



## Honey Assisted Chemical Synthesis of various Metal Oxide Nanoparticles: A Study on their Structural, Vibrational, Morphological and Compositional Analysis

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DOI: <https://doi.org/10.54392/irjmt25116>

Received: 06-11-2024; Revised: 14-01-2025; Accepted: 22-01-2025; Published: 30-01-2025



**Abstract:** This study documented the creation of metal oxide nanoparticles utilizing honey's biomolecules as an alternative to environmentally harmful chemicals. The produced nanoparticles were analyzed utilizing techniques such as XRD, FT-IR, SEM, TEM, and EDAX to examine their properties. The diffraction pattern derived from XRD analysis corresponded with conventional JCPDS data, and the existence of components in the synthesized nanomaterials was confirmed through elemental analysis using EDX. The XRD pattern indicated that cobalt oxide nanoparticles possess a cubic structure with an average crystallite size of approximately 31.2 nm, nickel oxide nanoparticles exhibit a cubic structure with an average crystallite size of about 29.6 nm, and copper oxide nanoparticles display a monoclinic structure with an average crystallite size 27.69 nm. The FTIR characterisation with infrared rays facilitates the identification of functional groups present in produced nanoparticles. SEM and TEM analyses verified the produced nanoparticle's surface shape and its particle size that was within the range of 35 - 45 nm. This work demonstrates that honey can be utilized in the green synthesis of Cobalt oxide, Nickel oxide, and Copper oxide nanoparticles.

**Keywords:** Natural Fuel, Metal Oxide Nanoparticles, Structural Properties, TEM

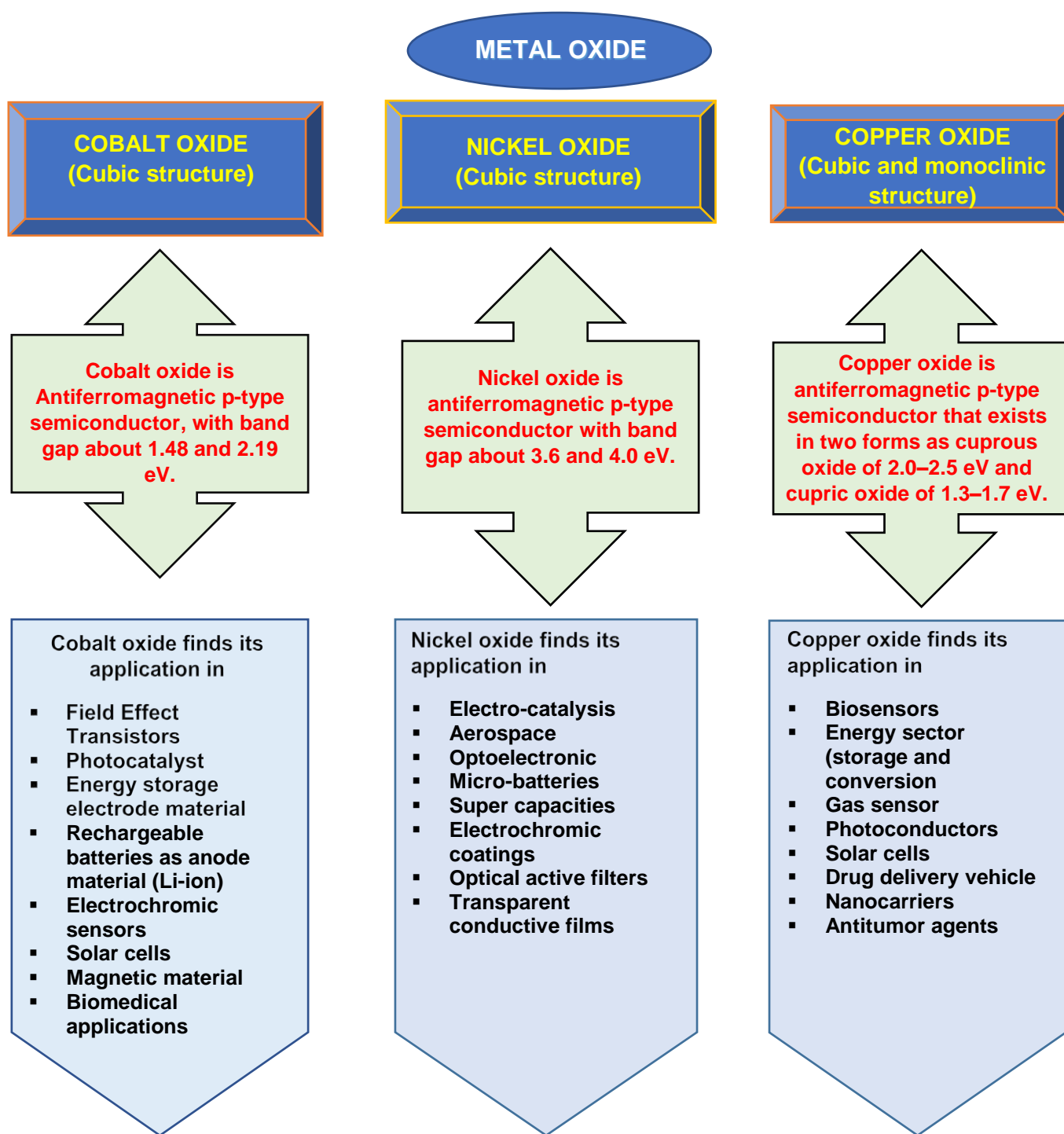
### 1. Introduction

Nanotechnology has emerged as a transformative discipline primarily centred on the synthesis of nanomaterials, which range in size from one to one hundred nanometers. Its applications span numerous sectors, including the food industry, agriculture, pharmaceuticals, cosmetics, electronics, textiles, optics and optical devices, the space industry, fuel cells, and environmental remediation [1-5]. The nanoparticles demonstrate the capacity to display diverse characteristics through alterations in its morphology, shape, and phase [6]. Nanomaterials exhibit unique characteristics such as their nanoscale dimensions and elevated surface-to-volume ratio, which enhance chemical stability and thermal conductivity [5] [8-9]. Two methodologies exist for the production of nanoscaled materials. The top-down strategy entails deriving nanostructures from bigger materials, while the bottom-up approach results in nanostructures through the self-assembly of atoms and molecules [8, 10-11]. These methodologies encompass various techniques such as laser ablation, vapour deposition, sputtering deposition, chemical vapour deposition, laser pyrolysis,

sol-gel, lithography, pulsed electrochemical etching, and micro emulsion for nanoparticle synthesis; however, they present disadvantages like elevated costs, toxicity, generation of hazardous by-products, instability, necessity for high temperature and pressure, and low yield [6-8, 12]. The process of green synthesis utilizing biomolecules has become significant in research due to its environmental friendliness, reduced energy consumption (temperature, energy, and pressure), cost-effectiveness, and the production of biocompatible products and by-products that contribute to environmental stability and suitability [2, 4, 8, 13-14]. The green synthesis of nanomaterials is conducted with natural bioactive agents such as microbes, plant extracts, and organic molecules as its precursors [2] [8] [14-16]. The substance's chemical composition functions as both reducing and capping agent, eliminating the need for supplementary agents [11, 17]. Green synthesis is a straightforward process that involves the reduction of metal ions while simultaneously stabilizing their size, shape, and preventing aggregation [18]. Metal oxides are a unique category of nanomaterials that exist at the nanoscale, characterized by their ability to manipulate properties like as size,

shape, and morphology [2]. Metal oxides are chemical compounds containing a minimum of one oxygen atom along with metal atom, utilized in various fields due to their attributes such as diminutive size, biocompatibility, high adsorption capacity, surface plasmon resonance, and superparamagnetic characteristics [2, 9, 19]. Recently, several researchers on green synthesis of metal oxide has been reported that used diverse biomolecules in the synthesis of nanoparticles. Figure 1 exhibits the properties and applications of synthesized metal oxides cobalt oxide, nickel oxide and copper oxide's nanoparticles from reviewed articles. Murshed et al. produced copper oxide nanoparticles with bee propolis and examined the influence of pH on their structural and optical properties for UV blocking and photodegradation. It demonstrated efficacy in UV blocking at pH 8 and exhibited 94.15% dye degradation at pH 6.4 [20]. Vasantharaj et al. Demonstrated the potential of copper oxide nanoparticles through biomedical activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and lung cancer cell line and also demonstrated efficient degradation of methylene blue dye, synthesized using *Tecoma Stans* flower extract. Its Characterization revealed a peak at 360 nm in the UV spectrum that confirmed the formation of cobalt oxide nanoparticles with spherical morphology in the FE-SEM analysis [21]. Sahithya.K *et al.* produced grain-shaped copper oxide nanoparticles measuring 77 nm from a cell-free extract of *Aspergillus niger*, which contained alcohol, alkene, and amine functional groups. The produced nanoparticles exhibited antibacterial efficacy against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, as well as antifungal activity against *Aspergillus fumigatus* and *Aspergillus versicolor* [22]. Nickel oxide nanoparticles derived from *Punica granatum* seed extract were synthesized by Hussein et al. with an average size of 10 to 60 nm. The bioactivity of synthesized nanoparticles demonstrated antibacterial efficacy against both gram-positive (*Staphylococcus aureus* and *Aerococcus viridans*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria, with a greater effect observed on gram-positive strains. Additionally, these nanoparticles exhibited DPPH, ABTS, and  $H_2O_2$  radical scavenging activities, along with non-hemolytic properties at low concentrations (< 250 mg/L) and its biocompatibility at various concentrations of nickel oxide was demonstrated through cytotoxicity study [23]. Alghamdi et al. documented the synthesis of nickel oxide nanoparticles from discarded olive leaf extract and its characterization revealed that the NiO nanoparticles, measuring 32.94 nm with an asymmetrical structure, exhibited 95% mortality on antiparasitic activity and demonstrated significant antimicrobial efficacy against the *Candida albicans* strain, with a substantial inhibition zone of  $33 \pm 0.2$  mm [15]. Abdallah et al. utilized nickel oxide nanoparticles, which demonstrated a significant reduction in the leaf area of plants affected by

*Xanthomonas oryzae* pv. *oryzae*, alongside a notable enhancement in growth and biomass, achieving reductions of 88.68% and 83.69% in the growth of *Xanthomonas oryzae* pv. *oryzae* when treated with 200  $\mu$ g / mL of nickel oxide nanoparticles synthesized using an aqueous extract of *Crocus sativa*. L with an average size of 41.19 nm with spherical morphology [24]. Alemu et al. used the aqueous extract of *Croton Macrostachyes* leaves to synthesize cobalt oxide nanoparticles, which were subsequently showed antibacterial efficacy against gram-positive (*S. aureus* and *E. faecalis*) and gram-negative (*E. coli*, *S. typhimurium*) bacteria. Cobalt oxide derived from *Croton Macrostachyes* leaf extract exhibited a cubic spinel crystal structure with an average crystalline size of 12.75 nm, displaying both round and irregular morphologies in SEM pictures [25]. Chole et al. conducted green synthesis through *Punica granatum* L. seed oil for the production of cobalt oxide nanoparticles, which was validated by UV-visible spectroscopy. The study demonstrated its efficacy against eukaryotic cancer cells and microbial pathogens [26]. Kolahalam et al. showed that Cobalt oxide nanoparticles can be produced from *Lawsonia inermis* plant extract as a natural reducing agent, and confirmed their synthesis using EDX analysis, that was with an average size of 98 nm as determined by XRD analysis. It demonstrated promising antibacterial action against *Staphylococcus* and *Streptococcus aureus*, as well as antifungal activity against the *Meyerozyma guilliermondii* strain, at a concentration of 170 ppm of cobalt oxide nanoparticles [27]. Here usage of honey in green synthesis was the method adopted to synthesis metal oxide nanomaterials this concept derived from previously documented synthesis involving honey and its constituents [1, 5, 28]. In humans, honey serves not only to enhance nutritional value but also to heal ailments [29]. Honey is a functional food possessing properties of wound healing, antibacterial, antioxidant, anti-inflammatory, analgesic, debriding, and tissue regeneration characteristics [7] [17]. Honey is a globally consumed food product [29] that is a sweet in taste, viscous organic compound produced by bees (*Apis mellifera* L.) and stored in honeycombs, derived from the collection of nectar from plants which comprises of phytochemicals, primarily carbohydrates, proteins, water, and minerals, including trace amounts of iron, calcium, manganese, copper, zinc, sulphur, magnesium, and sodium [10]. Potassium constitutes approximately one-third of the overall mineral content; nevertheless, honey's chemical makeup can fluctuate based on geographical origin, floral sources, and seasonal variations, with elements also subject to change during storage [29, 30]. Fructose and glucose constitutes the major carbohydrates with others like disaccharides, oligosaccharides and fructooligosaccharides [10]. The water content influences the quality of honey in preventing spoilage [30].



**Figure 1.** Flowchart of Cobalt Oxide, Nickel Oxide and Copper Oxide's Properties and Application

The pH of honey varies between 3.2 and 4.5 [29]. In honey, protein functions as a stabilizing agent, whereas fructose and glucose serve as reducing agents [16, 31]. This study concentrates on the green synthesis approach for the production of metal oxides copper oxide, nickel oxide, and cobalt oxide nanoparticles and characterizations to investigate their properties in the presence of honey.

## 2. Materials and Method

This study utilized honey sourced from the Sathyamangalam forest and Cobalt nitrate, Nickel nitrate, and Copper nitrate that were of analytic grade which required no additional purification. Cobalt oxide, Nickel oxide, and Copper oxide nanoparticles were prepared at room temperature by combustion approach that utilized honey as an organic ingredient and double distilled water as solvent.

## 2.1 Synthesis of $\text{Co}_3\text{O}_4$ , $\text{NiO}$ , $\text{CuO}$ Nanoparticles

The synthesis of metal oxide via the combustion method is represented in Figure 2 represents experimental procedure carried out to synthesis metal oxide that commenced with the dissolution of 0.5 M cobalt nitrate  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (Merck 99%) in 50 ml of double-distilled water, using a magnetic stirrer until a transparent solution was achieved. Subsequently, 50 ml of honey was introduced dropwise as a reducing and stabilizing agent into the metal nitrate solution while maintaining steady stirring for 1 hour and 30 minutes, resulting in a homogeneous solution. Immediately, the agitated solution was promptly transferred to a hot plate set at  $100^\circ\text{C}$  that ended in combustion after evaporation of water. Following combustion, the remaining residue—burnt ash—was collected and ground using a pestle and mortar. Ultimately preserved in hermetically sealed containers for subsequent characterisation procedures. Nanoparticles are produced through the reduction of metal salts facilitated by the phyto-chemicals present in honey. The identical synthesis procedure was employed to produce other metal oxides instead of cobalt nitrate with nickel nitrate  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (Merck 99%) for nickel oxide and copper nitrate  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (Merck 99%) for copper oxide, while all other processes remained unaltered to execute the synthesis.

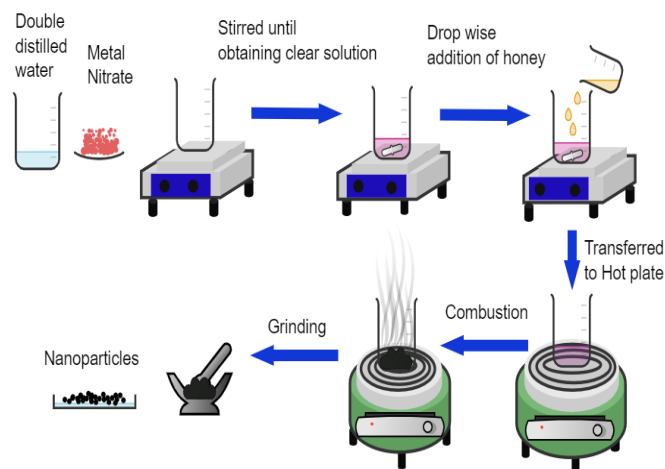
## 3. Results and Discussion

### 3.1 XRD Analysis

XRD analysis was carried out using Instrument Bruke D8 Advance with operating current 30 mA and voltage 40 kV in the range of  $20^\circ$  to  $80^\circ$  ( $2\theta$ ). By exposing X-ray of wavelength  $1.5418 \text{ \AA}$  on synthesized samples and recording its diffraction in a different direction, the crystal structure can be studied. The XRD pattern in Figure 3 of Cobalt oxide nanoparticles showed peaks 31.34, 36.93, 38.91, 44.93, 55.65, 59.44, and 65.37 that were indexed at (220), (311), (222), (400), (422), (511), (440) planes. The peaks of Nickel oxide nanoparticles showed peaks 37.24, 43.29, 44.48, 51.83, 62.89, 76.23 that corresponds to (111), (200), (232), (002), (311), (222) planes and copper oxide nanoparticles showed peaks 32.68, 35.72, 38.88, 49.01, 53.61, 58.41, 61.71, 66.45, 68.22, 72.44, 75.31 that were indexed at (110), (002), (111), (202), (020), (20-2), (11-3), (31-1), (113), (220), (311) planes. The obtained diffraction pattern of cobalt oxide, nickel oxide, and copper oxide nanoparticles revealed the formation of cubic structure with lattice parameter  $a = 8.145$  (JCPDS card no: 76–1802), cubic structure with lattice parameter  $a = 4.114$  (JCPDS card no: 04-083) and monoclinic structure with lattice parameter  $a = 4.552$ ,  $b = 3.392$ ,  $c = 5.216$  (JCPDS card no: 80-0076) and that matched with standard XRD pattern. Deduced average crystalline size of cobalt oxide nanoparticles, nickel oxide, and copper oxide nanoparticles are 31.214 nm, 29.6 nm and 27.69 nm,

from Scherrer's formula based on full-width half maxima of peak from main intensity peak. The smaller the crystal size produces increased surface area of the particle [18].

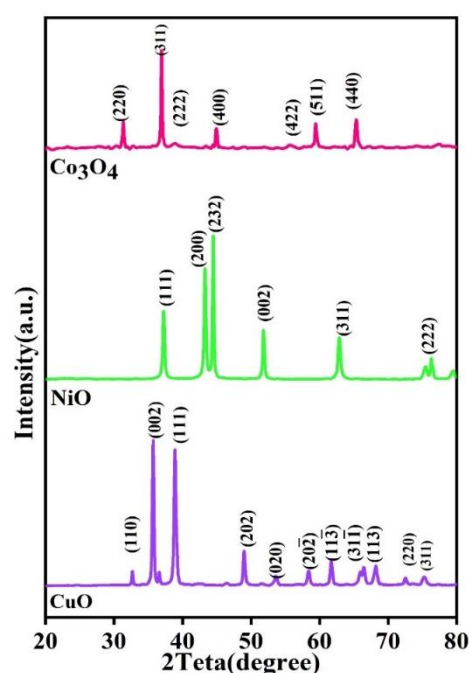
SYNTHESIS OF METAL OXIDE BY EMPLOYING HONEY



**Figure 2.** Represents Experimental procedure in synthesis of metal oxide.

**Table 1.** Calculated Lattice parameter and Average crystallite size of synthesised nanoparticles.

Nanoparticle	Structure	Lattice parameter Å	Average crystallite size nm
$\text{Co}_3\text{O}_4$	Cubic	$a = 8.145$	31.214
$\text{NiO}$	Cubic	$a = 4.114$	29.6
$\text{CuO}$	Monoclinic	$a = 4.552$ , $b = 3.392$ , $c = 5.216$	27.69



**Figure 3.** XRD of  $\text{Co}_3\text{O}_4$ ,  $\text{NiO}$ ,  $\text{CuO}$  nanoparticles.

### 3.2 FTIR Analysis

To discover the functional group on formed nanoparticles synthesized by using honey Themofisher–Nicoletis10 was used and spectra were recorded between the wave number range 4000 to 500  $\text{cm}^{-1}$  in Figure 4. In spectrum of cobalt oxide nanoparticles peaks the peak appeared at 657 $\text{cm}^{-1}$  is due to stretching vibration of halo compound. The nickel oxide nanoparticles recorded spectrum revealed the peak at 731  $\text{cm}^{-1}$  is assigned to C-H bending indicating a halo compound. The spectra of copper oxide nanoparticles showed the weak peak at 817 $\text{cm}^{-1}$  causes stretching of C-Cl of halo compound. The noticeable peaks at 1377 $\text{cm}^{-1}$ , 1987  $\text{cm}^{-1}$  is related to bending of O-H for phenol group and C-H of aromatic compound. The peak at 1590  $\text{cm}^{-1}$  was of N-H bending of the phenol amine. The peak obtained at, 2057 $\text{cm}^{-1}$ , 2243 $\text{cm}^{-1}$  2317 $\text{cm}^{-1}$  represented stretching in N=C=S, C $\equiv$ C, O=C=O bond of isothiocyanate, alkyne, carbon dioxide functional group. The metal oxide formation was confirmed through the peaks range lying between 450-650  $\text{cm}^{-1}$  [34]. So, peaks at 549 $\text{cm}^{-1}$ , 572 $\text{cm}^{-1}$ , 541  $\text{cm}^{-1}$  corresponding to cobalt oxide, nickel oxide and copper oxide corroborated the formation of metal oxides.

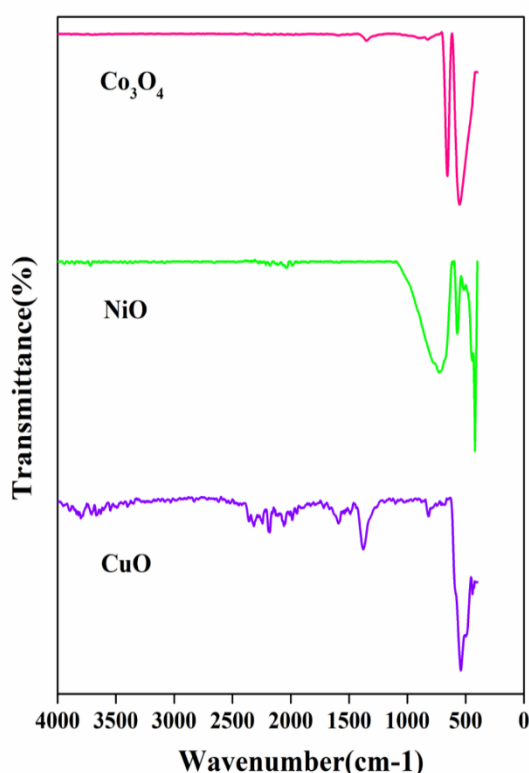


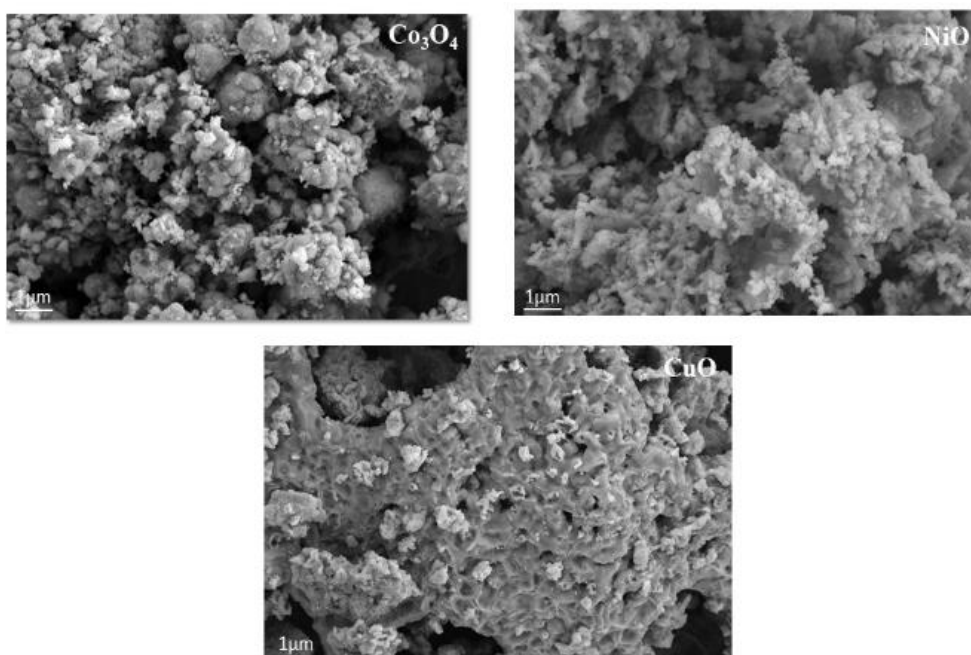
Figure 4. FTIR of  $\text{Co}_3\text{O}_4$ , NiO, CuO nanoparticles.

### 3.3 SEM Analysis and EDAX Analysis

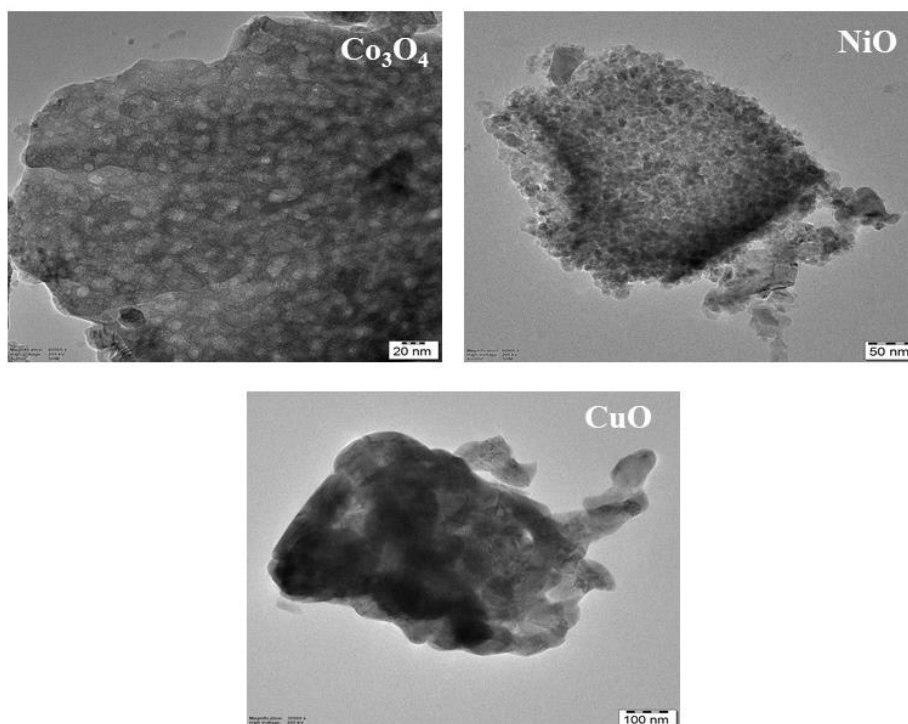
In order to obtain surface morphology of synthesized nanoparticles SEM analysis was performed and results are displayed in Figure 5, by Gemini 300 SEM at various magnification. Mostly appeared to be of clustered spherical particles in different sizes in the

image of cobalt oxide nanoparticle. The SEM image obtained for cobalt oxide nanoparticles matched from prior work that reported synthesis Cobalt oxide nanoparticles of spherical shape with aggregation and mixed spherical morphology from the precursors of Cobalt nitrate hexahydrate with Curcuma longa plant extract [18] and in other research article obtained 99.16 nm sized spherical shaped cobalt oxide employing same bio-molecule [18] but employed root extract of Curcuma longa [32, 33]. Even  $\text{Co}_3\text{O}_4$  nanoparticles showed clustered irregularly shaped nanoparticles in synthesis with M.calabura leaf extract with bandgap of 2.05 eV, where its elemental composition was confirmed through EDX and XPS analysis spectrum [34]. The Synthesized nickel oxide showed irregular shaped particles agglomerated and formed sponge like structure. The previously published work, synthesized nickel oxide particles with average crystallite size 38.5 nm of aggregated cubical morphology that employed Anagallis arvensis L's aqueous leaf extract in which its compounds that act as stabilizing and reducing agent [35] and Green tea mediated synthesis on Nickel oxide on characterization showed flattened flake like particles of size about 29.75 nm [36]. Image of Copper oxide nanoparticles exposed particles to be of interconnected honey comb like structure. Similarly, to interconnected nanosheet structures was observed in the SEM analysis in which C. oblonga seed hydrogel with  $\text{CuSO}_4$  were employed for environmental benign synthesis of CuO with average particle size 69 nm and its formation was confirmed by adsorption peak at 279 nm and 295 nm for UV-visible spectrum [32]. Whereas using Macroptilium lathyroides extract for CuO nanoparticles synthesis resulted in particles of agglomerated granular and spherical shaped with average size of 6 nm to 32 nm and the reason for aggregation and overlapping of synthesized nanoparticles has been reported due to the oxidation that occurred [37] and another experiment on copper oxide synthesis using Melia azedarach as reducing agent with copper chloride produced particles of spherical structure with few agglomeration [19]. The microscope used for SEM was used to record the EDAX spectra to identify and quantify elements present in the synthesized sample (Table 2).

The presence of peaks for Co and O elucidates the formation of Cobalt oxide nanoparticles. The EDAX of nickel oxide nanoparticles reveals the presence Ni and O peaks and the presences of spectrum for Cu and O in Copper oxide nanoparticles confirmed its formation. The published work that employed an aqueous extract of Melia azedarach fruit and copper chloride dihydrate under constant stirring and heating lead to the formation of CuO that was followingly centrifuged, washed, and calcinated, and its formation was confirmed with prominent peaks at the EDS spectrum for Cu, O with other peaks for C, Cl, and K due to interaction with extract during the synthesis [19].



**Figure 5.** SEM of  $\text{Co}_3\text{O}_4$ ,  $\text{NiO}$ ,  $\text{CuO}$  nanoparticles.



**Figure 6.** TEM of  $\text{Co}_3\text{O}_4$ ,  $\text{NiO}$ ,  $\text{CuO}$  nanoparticles

**Table 2.** Elemental composition of synthesized nanoparticles

Nanoparticle	Element	Weight%
$\text{Co}_3\text{O}_4$	Co	84.20
	O	15.80
$\text{NiO}$	Ni	88.38
	O	11.62
$\text{CuO}$	Cu	81.20
	O	18.80

### 3.4 TEM Analysis

The TEM image seen in Figure 6 was obtained from JEM-2100 Plus that helped to monitor morphology and to find size distribution of nanoparticles this revealed that Cobalt oxide nanoparticles showed spherical shaped particles with size 47.6 nm, that matched with already reported synthesis for Cobalt oxide showed spherical shapes and conjoint spherical shapes due to encapsulation ability of plant extract of *Curcuma longa*. These particles synthesized using *Curcuma longa* showed enhanced activity for dye degradation of Methylene blue and against gram-positive bacteria *Staphylococcus aureus* [18]. Cobalt oxide prepared using lemon juice subject to analysis gave uniformly distributed particles almost of spherical shape [38]. Nickel oxide nanoparticle showed well dispersed anomalous particles that were 39.2 nm sized, from literature review, synthesised nickel oxide gave narrowly dispersed spherical particles with black and white areas in TEM that indicates hydrophobic and hydrophilic nature that was synthesized from Banana peel extract as stabilizing agent for method of green synthesis [39] and another study on employing *Ocimum sanctum* leaf extract with Nickel nitrate gave Nickel oxide Nanoparticles of different sizes of spherical and sub-spherical structures [40] and overlapped structure was disclosed from image of copper oxide nanoparticle of with particle size of 34.3 nm. The reported study on Copper oxide nanoparticles from *Macropitilium lathyroides* extract showed effective antioxidant, antibacterial, cytotoxicity, anticancer and antifungal activity that were of spherical shaped and exhibited its potential on degradation of Methylene blue under solar irradiation [37]. Similarly, obtained spherical shape for substitution of *Mangifera Indica* aqueous extract as a reducing agent instead of traditional one in preparation of copper oxide [41].

### 4. Conclusion

There still occurs a challenge for reliable approaches to produce nanoparticles that would pay a pathway of switching to environmentally friendly technologies that doesn't involve any hazardous substance. The findings depict that Honey is used as an effective reducing and stabilizing agent in the synthesis of synthesis cobalt oxide, nickel oxide, and copper oxide nanoparticles via the combustion method. The structural, vibrational, morphological and compositional analysis was carried out through XRD, FTIR, SEM, TEM and EDAX techniques. The crystal parameter analysis of synthesized nanoparticles confirmed the crystalline nature with no extra peaks indicating phase purity, in the analysis of functional group identified peaks other than metal oxide that was ascribed to honey's nature, the synthesized metal oxide's respective elements Co, Cu, Ni and O presences is proved through elemental analysis and agglomerated spherical particles, sponge

like structure and interconnected honey like comb structure for cobalt oxide, nickel oxide and copper oxide was affirmed on morphological analysis. This study establishes a simple, cost-effective, and ecofriendly, effective production method for cobalt oxide, nickel oxide and copper oxide nanoparticles using bio molecule. It's evident that synthesis using Honey is less-toxic and environmentally benign contributes to the decrease in size of nanoparticle, with increased speed of reaction in synthesis of metal oxide nanoparticles. This work provides a basement for the future research as the present work resulted in successful synthesise of metal oxide nanoparticles with honey and this would render its potential in copious application.

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#### Authors Contribution Statement

K. Mathu Metha: Conceptualization, Methodology, Writing - Original draft. I. Pradeep: Validation, Investigation. S.J. Nilofur Fathima: Formal analysis. E.Ranjith Kumar: Writing - Review & Editing, Visualization, Supervision.

#### Funding

The authors declare that no funds, grants or any other support were received during the preparation of this manuscript.

#### Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

#### Data Availability

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

#### Has this article screened for similarity?

Yes

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