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GREENER SYNTHESIS OF CUO NANOCRYSTALS USING *RAMBUTAN NEPHELIUM LAPPACEUM* L. PEEL EXTRACT AND ITS ANTIBACTERIAL APPLICATIONS

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Keywords:

Green synthesis, Copper oxide, *Rambutan* peel waste, *Nephelium lappaceum* L., Antibacterial activity

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ABSTRACT: Green synthesis of copper oxide nanocrystals was carried out *via* hydroxide precipitation using *rambutan* peel wastes. The successful formation of copper oxide nanocrystals was confirmed by X-Ray diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) analysis. XRD analysis confirms the calculated average crystallite size of higher intense plane peak (111) was 35.74 nm. SEM and TEM show the surface morphology and look flake-shaped. The AFM analysis shows needle formation of CuO nanoparticles. A possible mechanism for the formation of CuO nanocrystals with *rambutan* peel extract was also proposed. The prepared nanocrystals were coated on the cotton fabric, and their antibacterial activity was analyzed. Both CuO and CuO nanocrystals coated cotton fabric showed good antibacterial activity towards Escherichia coli *E. coli* and *Staphylococcus aureus S. aureus*. Wash durability test was carried out on the test fabrics showed that the significant antimicrobial activity was actively retained in the fabrics treated with CuO nanocrystals.

INTRODUCTION: Transition metal oxides such as copper oxide nanoparticles have potential industrial applications in the areas such as catalysis, gas sensors, magnetic phase transitions, and biocidal activity; and hence more attention is paid to study copper oxide nanoparticle. Copper oxide nanoparticles can be synthesized by employing various methods, including sol-gel. chemical vapor deposition, precipitation, pyrolysis, Sonochemistry, electrochemistry, one-step solid-state reactions, cathodic vacuum arc and solvothermal reactions. The utilization of plant extracts, microorganisms, or plant biomass has been a recent research topic for the alternative of physical and chemical methods for the production of nanoparticles.

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Recently, several groups have successfully achieved nanoparticles synthesis using unicellular organisms like bacteria and fungi as well as plant extracts such as *acalypha indica*, coffee powder, Bauhinia tomentosa leaves extract, and so on [1, 18]. This new green chemistry approach is also in consequence of its simplicity, eco-friendly, inexpensive price, and environmentally acceptable nature. Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be scaled up for nanomaterials synthesis. To ience and to reutilize the biachieve the above-mentioned aims of green nanoscological waste as a natural resource, the preparation of nanomaterials have been preferred employing the agricultural waste includes seeds and peels of fruits and so on. In this, the *rambutan* fruit peels are considered as one of the biological waste materials. *Rambutan* is an evergreen bushy tree growing up to a height of 20 meters, and its fruit is oblong, 4 to 5 cms long; colour is red to yellow, covered with thick, coarse hairs or soft spines. *Rambutan* pulp is edible, white, opaque, translucent, juicy, and sweet.

The principal components of *rambutan* peels such as ellagic acid, corilagin, geranin, and ellagitannins are important constituents responsible for the potential utilization in both the food and medical industry. The higher amount of phenolic compounds in the methanolic extract of *rambutan* peels exhibit higher antioxidant activity. In addition, *rambutan* peel extract also possesses antibacterial activity against pathogenic bacteria. Very recently, ellagic acid promoted biomimetic synthesis of shape-controlled silver nano chains has been reported. In this work, ellagic acid, a naturally occurring plant polyphenol, was utilized for the biomimetic synthesis of silver nanoparticles.

It was found that ellagic acid not only has the capability of reducing silver ions, resulting in the formation of Ag nanoparticles, due to its extended polyphenolic system, leading to the formation of hexagon-shaped Ag nanocrystals. Although the exact mechanism of formation of the nanocrystals is not known, this biomimetic approach may be developed as a green synthetic method to prepare building blocks with tunable properties for the development of nanodevices. Further, the ellagic acid-assisted silver nanochains substantially enhanced the antibacterial properties of both gram-positive and gram-negative bacteria [19]. Based on the above two reported literature, we hypothesized the preparation of copper oxide nanocrystals using *rambutan* peel extract, and a possible mechanism for the formation of nanocrystals has also been proposed. In the present investigation, a novel, eco-friendly green chemistry route is adopted for the synthesis of CuO nanocrystals using *Nephelium lappaceum* L., peel wastes. *Rambutan* fruit peel wastes are used as a natural reducing agent to prepare copper oxide nanocrystals. The motivation of the present feasibility study is to bring out a novel green synthetic strategy to prepare CuO nanocrystals using *rambutan* peel waste which will facilitate the industry to introduce new innovations in material synthesis. To the best of our knowledge, it is the first report on the synthesis of CuO nanocrystals using *rambutan* fruit peel extract, and no other attempts have been made earlier so far. The prepared CuO nanocrystals coated on cotton fabric have been characterized employing SEM, and EDX analysis and their antibacterial activity has been examined using agar diffusion test. The prepared CuO nanocrystals on cotton fabric treatment show good antibacterial activity towards E. coli, a gram-negative bacteria, and *S. aureus*, a gram-positive bacteria.

MATERIALS AND METHODS:

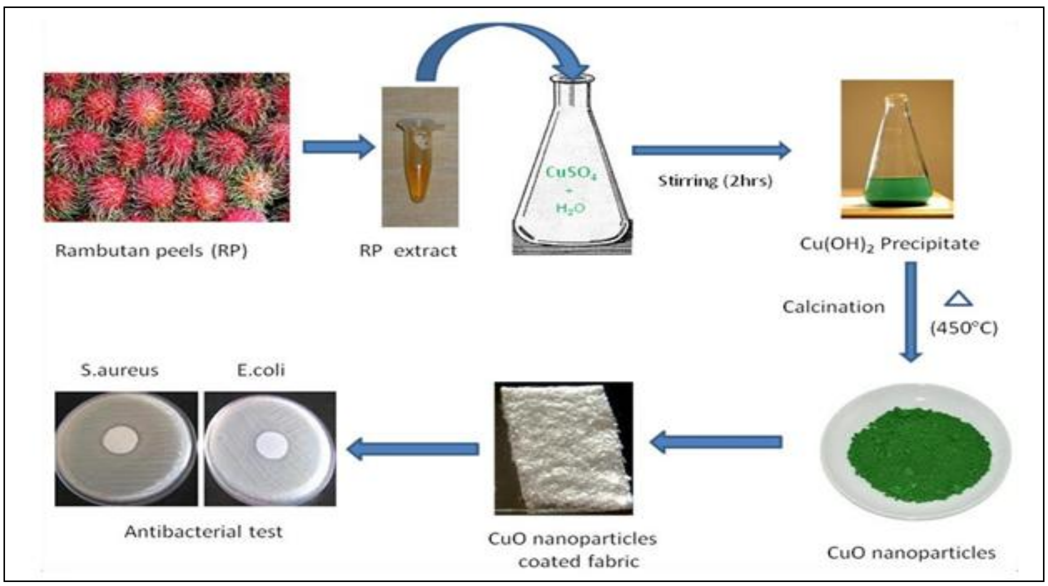
Materials: The *rambutan* fruit peels (*Nephelium lappaceum* L.) were collected from a fruit stall at Ooty, Tamil Nadu, India. Copper sulphate pentahydrate [$Cu(SO_4)2.5H_2O$], ciric acid, sodium lauryl sulphate, and ethanol were purchased from Merck chemicals Ltd, India, and used as received without any further purification. The bleached cotton fabric was purchased from Ayyappa textiles, Karaikudi. Double distilled water was used throughout the experiment.

Instrumentation: JASCO V-530 UV spectrometer was used to characterize the CuO nanocrystals. The powder X-ray diffraction (XRD) analysis of prepared CuO powders was carried out on a PANalytical X'pert Pro X-ray Diffractometer operating at 40 kV with a current of 30 mA using Cu-Kα radiation in the 2θ range of 10 °C to 80 °C. Scanning electron microscopy (SEM) images were recorded using a JEOL JSM 6390 SEM system. TEM analysis was undertaken on Philips 200 model at an acceleration voltage of 200kV. The surface morphology was studied by diCPII veeco USA model AFM. Energy dispersive spectroscopy (JED 2300, JEOL) analysis was carried out on the prepared samples for qualitative elemental analysis. *E. coli* (gram-negative TACC 10536) and *S. aureus* (gram-positive ATCC 11632) bacterial test were done employing qualitative measurement using agar diffusion test (Kirby Bauer disc diffusion method).

Preparation of the Extract: Manually separated fruit peels of *rambutan* were washed thoroughly with running water and subsequently incised into small pieces and then placed in a circulating oven at 50 °C until complete dryness. About 3 g of finely dried *rambutan* peels were boiled with a mixture of ethanol and double distilled water (1:2 ratio) for 10 min. The extract obtained was filtered through Whatman No. 1 filter paper and the filtrate was collected in a 250 mL Erlenmeyer flask and stored in a refrigerator for further use.

Synthesis of Copper Oxide Nanocrystals: For the synthesis of copper oxide nanocrystals, 0.1 M of copper sulphate pentahydrate ($Cu(SO_4)2.5H_2O$) was prepared in 50 mL double-distilled water and then 10 mL of *rambutan* peel extract was slowly added dropwise into the solution under magnetic stirring at 80 °C for about 2 h to form a copper hydroxide precursor. The pH of the reaction mixture was found to be [6].

The light green precipitate formed after an adequate time of stirring was collected by centrifuge at 10000 rpm for 10 min. Then the centrifuged crystals were washed with water and again subjected to centrifugation at 5000 rpm for 10 min. The separated light green precipitate was dried in an oven at 40 °C for 8 h followed by grinding using mortar and pestle. This powdered sample was calcinated in a muffle furnace at 450 °C to get pure copper oxide nanocrystals. The diagrammatic representation of the synthesis of copper oxide nanocrystals is shown in **Scheme 1**.



SCHEME 1: DIAGRAMMATIC REPRESENTATION OF CUO NANOCRYSTALS SYNTHESIS AND ITS ANTIBACTERIAL APPLICATION

Fabric Treatment with Cuo Nanocrystals: A fine, medium weight 100% cotton fabric was used for this treatment. Copper oxide nanocrystals were applied to the fabric employing the pad-dry-cure method. The cotton fabric, cut to a size of 12 cm × 12 cm, was immersed in a solution containing 2% of CuO nanocrystals and 1% of citric acid as a cross linker for 5 min and then passed through the padding mangle run at a speed of 15 m/min with a mangle pressure of 15 kgf/cm^{-2}. The padded fabric was air-dried and then cured for 3 min at 140 °C. The coated fabric was immersed for 5 min in 2g/l of sodium lauryl sulphate to remove any unbound nanocrystals. Further, the fabric was rinsed several times to remove any traces of soap completely. The fabric was finally dried in ambient air.

Antibacterial Test: Antibacterial activities of CuO nanocrystals treated and the untreated fabric was tested on both gram-positive *S. aureus* and gram-negative *E. coli* bacteria. Antibacterial activity towards bacteria ATCC 10536 and ATCC 11632 against copper oxide nanocrystals was performed using the disc diffusion method. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. Finally, we measured diameters (mm) of zone of inhibition of the control strain and test with a ruler and callipers.

Wash Fastness: Wash fastness test of treated samples was conducted according to the standard ISO Test 3 method (ISO 105-C03:1989, Geneva). In this approach, each cycle of the washing process was equivalent to five home laundries in an ambient condition at 38 ± 3 ºC. The stability of nanocrystals on the surface of cotton was studied through antimicrobial test after 10, 20 and 30 home launderings in the presence of non-ionic detergent.

RESULTS AND DISCUSSION: In the present investigation, for the first time, the synthesis of copper oxide nanocrystals has been carried out employing a novel, environmentally benign synthetic strategy using *rambutan* peel waste extracts. It aims to reduce risk in the laboratory employing less hazardous synthesis methods and to minimize the environmental footprint *via* waste minimization and prevention.

XRD Analysis: The structural information and crystallinity of the prepared CuO nanocrystals were given in the XRD pattern, as shown in **Fig. 1.** The obtained XRD pattern clearly shows the presence of sharp peaks and the broad peaks suggesting the formation of the resulting CuO products were of mixed-phase of an amorphous and crystalline state.

According to JCPDS data (89-2529), the distinct diffraction peaks at 2θ = 32.06 °C, 35.48 °C, 38.74 °C, 48.71 °C, 61.53 °C, and 66.07° are assigned to (110), (111), (200), (202), (113) and (311) crystal planes of cubic phase CuO. The crystallite size of the CuO nanocrystals was estimated from the higher intense peak of XRD spectrum using Debye Scherrer's equation. The calculated crystallite size from the intense high plane from XRD pattern and their parameters are summarized in Table 1. The calculated average crystallite size of a higher intense plane of (111) was 35.74 nm.

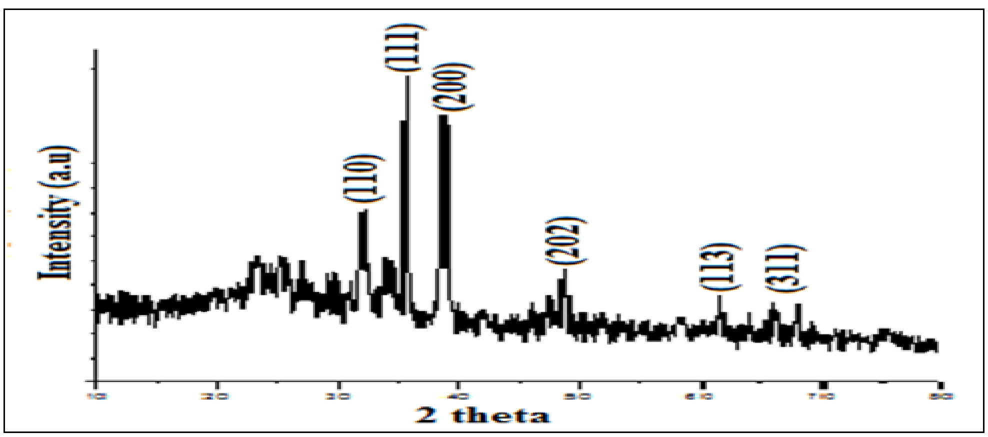


FIG. 1: X-RAY DIFFRACTION PATTERN OF COPPER OXIDE NANOCRYSTALS

TABLE 1: CRYSTALLINITY PARAMETERS OF THE CuO NANOCRYSTALS

Pos. [°2Th.]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Mean particle size (nm)
32.0637	0.5087	2.7892	41.99	17.13
35.4893	0.2441	2.5274	100.00	35.74
38.7427	0.3426	2.322	91.43	25.71
48.7139	0.3852	1.8677	22.58	23.69
61.5325	0.3196	1.5058	19.83	30.66
66.0728	1.1237	1.4129	12.85	11.86

SEM and TEM Analysis: Typical scanning electron microscopic analysis was employed to study the morphology of the nanocrystals. **Fig. 2A** shows the representative SEM image carried out at different magnifications such as 30,000X and 60,000X. It is evident from the SEM images that the synthesized crystals was distributed in the form of aggregation of small individual nanocrystallites of about 20-50 nm in size. Although they agglomerate, the boundaries between single crystallites were clearly observable. Agglomerates were composed of several individual small nanocrystals with an average size of 50 nm. Transmission electron microscopic (TEM) analysis was carried out to further confirm the size of copper oxide nanocrystals. **Fig. 2B.** shows the representative TEM images of CuO nanocrystals. The uniform weak agglomeration of CuO nanocrystals with reasonable distribution has evidence from the obtained TEM images. The average crystallite size obtained from TEM images was around 40 nm which agrees well with the size calculated from XRD analysis.

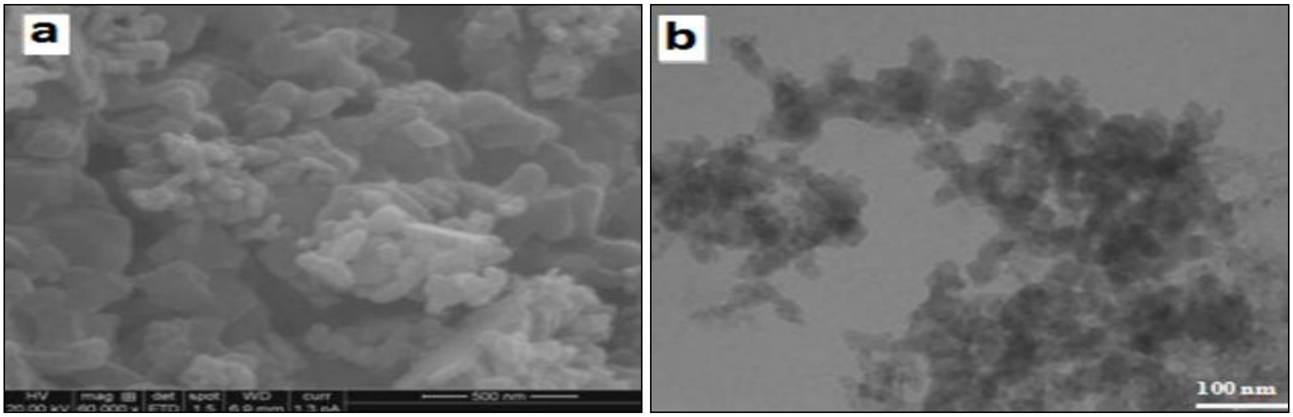


FIG. 2A AND B: SEM AND TEM IMAGES OF COPPER OXIDE NANOCRYSTALS

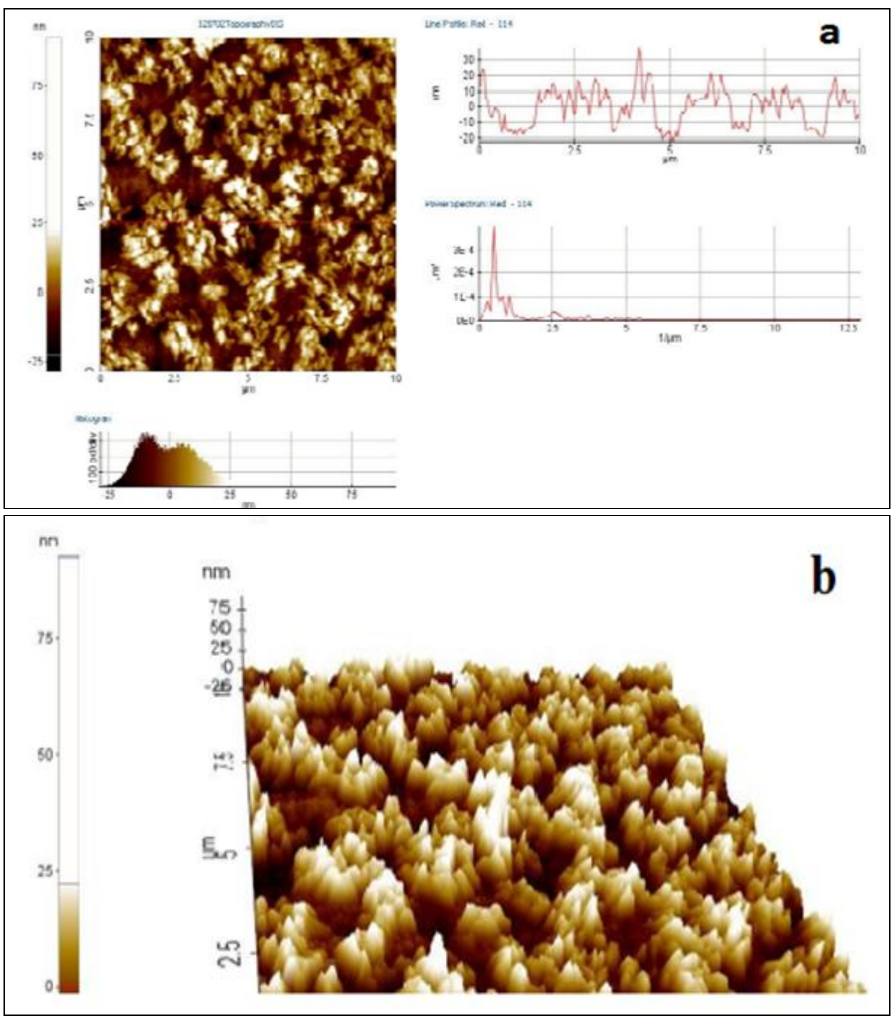
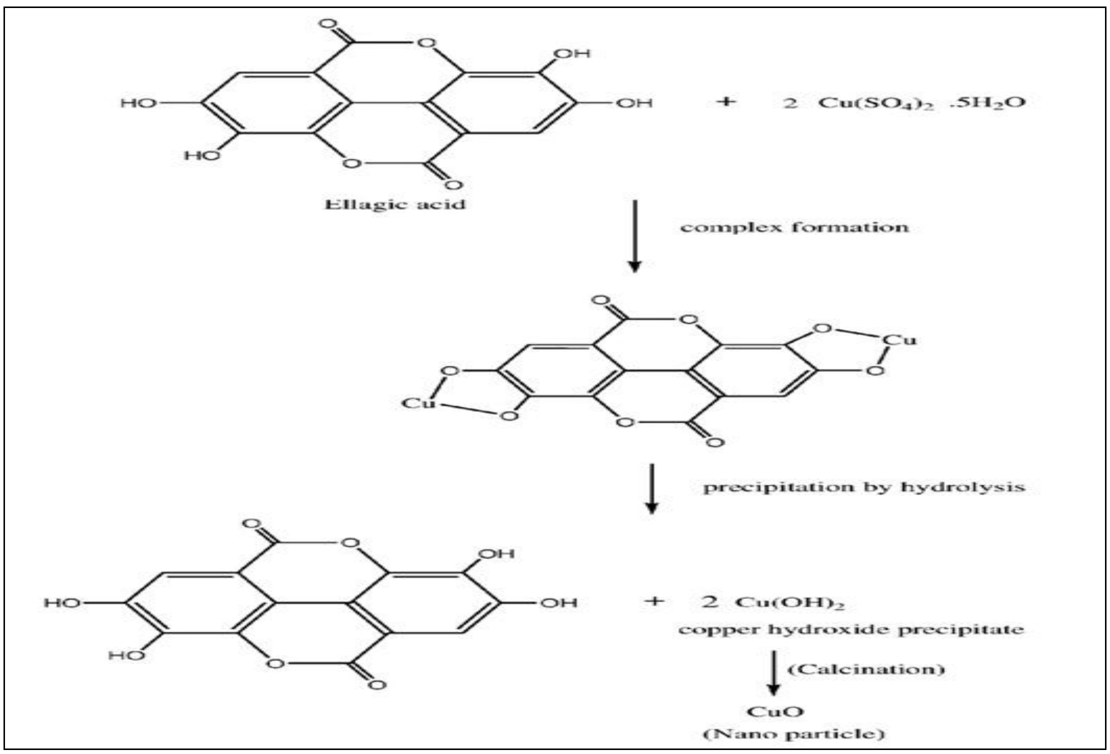


FIG. 3: AFM IMAGES OF CUO NANOCRYSTALS (A) 2D IMAGE AND ITS LINE PROFILE (B) 3D IMAGE

AFM Analysis: The atomic force microscopy images of the copper oxide nanocrystals in 2D and 3D forms and height distribution are shown in **Fig. 3A** and **B.** For imaging by AFM, the samples were suspended in acetone and spin-coated on a silicon wafer. The size of the copper oxide nanocrystals was measured in contact mode with silicon cantilevers with a force constant of 0.03-0.77 Nm^{-1} and tip height of 10-15 m. In **Fig. 3A**, most of the copper oxide nanocrystals are in the range of 0-25 nm diameter with a smooth surface and compacted structure. The nanocrystals with sizes between 25 and 50 nm are due to the aggregation of nanocrystals. The AFM image clearly reveals the high symmetric size of crystals, as evident from three-dimensional images. Particle profiles show the indication of agglomeration of crystals as a distorted line profile. The copper oxide nanocrystal exhibits a remarkable tendency to form uniform-sized and shaped nanocrystals.

Possible Mechanism of Cuo Nanocrystals: Scheme 2. shows the possible mechanism of copper oxide nanocrystals using *rambutan* peel extract. The ester oxygen atom and the phenolic hydroxyl groups of polyphenols from the p-track conjugation effect when the hydroxyl groups form binding with the metal as metal-phenolate complex by the chelating effect. These complexes undergo hydrolysis to precipitate metal ions as metal hydroxide or metal oxide, depending upon the reaction pH. The pH value varies depending upon the metal ions. Moreover, polyphenols tend to lose electrons easily, resulting in the formation of H+ radicals by reducing the size of nanocrystals. In our synthesis, *rambutan* peel extract permits the copper sulphate to precipitate the copper hydroxide at pH [5, 7]. The precursor copper hydroxide was calcinating at 450 °C yields copper oxide nanocrystals by treatment on heat.

The utilized *rambutan* peel extracts have rich sources of active ingredients such as polyphenols, alkaloids, flavonoids, and vitamins. These active components are highly responsible for performing antioxidant, radical scavenging, antiviral effects, *etc*. Among the functional, active ingredients, polyphenolic compounds such as ellagic acid, corilagin, and geranin are major components with high proportions. These natural phenolic antioxidant compounds have favourable effects on the synthesis of copper oxide nanocrystals and have anti-carcinogenic nature [20].



SCHEME 2: POSSIBLE MECHANISM OF COPPER OXIDE NANOCRYSTALS

SEM and EDX Studies of Cuo Nanocrystals Treated and Untreated Fabrics: The morphological changes of cotton samples before and after treatment with nanocopper substances can be clearly observed from the SEM images. **Fig. 4 A1, A2,** and **B1, B2, B3** show the morphology of untreated and treated with copper oxide nanocrystals treated cotton samples. The observed

SEM images doesn't clearly show any significant difference between the untreated and treated cotton fibers due to the imposition of small-sized copper oxide nanocrystals onto the surface of the fibers. From the micrograph of **Fig. 4B2**, it is clear that the size of the cotton fibers was in the range of micrometer [10, 20]. However, the size of the nanocrystals in the range of 50-100 nm was utilized to coat on the fabric, and hence it cannot be seen the well-distributed copper oxide nanocrystals onto the fabric due to the small crystallite size. Energy-dispersive X-ray spectroscopy (EDX) was employed to establish the chemical identity of the deposited nanocrystals. **Fig. 5A** and b show the EDX images of untreated and CuO nanocrystals treated cotton samples. It can be clearly seen that in untreated cotton fabrics **Fig. 5A,** there was no evidence for the existence of any characteristic material, especially copper ions, whereas the presence of copper ions which we can't distinguish on the surfaces of CuO nanocrystals treated cotton fabric **Fig. 5B,** due to the smaller size of CuO crystals which is clearly exhibited from the EDX image **Fig. 5B.** The observed result clearly reveals that the fabric has strong adsorption of CuO nanocrystals on the surface of the fibers due to the citric acid as a cross-linker and change the surface charge on the cellulose fibers to bind into the fabric.

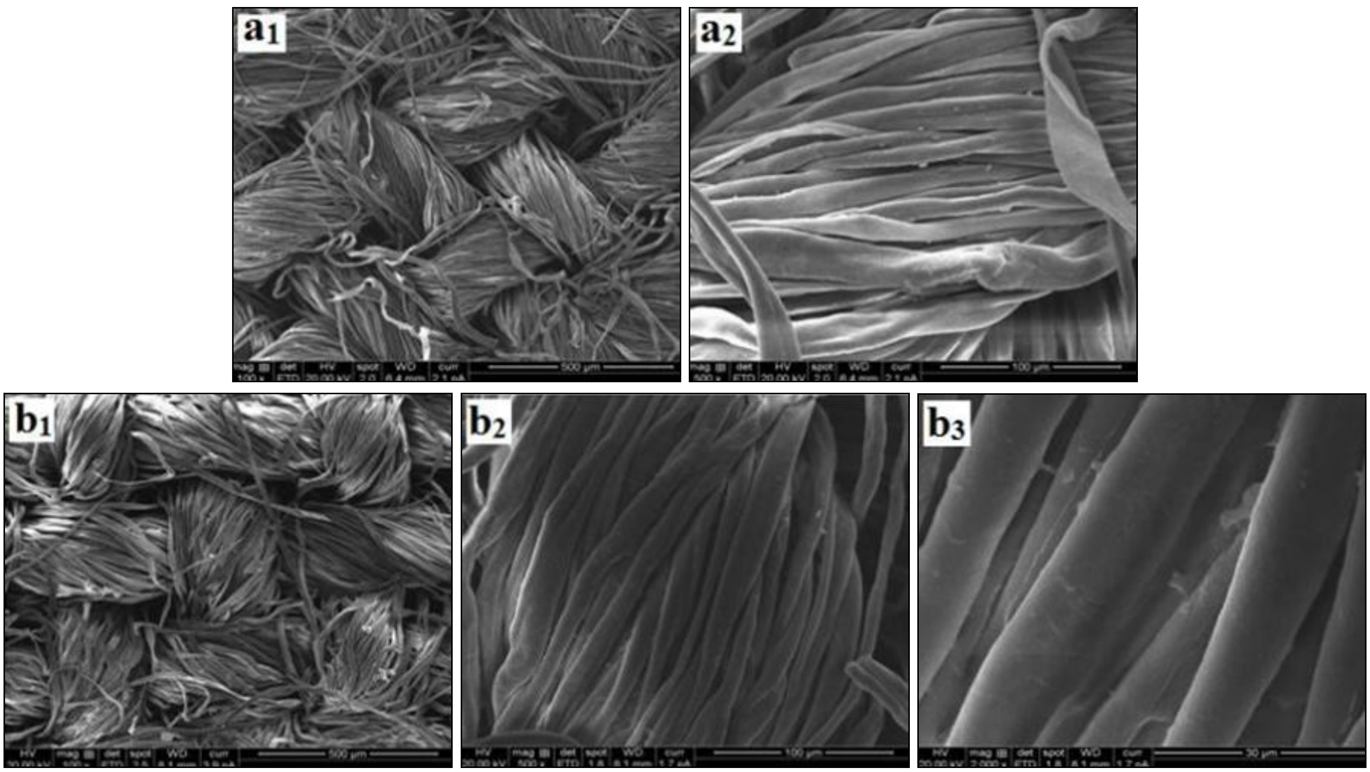


FIG. 4: SEM IMAGES OF CUO NANOCRYSTALS UNTREATED (A1, A2) (500µM, 100µM); TREATED FABRICS (B1, B2, B3) WITH DIFFERENT MAGNIFICATION (500 µM, 100 µM AND 30 µM)

Antibacterial Studies of Cuo Nanocrystals Treated and Untreated Fabric: Fig. 6. shows the representative zone of inhibition that occurred by CuO nanocrystals. The average zone of inhibition was found to be more towards gram-positive bacteria compared with gram-negative bacteria. Our results were well correlated with a study by Tian *et al* [21]. The structural difference of bacteria plays an important role in their susceptibility and includes cytoplasmic membrane and cell wall component, which are different between gram-positive and gram-negative bacteria. Gram-negative bacteria possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering, and without an outer membrane, the cell wall of gram-positive bacteria can be permeated more easily [21]. **Fig. 7A** and b show the antibacterial activity of treated and untreated copper oxide nanoparticles on cotton fabrics. *Escherichia coli* and *S. aureus* were used as test microorganisms to detect the antimicrobial activity of CuO nanocrystals using the disc diffusion method. **Table 2** shows the antibacterial activity results of copper oxide nanocrystals The results of the qualitative antibacterial assessment by agar diffusion method show that the fabric sample treated with CuO nanocrystals showed an inhibitory effect against both *E. coli* and *S. aureus*. The observed results clearly reveal that there was also a zone of intermediate resistance, indicating that some inhibition occurs using this antimicrobial activity, but it may not be sufficient enough to eradicate the organism completely. Once CuO kills or captures the cell membrane, the copper oxide nanocrystals presumably remain tightly adsorbed on the dead bacteria preventing further bacterial action and thereby showing high bacterial efficiency.

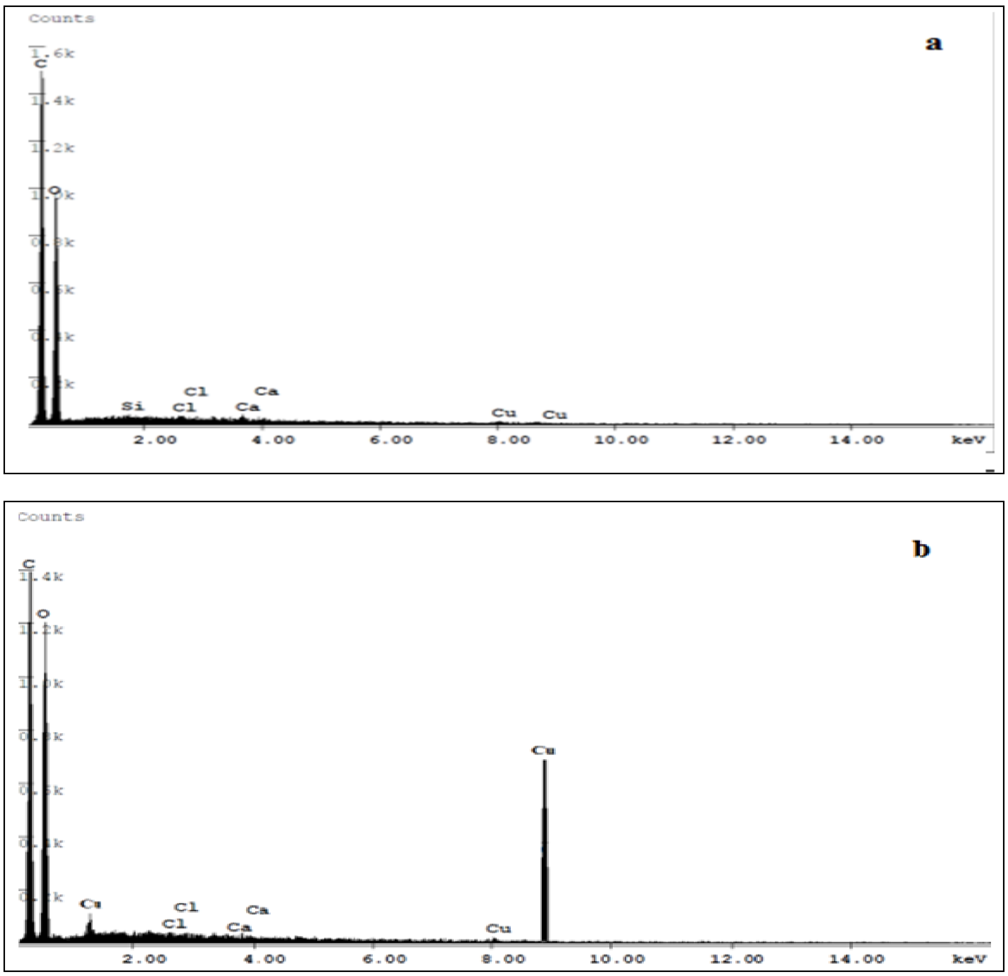


FIG. 5: EDX IMAGE OF (A) UNTREATED COTTON FABRIC (B) TREATED COTTON FABRIC WITH CUO NANOCRYSTALS

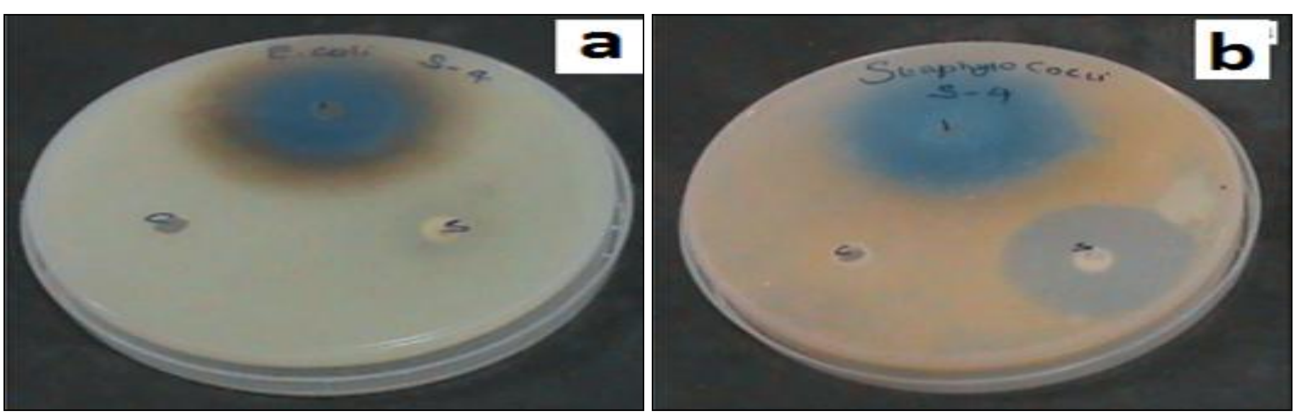


FIG. 6: ANTIBACTERIAL ACTIVITY OF COPPER OXIDE NANOCRYSTALS AGAINST (A) E.COLI (B) *S. AUREUS*

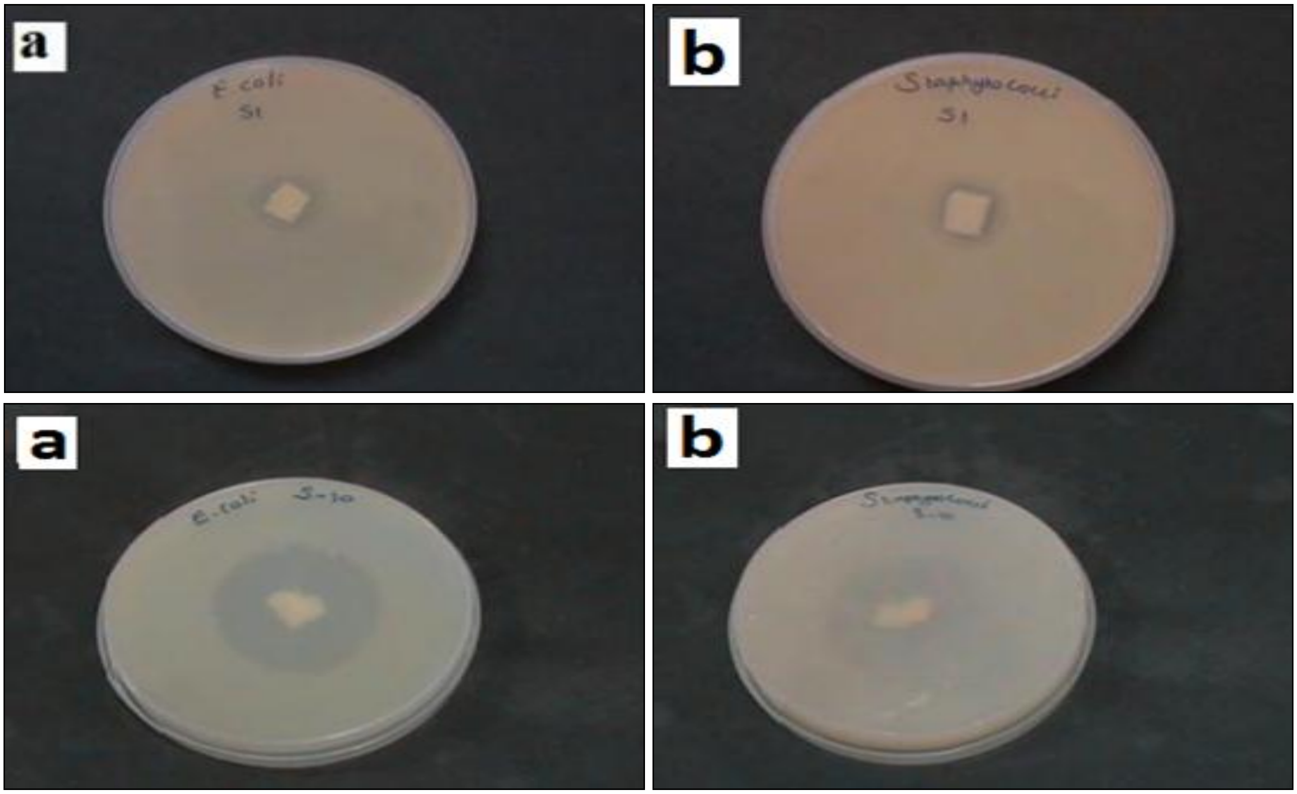


FIG. 7: ANTIBACTERIAL ACTIVITY OF COPPER OXIDE NANOCRYSTALS (A AND B) UNTREATED (C AND D) TREATED COTTON FABRICS AGAINST *E. COLI* AND *S. AUREUS*

TABLE 2: ANTIBACTERIAL ASSESSMENT BY AGAR DIFFUSION METHOD

Fabric treated	Organism	Zone of Inhibition (in mm)
Fabrics without CuO nanocrystals (Control)	*E .coli*	0
	S. aureus	0
Fabrics treated with CuO nanocrystals (Test)	*E. coli*	23
	S. aureus	25

Washing Durability: Wash durability test was carried out on the test fabrics showed that the significant antimicrobial activity was actively retained in the fabrics treated with CuO nanocrystals even after repeated wash cycles, as shown in **Table 3.**

After 10 washes, the % bacterial reduction was reduced significantly and there was no activity found in the fabrics after 20 washes. The control untreated fabrics were not subjected to any wash durability test as it has no antibacterial activity. Based on the above studies, we concluded that the present investigation leads to many advantages, such as the use of the new possibility of a green approach employing process flexibility, reutilisation of the fruit waste as a natural resource, ease in scaling up, and not adverse to the environment. This is the first report of using a natural *rambutan* peel waste to synthesize CuO nanocrystals and the synthetic strategy is adoptable to other metal oxides. The prepared CuO nanocrystals using *rambutan* peel extract have excellent antimicrobial activity and could be applied to the fabrics of interest.

TABLE 3: WASHING DURABILITY TEST OF COTTON FABRICS TREATED WITH CUO NANOCRYSTALS

Cycles	% Bacterial Reduction	Cycles
	E. coli	***S. aureus***
1	77.10	82.11
2	73.50	80.21
5	70.15	79.02
10	68.50	71.50
15	34.50	41.20
20	10	12.85
25	0	0

CONCLUSIONS: The reported novel green synthetic strategy explores the possibility of utilizing simple, inexpensive and abundantly available green waste to synthesize copper oxide nanocrystals.

The mechanism for the formation of ZnO nanocrystals using rambutan peel extract was discussed. The rich source of polyphenols such as ellagic acid facilitates the formation of copper oxide nanocrystals. The structure and size of synthesized copper oxide nanocrystals were confirmed by spectrophotometric analysis. SEM and EDX images of CuO nanocrystals coated cotton fabric confirmed the adsorption of CuO. The CuO nanocrystals treated cotton fabric exhibited stronger antibacterial activity due to the CuO nanocrystals adsorption on the surface of cellulosic fibers. More importantly, the strategy present in this work can be extended to other metal oxide nanoparticles.

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CONFLICTS OF INTEREST: There is no conflict of interest related to this work by any authors.

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