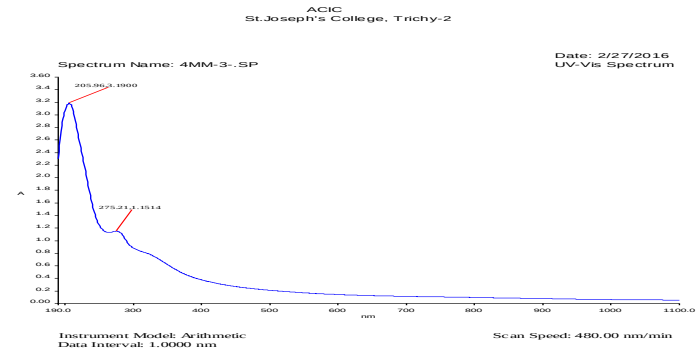


Fig 4: UV report of synthesized CuNP'S

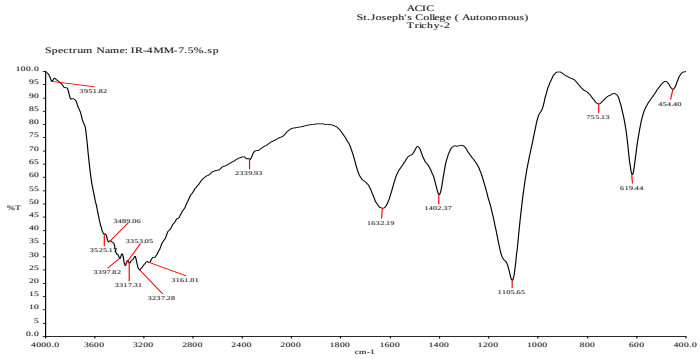


Fig 5: FTIR spectra for copper nanoparticle

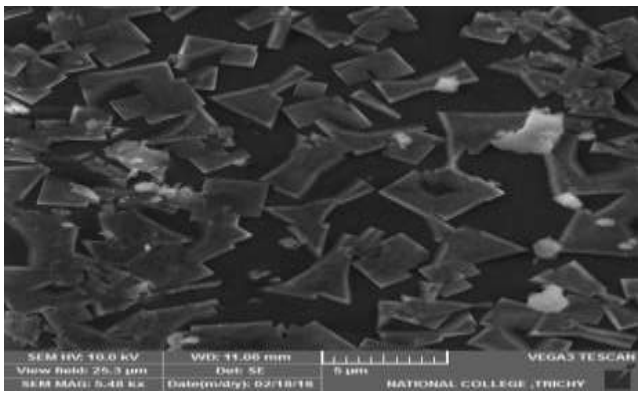


Fig 6:SEM image of CuNP's

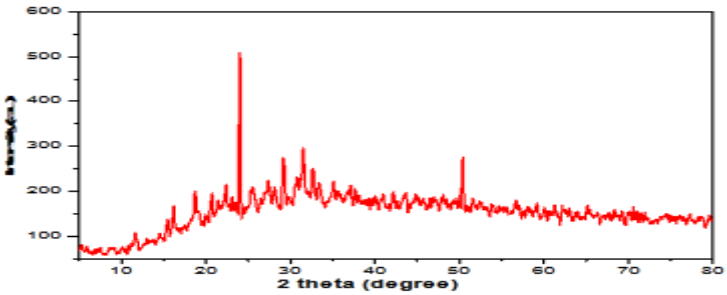
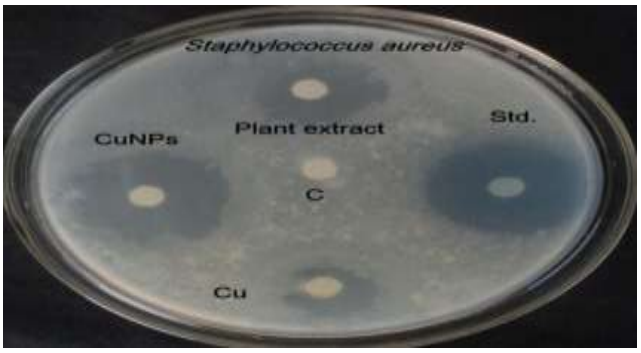


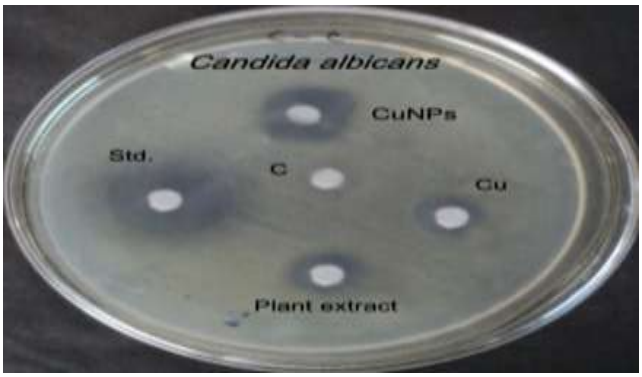
Fig. 7 XRD pattern of copper nanoparticles

Antimicrobial activity of copper nanoparticle



Staphylococcus aureus

Fig 8a:Antibacterial activity of CuNP'S



Candida albicans

Fig 8b:Antifungal activity of CuNP'S

Table 2: antimicrobial activities of copper nanoparticles

Microorgan Isms	Cu (30µl)	Plant extract (µl)	CuNPs (30µl)	Standard (µl)	Control (solvent) (µl)
Staphylo coccusaureus(mm) (mm)	0.64± 0.04	2.61± 0.18	4.85± 0.33	8.12± 0.56	0
Candida albicans(mm)	0.42± 0.02	2.58± 0.17	4.23± 0.29	6.49± 0.45	0

Values were expressed as Mean ± SD

Bacterial standard - Chloromphenical

Fungal standard - Fluconazole

CONCLUSION

Green synthesis of copper nanoparticles by the help of green plants is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale. *Aegle marmelos* showed great capability to synthesis CuNPs at optimum temperature

conditions. The UV absorption peak at 275.00 nm clearly indicates the synthesis of CuNPs. The SEM studies was helpful at deciphering their morphology and distribution. FTIR studies confirmed the biofabrication of the CuNPs by the action of different phytochemicals with its different functional groups present in the extract solution. The XRD patterns confirmed the purity, phase composition and nature of the synthesised nanoparticles. The CuNPs have great antimicrobial activity against *Staphylococcus aureus* and *candida albicans.*

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