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Biogenic fabrication of copper oxide nanoparticles using *Merremia tridentata* (L.) Hallier f. and its antibacterial activity

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Abstract
In this article, we demonstrate the green synthesis of copper oxide (CuO) nanoparticle using the aqueous extract of *Merremia tridentata* (L.) Hallier f., which serves as the capping and reducing agent. The biosynthesized CuO nanoparticles were characterized by UV-Visible spectroscopy and X-ray diffraction (XRD). The *in vitro* antibacterial activity of biosynthesized nanoparticle was compared with the ethanol and ethyl acetate extracts of *Merremia tridentata.* Biosynthesized CuO nanoparticles shows significant inhibition activity against *Bacillus subtilis* (MTCC No.2413), *Klebsiella pneumoniae* (MTCC No.3384), *Staphylococcus aureus* (MTCC No.87) and *Escherichia coli* (MTCC No.443) with an inhibition zone of 14 mm, 13 mm, 12 mm and 11 mm respectively, when compared to the other extracts. The potent antibacterial activity of eco-friendly, plant based CuO nanoparticles from *Merremia tridentata* can be exploited as a remedy against the tested pathogens.

Keywords: *Merremia tridentata*, green synthesis, nanoparticles, copper oxide, antibacterial activity

Introduction
Nanotechnology is an advancing field of science, which incorporates the extraordinary activity of nanoparticles with the size range of 1-100 nm (Simon *et al*., 2022) [19]. For the synthesis of nanoparticles, biological or green method have been suggested to address the limitations of physical and chemical methods. The plant parts such as leaves, fruits, flowers, roots, etc. are used for the preparation of extract to carry out the green synthesis (A. M. Al-Faouri *et al*., 2021) [1]. Nanoparticles synthesized from medicinal plant extract will benefit their application in the field of biomedicines (Bhavyasree *et al*., 2022) [4].
The nanomedicines produced by biogenic fabrication or green synthesis enhances the safety profile of the drug (Mittal *et al*., 2022) [11]. The potential benefits of nanomedicines including improved efficacy, bioavailability, active targeting ability, greater dose response, drug delivery, enhanced solubility, retention effect and less toxicity leads to the development of treatments such as chemotherapy, radiotherapy, targeted therapy and surgery using nanoparticles (Sevastre *et al*., 2019) [16] (Ventola, 2012) [23].
Nanoparticles are currently utilized to target the multi drug resistant (MDR) strains of bacteria, which shows resistance against almost all mode of actions of antibiotics. Unlike antibiotics, the action of nanoparticles occurs by cell wall contact and not by penetrating the cell. This makes less bacterial resistance to nanoparticles and marks the importance of nanoparticle based material to treat bacterial infections effectively (Amin *et al*., 2021) [3].
In the area of biomedicines, biocompatible CuO nanoparticles exhibit potent antibacterial, antifungal, antiviral, antiparasitic, antidiabetic and antioxidant activity (Naz *et al*., 2023) [13]. Due to the large surface area and small size, low dose of CuO nanodrugs is sufficient to exhibits it potency compared to conventional drugs (Sulaiman *et al*., 2022) [20]. Green synthesis of CuO nanoparticles are reported in *Catharanthus roseus* (Dayana, K.S. *et al*., 2021) [7], *Gloriosa superba* (Naika *et al*., 2015)[12], *Lantana camara* (Chowdhury, R. *et al*., 2020) [5], *Camellia sinensis* (Jeronsia, J.M. *et al*., 2019) [8] *Calotropis gigantean* a (Sharma, J.K. *et al*., 2015) [17], *Psidum guajava* (Das, D. & Goswami, S., 2019) [6], *Eletteria cardamomum* (Venkatramanan *et al*., 2020) [22], *Saraca indica* (Prasad *et al*., 2015) [14], *Aloe vera* (Kumar *et al*., 2015) [10], *Ixora coccinea* (Yedurkar *et al*., 2017) [24], *Ocimum basilicum* (Altikatoglu *et al*., 2017) [2].

In the present study, the whole plant extract of *Merremia tridentata* was used for the green synthesis of CuO nanoparticles. *Merremia tridentata* (Convolvulaceae) commonly known as Prasarani, is a slender, perennial, prostrate herb (Sharma, 2022) [18]. Phytochemistry of *Merremia tridentata* reveals the presence of flavanoids such as luteolin 7-O-beta-D-glucosides, diosmetin 7-O-beta-D-glucosides; alkaloids like hygrine, nicotine; quinone; tannins; phenolic acids; saponins; p-hydroxy benzoic acid; vanillic acid and syrigic acid (Kandhasamy Sowndhararajan and Nyuk Ling Chin, 2014) [9] (Rajashekhara *et al*, 2012) [15]. In traditional medicines, *Merremia tridentata* was reported for its use in ailments such as urinary infections, ulcers, swellings, piles, wound healing and rheumatic affections (Sharma, 2022) [18].

Materials and Methods

Chemicals and reagents

Copper acetate [$Cu(CH_3COO)_2$], distilled water, de-ionized water, ethanol, ethyl acetate.

Collection and identification of plant material:

The whole plant of *Merremia tridentata* (L.) Hallier f. were freshly collected from Alappuzha district of Kerala, identified using Flora of the Presidency of Madras by J.S. Gamble and authenticated by experts.

Preparation of plant extracts

The whole plant of *Merremia tridentata* were surface cleaned and washed repeatedly with tap water followed by distilled water to remove extraneous matter. The plant material was shade dried for 10 days and were grind using a grinding machine to obtain fine powder.

- 10 g of the powdered plant material were soaked separately in 100 ml ethanol and ethyl acetate, taken in screw cap bottles. The container was closed tightly and kept at room temperature for 7 days with frequent agitation. Finally, the extracts were filtered out using Whatman No. 1 filter paper and evaporated to obtain the concentrated extracts (in ethanol and ethyl acetate).
- For aqueous extract, 10 g of the powder plant material were mixed with100 ml de-ionized and allowed to boil for 30 minutes in water bath. The mixture was then cool at room temperature and filtered out using Whatman No. 1 filter paper.

Green synthesis of copper oxide nanoparticles

About 4.99 g of copper acetate was dissolved in 250 ml de-ionized water (0.1 M copper acetate solution) with constant stirring for 5 min. Add 100 ml aqueous extract of *Merremia tridentata*, drop by drop in to it and mixed uniformly using a magnetic stirrer at 70ºC. Under continuous stirring for 1 hour, blue colour of copper acetate solution changes to deep green and then to brown- black colour. Afterwards, cool the brown-black mixture to attain the room temperature of 30ºC. Centrifuge the mixture at 10000 rpm for 5 minutes. Filter the resulting pellet using Whatman No.1 filter paper and allow to dry for an overnight. Scratch the filter paper to obtain the powder and grind it in a mortar and pestle. Finally, the powder is transferred into silica crucible for heating at 500 ºC in furnace for 3 hours to remove all the impurities (Fig.1).

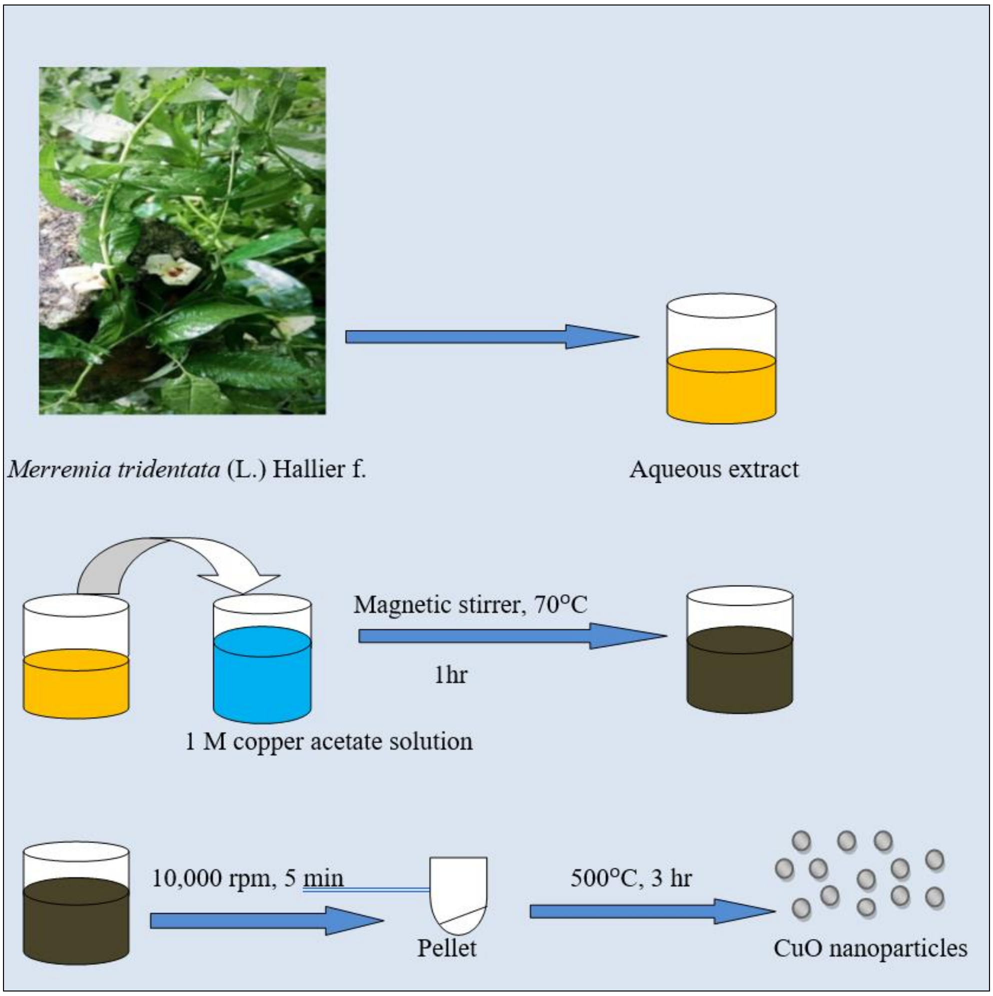

Fig 1: Schematic representation of biogenic fabrication of CuO nanoparticles

Characterization of copper oxide nanoparticles

The UV- Visible absorption spectrum of the synthesized CuO nanoparticles were characterized by JASCO V-760 UV-Visible spectrophotometer and wavelength in the range of 200-800 nm was recorded. The bandgap of the synthesized nanoparticles was calculated by Tauc plot method.

The structural analysis of CuO nanoparticle was carried out by the powder X-ray diffraction technique using Rigaku-Miniflex 600 with CuKα radiation in the range of 20° to 80°. The average crystalline particle size (nm) of the CuO nanoparticles was estimated using Scherrer equation.

$$D = \frac{k\lambda}{\beta \cos \theta}$$

where, D - the average particle size in nm, λ- wavelength (0.15406), k- Scherrer constant (0.9), β- full width at half maximum (FWHM) of the diffraction peak and θ- Bragg angle.

***In vitro* antibacterial assay**

Agar well diffusion method is used to evaluate the antibacterial activity [Valgas *et al*., 2007] [21]. Mueller-Hinton agar (15-20 ml) was poured on glass petri plates and allowed to solidify. Standardized inoculum of the test organisms- *Escherichia coli* (MTCC No.443), *Staphylococcus aureus* (MTCC No.87), *Klebsiella pneumoniae* (MTCC No.3384) and *Bacillus subtilis* (MTCC No.2413) was uniformly spread on the surface of the plates using sterile cotton swab. Four wells with a diameter of 8mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. The test sample (100 µl) was added into the wells from 10mg/ml stock. Gentamycin (40µl from 4 mg/ml stock) and the solvent used for sample dilution were added as positive and negative control respectively. The plates were incubated for 24 hours at 36 ºC ± 1 ºC, under aerobic conditions. After incubation, the plates were observed and the zone of inhibition around the wells was measured in mm.

Result

Characterization of green synthesized CuO nanoparticles: UV-Visible spectroscopic measurement:

Fig. 2 shows the UV-Visible spectra of CuO nanoparticles synthesized from *Merremia tridentata* in the range of 200-800 nm. The maximum absorption peak was observed at 406 nm and the bandgap was calculated as 1.29 eV through the Tauc plot method.

XRD analysis

The green synthesized CuO nanoparticles, subjected to X-ray diffraction analysis reveals the crystalline nature of CuO nanoparticles. The diffraction peaks at the 2θ angles 32.53, 35.5, 38.71, 46.15, 48.82, 53.44, 58.24, 61.57, 66.28, 67.93 corresponding to the lattice planes (110), (002), (200), (-112), (-202), (020), (202), (-113), (022), (220) respectively are shown in Fig. 3. Using Scherrer equation, the average crystalline size of the synthesized CuO nanoparticles obtained from the three intense peaks corresponding to (002), (200), (-202) was measured as 12.47 nm.

Antibacterial activity

To confirm the antibacterial activity, ethanol extract, ethyl acetate extract and CuO nanoparticle extract were analysed against the four MDR strains- *Bacillus subtilis* (MTCC No.2413), *Klebsiella pneumoniae* (MTCC No.3384), *Staphylococcus aureus* (MTCC No.87) and *Escherichia coli* (MTCC No.443) as shown in Table 1 and Fig. 4. The CuO nanoparticle extract shows better antibacterial activity when compared to the other extracts with an inhibition zone of 14 mm, 13 mm, 12 mm and 11 mm respectively.

Table 1: Antibacterial activity of the extracts mentioning the zone of inhibition (mm)

Sl. No.	Microorganisms	Sample	Zone of inhibition (mm)		
			Standard (Gentamycin)	Negative control	Test (1000 µg)
1	*Escherichia coli* (MTCC No.443)	A	19 mm	-ve	11 mm
		B			11 mm
		C			11 mm
2	*Staphylococcus aureus* (MTCC No.87)	A	25 mm	-ve	12 mm
		B			-ve
		C			10 mm
3	*Klebsiella pneumoniae* (MTCC No.3384)	A	19 mm	-ve	13 mm
		B			12 mm
		C			12 mm
4	*Bacillus subtilis* (MTCC No.2413)	A	23 mm	-ve	14 mm
		B			-ve
		C			-ve

* (Sample A- CuO nanoparticle extract, Sample B- Ethanol extract, Sample C- Ethyl acetate extract)

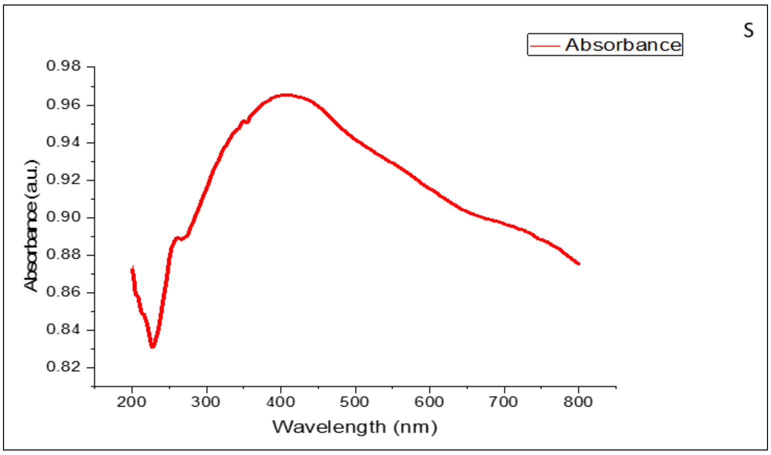

Fig 2: UV-Visible spectra of CuO nanoparticles synthesized from *Merremia tridentata*

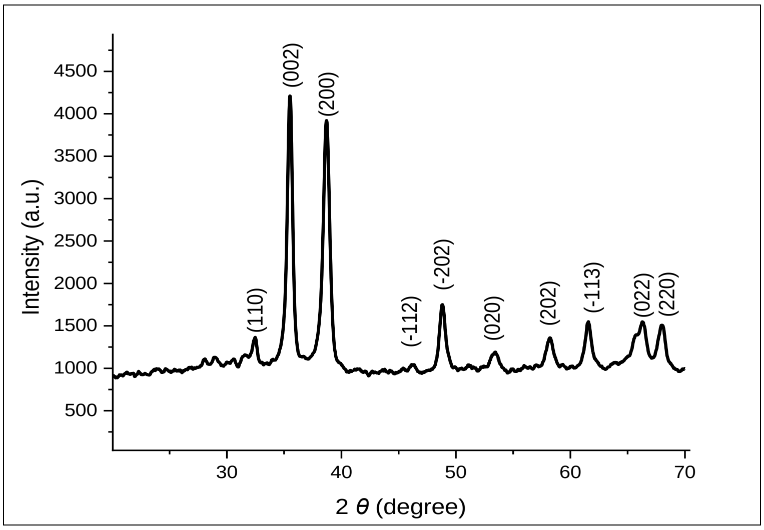

Fig 3: X-ray diffraction analysis showing the diffraction peaks at the 2θ angles

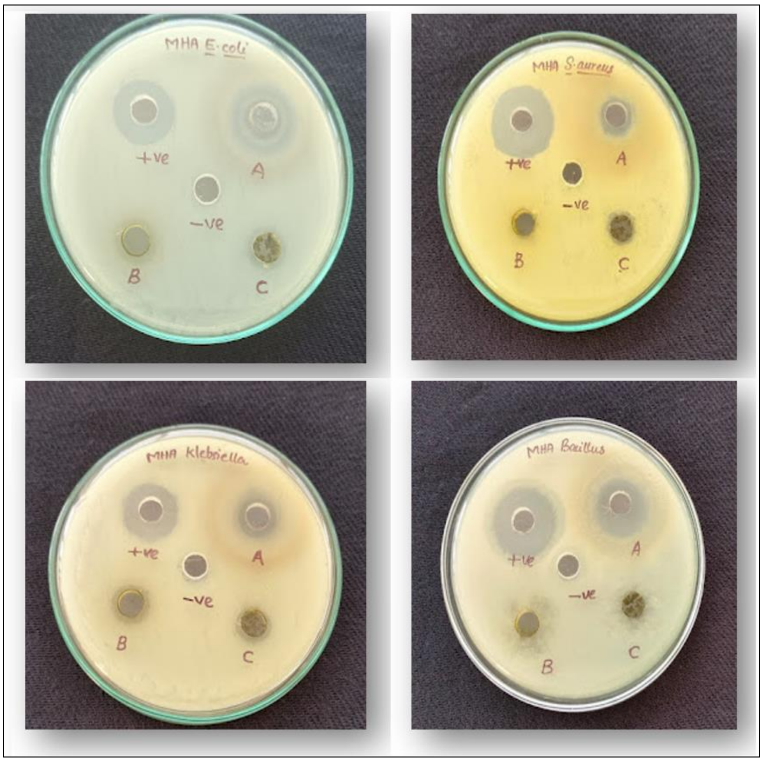

Fig 4: Inhibitory effect of extracts against *Escherichia coli* [1], *Staphylococcus aureus* [2], *Klebsiella pneumoniae* [3] and *Bacillus subtilis* [4]

Conclusion

Development of the green technology is an eco-friendly and low-cost method for the biological synthesis of nanoparticles. For decades, copper was used as an antimicrobial agent to decrease the concentration of microorganism to 99.9 % and revealed potential activity against infectious microbes. Research reports documented *Merremia tridentata* for its ethnomedicinal uses such as antimicrobial, anticancerous, antiinflammatory, antidiabetic and wound healing activity. During the green synthesis, phytochemical constituents acts as capping and reducing agent in the formation of copper nano from the plant extract. In this study, green synthesis of CuO nanoparticles using the aqueous extract of *Merremia tridentata*, followed by characterization of synthesized CuO nanoparticles by UV-Visible spectroscopy revealed the maximum absorption peak at 406 nm with a energy gap of 1.29 eV and the XRD analysis indicated a mean particle size of 12.47 nm. Finally, the antibacterial activity of the plant extracts were compared with the CuO nanoparticles. The wide inhibition zone of CuO nanoparticles against the pathogenic bacterial strains proved its immense use as a safe and potential remedy for bacterial infections.

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