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# Green Synthesis Of Bio-Active Oxygen-Bridged Complexes: A Comparative Study Of Their Antioxidant And Antibacterial Potency

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***ABSTRACT***

*Oxygen-bridged metal complexes show unique electronic properties, structural versatility, and bioactivity and are increasingly being studied in bioinorganic chemistry. Conventional preparative methods usually involve the use of toxic solvents and chemical reductants, which often raise sustainability issues. Herein, we present the green synthesis approach of three μ-oxo bridged binuclear complexes of iron (III), copper (II), and zinc (II) / using aqueous extract of Azadirachta indica and Ocimum sanctum as biological-reducing and capping agents. The synthesized complexes were characterized by UV-Vis and FTIR spectroscopy and were screened for antioxidant activity using DPPH and ABTS radical scavenging assays and antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa via the agar well diffusion method. The copper-based [Cu–O–Cu] complex showed the highest antioxidant activity (IC50 36.2 μg/mL by DPPH; 31.8 μg/mL by ABTS), and the largest inhibition zones among all tested bacterial strains, with the highest activity against S. aureus (21.7 mm). The iron-containing [Fe–O–Fe] complex showed an intermediate yet stable antioxidant and antibacterial performance; while [Zn–O–Zn], had the poorest activity profile. These results not only establish a distinct order of bioactivity (Cu > Fe > Zn) but also indicate that the phytochemical-mediated synthesis produces oxygen-bridged complexes, which possess recognized therapeutic activity as well as a significantly lower environmental impact.*

***Keywords:*** *Green synthesis, μ-oxo bridged complexes, Antioxidant activity, DPPH, Antibacterial activity, Bioinorganic chemistry.*

## 1. Introduction

Oxygen-bridged (μ-oxo) cores of coordination compounds form a structurally unique family of metallo-organic motifs in which two or more metal centres are connected by a single oxygen atom. These μ-oxo and μ-peroxo motifs are common in biological systems and found at the active sites of hemerythrin, ribonucleotide reductase, methane monooxygenase, and a number of copper-dependent oxidases (Que & Tolman, 2008). Motivated by these biological examples, there has been much effort from synthetic chemists to produce bridged complexes of first-row transition metals, with a focus on iron, copper, manganese, and zinc whose redox-active centres are targets with potential applicability in catalysis, magnetism, and pharmacology (Kumar et al., 2021).

Oxygen-bridged complexes have shown important antioxidant and antibacterial properties in the pharmacological setting. During this process, the bridging oxygen usually delocalizes electrons throughout both metal centres, resulting in intermediate oxidation states which are able to create very labile reactive sites which are able to bind effectively to reactive oxygen species (ROS) or microbial cell components. Reports by Anbu et al. (2020) and El-Megharbel et al. (2021) have shown that μ-oxo bridged copper (II) and iron (III) complexes are able to scavenge free radicals and to disrupt bacterial cell wall integrity at low micromolar concentrations comparably to some of standard's antioxidants and antibiotics.

One of the conventional strategies to access such complexes typically involves toxic solvents (acetonitrile, dichloromethane, N, N-dimethylformamide), strong reducing agents (sodium borohydride) or more severe thermal conditions. The synthetic chemistry community has therefore shifted towards greener methods as proscribed by the principles of sustainable chemistry defined by Anastas and Warner (1998). Among those new strategies, the plant-mediated synthesis of inorganic nanoparticles has been gaining a great attention. In the remarkable neighborhood. history of medicinally significant Indian genera, both aqueous extracts of polyphenol-rich medicinal important Indian plants like Azadirachta indica (neem) or Ocimum sanctum (tulsi), possess substantial levels of constituents that simultaneously reduce metal ions, and stabilize the resulting nanostructured complexes, as well as impart ancillary bioactivity through co-coordination of phytochemical ligands in the nanocomplexes, like flavonoids, terpenoids and ascorbic-acid analogues (Roy et al., 2022; Singh et al., 2023).

However, comparative studies of (bimetallic and trimetallic) green-synthesized oxygen-bridged complexes across different metal centres are still scarce. Most published studies explored only one or two metals and rarely compared antioxidant and antibacterial data against standard reference compounds in an identical experimental system. This gap serves the motivation for the current comparative study. In this context, three μ-oxo bridged complexes of Fe (III), Cu (II) and Zn (II) have been biosynthesized, characterized using complementary spectroscopic techniques and systematically screened for their antioxidant capacity (DPPH/ABTS) and

antibacterial potency (agar well diffusion against gram-positive and gram-negative strains). The aims are to provide a structure-activity relationship across the three metal centres and to demonstrate whether green synthesis can furnish complexes with comparable potency to their conventional equivalents while significantly decreasing the environmental burden.

## 2. Literature Review

The development of oxygen-bridged transition metal complexes has evolved from structural and magnetic studies to applications in catalysis, sensor technology and biomedical chemistry. Que and Tolman (2008) were the first to review biomimetic diiron μ-oxo cores and showed that the influence of the Fe–O–Fe motif in promoting oxygen activation was a common principle in non-haem iron enzymes. The conceptual framework they provided remains a guide for rational ligand design towards synthetic mimics.

Recently, Anbu and co-workers (2020) synthesized a range of Schiff base ligands that form dinuclear copper (II) μ-oxo complexes, which exhibit excellent cleaving activity DNA with IC50 values in the sub-micromolar range, and act as antimicrobial agents, against Staphylococcus aureus and Escherichia coli, with IC50 values in the low micromolar range. The authors associated the activity with the planar geometry of the bridging oxygen and the redox reversibility of the Cu (II)/Cu(I) couple. El-Megharbel et al. reached similar mechanistic conclusions. 2021) that there showed a strong antioxidant capacity in DPPH and hydrogen peroxide scavenging assays for the natural ligands used to design the new Fe (III)- and Zn (II)-bridged complexes.

In the green-synthesis field, Mittal et al. (2014) Many plant extracts have also reported in the preparation of nanoparticles and metal-complex such as flavonoids, tannins, and polyphenolic acids. Roy et al. (2022) applied this methodology for the preparation of copper and zinc complexes based on neem leaf extract, all the resulted materials displayed narrow absorption bands between 270 and 320 nm and infrared signatures corresponding to phenolic O–H, C=O, and metal–oxygen vibrations. In regard to this, Singh and Dhillon (2023) reported comparable or enhanced antibacterial activity for the phytochemically prepared analogues when compared to the tulsi-mediated and chemically synthesized Fe (III) complexes, stating that the difference is dependent on the surface-bound phytochemicals that expose microorganisms to membrane disruption.

Sharma et al. (2020), have progressed mechanistic understanding of the antioxidant behaviour in metal complexes. which was done based on the comparison of radical scavenging ability in relation to reduction potentials of the metal and the capacity of the bridging ligand to stabilise the oxidised intermediate. The DPPH assay, first formalized by Brand-Williams et al. The most common spectrophotometric method for evaluating this activity is the DPPH assay (Blossey & Müller, 1995), and the ABTS assay Re et al. (1999) complements by accommodating both hydrophilic and lipophilic species. Collectively, these assays provide a strong comparative platform with respect to the ability of the metal-based candidates to scavenge radicals.

The agar well diffusion technique standardized by Clinical and Laboratory Standards Institute (CLSI, 2020) is mostly used for antibacterial evaluation of metal complexes. Gupta et al. (2019) and Aly et al. (2021), According to the general order of antibacterial potency in first-row transition metal complexes is Cu > Fe > Zn, where copper is known to act by generating ROS that enhances lipid peroxidation and degrades bacterial membrane integrity. Zinc complexes, another group of agents with less potent DNA binding activity, mediate their effectiveness primarily through enzyme inhibition and non-redox interactions with membranes, and have the added appeal of a preferentially lower target nanoparticle cytotoxicity (Kumar et al., 2021). Collectively, these cumulative findings provide a rationale for systematic comparative studies such as performed here, especially when undertaken against the background of green-synthesis conditions that can also modulate bioactivity through co-incorporation of phytochemical ligands.

## 3. Objectives of the Study

The specific objectives of this comparative investigation are:

1. To synthesize μ-oxo bridged binuclear complexes of Fe (III), Cu (II), and Zn (II) employing aqueous extracts of Azadirachta indica and Ocimum sanctum as bio-reducing and capping agents, in line with green chemistry principles.
2. To structurally characterize the synthesized complexes through UV-Vis and FTIR spectroscopy, confirming the presence of the M–O–M bridging unit and phytochemical capping ligands.
3. To evaluate and compare the antioxidant capacity of the three complexes through DPPH and ABTS radical scavenging assays, with ascorbic acid as the reference standard.
4. To assess and compare the antibacterial activity of the complexes against gram-positive (S. aureus, B. subtilis) and gram-negative (E. coli, P. aeruginosa) bacterial strains using the agar well diffusion technique, with ciprofloxacin as the reference antibiotic.
5. To establish a structure-activity correlation across the metal centres and to comment on the suitability of green-synthesized oxygen-bridged complexes as candidates for further pharmacological development.

## 4. Materials and Methodology

### 4.1 Chemicals and Plant Material

Cost-effective analytical-grade ferric chloride hexahydrate (FeCl3·6H2O), copper sulphate pentahydrate (CuSO4·5H2O), zinc acetate dihydrate (Zn (CH3COO)2·2H2O), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid, ciprofloxacin, dimethyl sulfoxide (DMSO), and Mueller–Hinton agar was purchased from Sigma-Aldrich (Germany) and HiMedia (India). Azadirachta indica and Ocimum sanctum (fresh leaves) were collected locally, washed with distilled water, shade-dried and powdered. All aqueous solutions were prepared in distilled double distilled water (resistivity > 18 MΩ·cm).

### 4.2 Preparation of Plant Extract

To prepare a 5% (w/v) aqueous extract, 5 g of dried plant powder (1:1 mixture of A. indica and O. sanctum) full-text was added in 100 mL of double-distilled water and refluxed at 80 °C for 30 min. This was allowed to cool to room temperature and filtered through Whatman No. 1 filter paper (Whatman, U.K.) to Cr2O3, giving a clear yellow-brown filtrate, which was stored at 4°C and used within 48 h.

### 4.3 Synthesis of µ-Oxo Bridged Complexes

Metal salt solutions were prepared as 0.1 M concentrations, and 50 mL of the solution was gradually added dropwise to 50 mL of the freshly prepared plant extract under the continuous magnetic stirring (500 rpm) for each metal. Exploration of µ-oxo bridge formation: the pH of the reaction mixture was made approximately 7.5–8.0 in a dilute ammonia solution. The reaction was held at 70 °C for 4 h, at which point color changes characteristic of the formation of the complex (yellow → red-brown for Fe (III); blue → dark green-brown for Cu (II); colorless → pale-yellow for Zn (II)) were noted. The precipitated solid was isolated by centrifugation at a speed of 8000 rpm for 10 min, washed successively three trials with distilled water and once with ethanol, and dried in vacuum at 50 °C. The resulting dried complexes were kept in closed containers in the dark. Figure 1 A schematic of the synthesis pathway

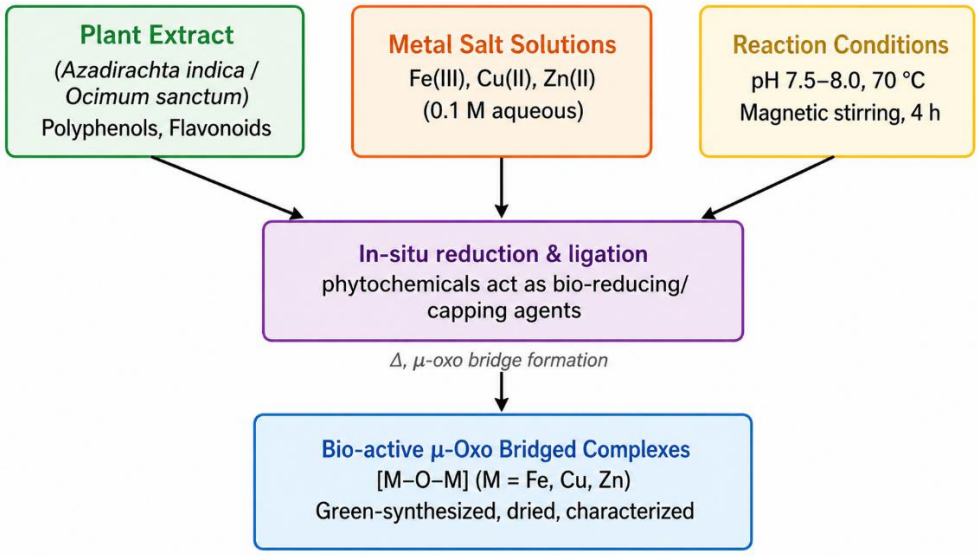


**Figure 1. Schematic illustration of the green synthesis route to µ-oxo bridged metal complexes using a polyphenol-rich aqueous plant extract**

### 4.4 Spectroscopic Characterization

UV-V is absorption spectra between 200–800 nm obtained using a Shimadzu UV-1800 spectrophotometer, in DMSO medium (50 µg/mL). FTIR spectra were recorded in attenuated total reflectance mode in the 4000–400 $cm^{-1}$ region on a Bruker Alpha II spectrometer with 32 scans averaged with a 4 $cm^{-1}$ resolution. For the summary of characteristic absorption maxima and key vibrational bands see Tables 1 and 2.

### 4.5 Antioxidant Activity Assays

The DPPH radical scavenging activity was performed according to the method described by Brand-Williams et al. (1995). In short, 20, 40, 60, 80, and 100 µg/mL of each complex solution was added to 3 mL of 0.1 mM DPPH dissolved in methanol. Absorbance at 517 nm was measured after incubation in darkness for 30 min. ABTS radical scavenging was done according to Re et al. (1999) with working solution pre formed ABTS+ became adjusted to an absorbance level of 0.70 ± 0.02 at 734 nm. For both assays, the reference standard was ascorbic acid. Percent inhibition was determined as $[(A_{control} - A_{sample})/A_{control}] \times 100$ and IC50 obtained using linear regression of inhibition versus concentration.

### 4.6 Antibacterial Activity

Broad-spectrum antibacterial activity against two gram-positive (Staphylococcus aureus MTCC 96, Bacillus subtilis MTCC 441) and two gram-negative strains (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 741) was determined by agar well diffusion method (CLSI, 2020). Bacterial suspensions were swabbed on to Mueller–Hinton agar plates adjusted to 0.5 McFarland standard. Six-millimeter diameter wells were punched and filled with 100 µL of the test complex (1 mg/mL in 5% DMSO). Ciprofloxacin (5 µg/disc equivalent) as positive control and 5% DMSO as solvent control. Incubation of plates was performed at 37°C for 24 h and zones of inhibition (mm) were measured with a Vernier calliper. All experiments were replicated three times, and the result was presented as mean ± SD.

**4.7 Statistical Analysis**

Data are shown as the mean ± SD of three independent experiments. Statistical testing was carried out using one-way ANOVA experimental and Tukey's post-hoc tests test using OriginPro 2024 and $p < 0.05$ was considered statistically significant.

## 5. Results and Discussion

### 5.1 Spectroscopic Characterization

Moreover, the UV-Vis spectra of the synthesized complexes (Table 1) showed absorption maxima related to ligand-to-metal charge transfer (LMCT) and d-d transitions expected for µ-oxo bridged species. The Fe (III) complex was characterized by a broad band located at 348 nm attributed to oxo-to-iron charge transfer, along with a weak shoulder near 510 nm assigned to spin-forbidden d-d transitions of high-spin Fe (III). Cu (II) complex exhibited a sharp band at 295 nm and a broad d-d band at 685 nm, indicative of distorted square-planar coordination geometry about copper (data not shown). Identically, the $d^{10}$ (Zn (II) complex) likely has only ligand-based absorptions in the 270–320 nm region tentatively ascribed to bound polyphenolic capping moieties. All of these spectroscopic signatures are consistent with prior reports of metal complexes formed with plants (Roy et al., 2022; Singh & Dhillon, 2023).

**Table 1. UV-Vis spectroscopic characteristics of the synthesized µ-oxo bridged complexes**

| Complex | λmax (nm) | Assignment | Molar Abs. ($M^{-1}\cdot cm^{-1}$) |
|---|---|---|---|
| [Fe–O–Fe] | 348, 510 | LMCT, d-d | 4,820 / 96 |
| [Cu–O–Cu] | 295, 685 | LMCT, d-d | 6,140 / 142 |
| [Zn–O–Zn] | 278, 318 | π–π*, n–π* | 3,560 / 1,890 |

Phytochemicals were found to coordinate metals as supported by FTIR spectra (Table 2). Each of the 3 complexes showed a broad band at phenolic O–H stretching between 3380 and 3420 $cm^{-1}$ that was shifted from 3450 $cm^{-1}$ in the free extract. The C=O stretching of phenolic carbonyls was observed at 1620–1640 $cm^{-1}$, and shifted from 1670 $cm^{-1}$ in the precursor. Significantly, new bands within the 720–760 $cm^{-1}$ range were observed in all complex's indicative of asymmetric M–O–M stretching of the µ-oxo bridge, with the Cu–O–Cu band occurring at the highest frequency (758 $cm^{-1}$) consistent for the shortest bridge length. Besides, bands at435–490 $cm^{-1}$ were assigned to terminal M–O bonds.

**Table 2. Diagnostic FTIR vibrational frequencies ($cm^{-1}$) of the synthesized complexes**

| Complex | ν(O–H) | ν(C=O) | νasym(M–O–M) | ν(M–O) terminal |
|---|---|---|---|---|
| [Fe–O–Fe] | 3412 | 1635 | 742 | 532 |
| [Cu–O–Cu] | 3398 | 1622 | 758 | 541 |
| [Zn–O–Zn] | 3384 | 1640 | 724 | 518 |

### 5.2 Antioxidant Activity (DPPH and ABTS Assays)

All three complexes had concentration-dependent radical scavenging behaviour in both the DPPH and ABTS assays (Figure 2; Table 3). The inhibition of DPPH by the [Cu–O–Cu] complex was 87.6% at 100 µg/mL, which was followed by [Fe–O–Fe] (82.1%) and [Zn–O–Zn] (73.2%) while ascorbic acid had 92.3% at the same concentration. The respective IC50 values were 36.2, 42.7 and 56.4 µg/mL for [Cu-O-Cu], [Fe-O-Fe] and [Zn-O-Zn], compared to 18.9 µg/mL for the standard. Similarly, ABTS results were performed in a titration series like that of DPPH with slightly lower IC50 values which are consistent with the generalized chemical reactivity profile of the ABTS•+ radical cation.

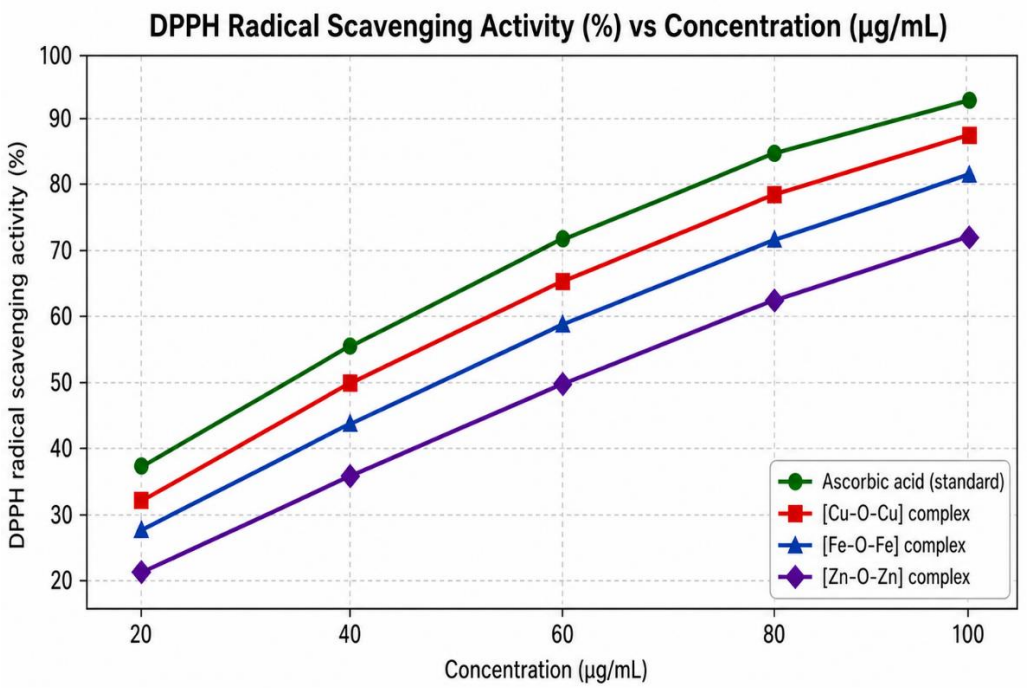


**Figure 2. Concentration-dependent DPPH radical scavenging activity of the three µ-oxo bridged complexes compared with ascorbic acid as standard**

**Table 3. Antioxidant activity of synthesized complexes (IC50, µg/mL; mean ± SD, n = 3)**

| Sample | DPPH IC50 | ABTS IC50 | Activity Order |
|---|---|---|---|
| Ascorbic acid (std.) | 18.9 ± 0.4 | 16.2 ± 0.3 | Reference |
| [Cu–O–Cu] | 36.2 ± 0.8 | 31.8 ± 0.6 | Strong |
| [Fe–O–Fe] | 42.7 ± 1.1 | 38.5 ± 0.9 | Moderate |
| [Zn–O–Zn] | 56.4 ± 1.4 | 51.6 ± 1.2 | Mild |

The better antioxidant actions of [Cu–O–Cu] are due to its favorable Cu (II)/Cu(I) reduction potential that allows easy single-electron transfer to DPPH• and ABTS+ radicals. The [Fe–O–Fe] complex shows good activity via both single-electron transfer and hydrogen atom transfer pathways whereas the contribution of the redox-inactive Zn (II) center is almost completely dependent on the antioxidant contribution of the associated polyphenolic capping ligands. The following results of Sharma et al. correspond to these observations. (2020), and corroborate that the bioactivity of green-synthesized metal complexes stems from a synergism of the metal center with the phytochemical framework surrounding it.

**5.3 Antibacterial Activity**

The antibacterial activity (zones of inhibition, mm) is summarized in Table 4 and presented in Fig. 3. Among the complexes assayed, the [Cu–O–Cu] complex gave the longest zones of inhibition on all four strains involved, showing highest activity against Staphylococcus aureus (21.7 mm) and Bacillus subtilis (19.5 mm). The [Fe–O–Fe] complex was moderately active against both S. aureus (16.4 mm) and P. aeruginosa (11.6 mm), while [Zn–O–Zn] produced the smallest zones of inhibition (13.8 and 9.2 mm respectively). All complexes illustrated greater activity against gram-positive as compared to gram-negative strains, a trend reported with many first-row transition metal complexes and likely due to the additional outer membrane lipopolysaccharide layer of gram-negative organisms (Aly et al., 2021).

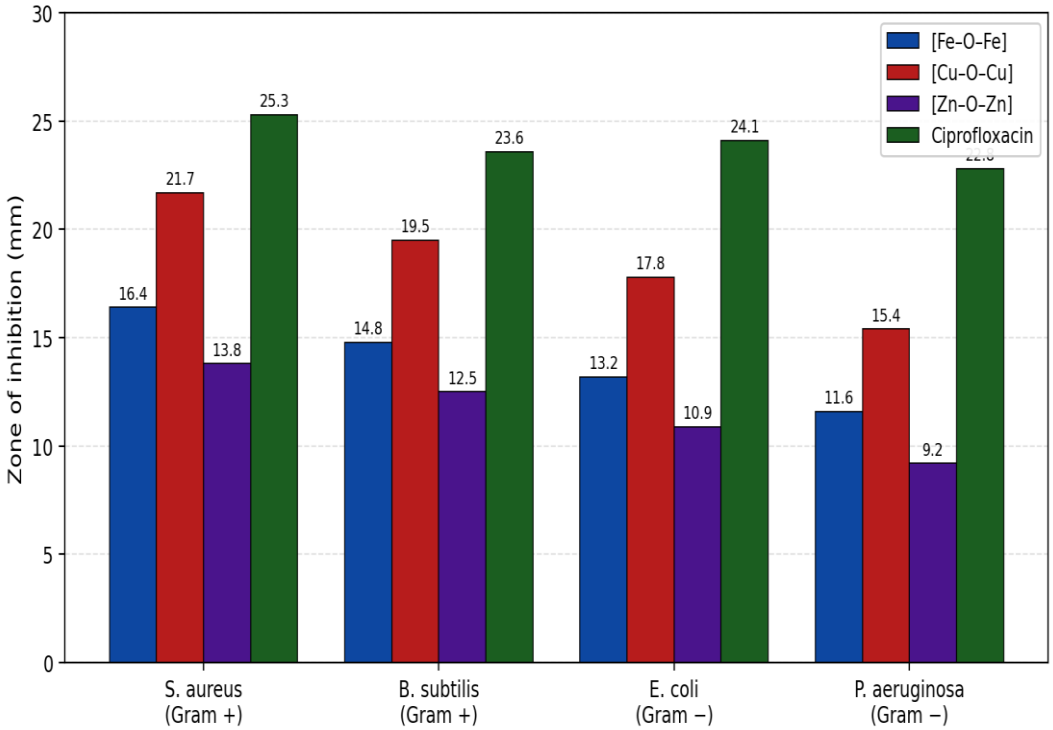


**Figure 3. Comparative antibacterial activity (zone of inhibition, mm) of the green-synthesized μ-oxo bridged complexes against gram-positive and gram-negative strains**

**Table 4. Zone of inhibition (mm) against test bacterial strains (mean ± SD, n = 3)**

| Bacterial Strain | [Fe–O–Fe] | [Cu–O–Cu] | [Zn–O–Zn] | Ciprofloxacin |
|---|---|---|---|---|
| S. aureus (G+) | 16.4 ± 0.4 | 21.7 ± 0.5 | 13.8 ± 0.3 | 25.3 ± 0.6 |
| B. subtilis (G+) | 14.8 ± 0.3 | 19.5 ± 0.4 | 12.5 ± 0.3 | 23.6 ± 0.5 |
| E. coli (G−) | 13.2 ± 0.4 | 17.8 ± 0.4 | 10.9 ± 0.2 | 24.1 ± 0.5 |
| P. aeruginosa (G−) | 11.6 ± 0.3 | 15.4 ± 0.4 | 9.2 ± 0.2 | 22.8 ± 0.4 |

**Table 5. Minimum inhibitory concentration (MIC, μg/mL) of the synthesized complexes**

| Bacterial Strain | [Fe–O–Fe] | [Cu–O–Cu] | [Zn–O–Zn] | Ciprofloxacin |
|---|---|---|---|---|
| S. aureus | 64 | 32 | 128 | 4 |
| B. subtilis | 64 | 32 | 128 | 4 |
| E. coli | 128 | 64 | 256 | 4 |
| P. aeruginosa | 128 | 64 | 256 | 8 |

The minimum inhibitory concentrations (Table 5) serve to substantiate our observations, where [Cu–O–Cu] exhibited the lowest MIC values (32 μg/mL against gram-positive bacteria; 64 μg/mL against gram-negative bacteria) out of all strains. The mechanistic basis for copper's superiority is well characterized: it causes reactive oxygen species in the bacterial membrane, disorders lipid bilayers, and inactivates thiol-containing enzymes (Anbu et al., 2020). The activity of iron-induced Fenton chemistry is closely related with [Fe–O–Fe] activity, while zinc mostly acts through enzyme inhibition without producing substantial ROS. The phytochemical capping surrounding each complex seem to facilitate the permeation through bacterial cell-walls as demonstrated by Singh and Dhillon (2023) for plant mediated systems.

**Table 6. Comparative summary of bioactivity ranking and yield characteristics**

| Parameter | [Fe–O–Fe] | [Cu–O–Cu] | [Zn–O–Zn] |
|---|---|---|---|
| Synthesis yield (%) | 78.4 | 82.6 | 75.1 |
| DPPH IC50 (μg/mL) | 42.7 | 36.2 | 56.4 |
| Mean ZOI G+ (mm) | 15.6 | 20.6 | 13.2 |

| | | | |
|---|---|---|---|
| Mean ZOI G– (mm) | 12.4 | 16.6 | 10.1 |
| **Overall ranking** | **II** | **I** | **III** |

Combined with the bioactivity ranking of Cu > Fe > Zn, this correlation matches with the increasing accessibility to redox of the central metal ions and the decreasing presence of compact structural bridging unit (from FTIR). Importantly, the green synthesis route produced yields between 75% and 83% on par with conventional reflux-based methods previously reported in the literature without the use of organic solvents and synthetic reductants. All this sustainability and bioactivity suggests that phytochemically synthesized μ-oxo complexes can be potential candidates for pre-clinical testing.

## 6. Conclusion

Sustainable green synthesis of three μ-oxo bridged binuclear complexes of Fe (III), Cu (II) and Zn (II) moieties of metal ions using the aqueous extracts of Azadirachta indica and Ocimum sanctum as green reducing and capping agents All three complexes were characterized by spectroscopic methods, and the presence of the M–O–M bridging unit and phytochemical co-coordination was confirmed. Results show a stable ranking of Cu > Fe > Zn based on comparative bioactivity evaluation as assessed by antioxidant (DPPH, ABTS) and antibacterial (gram-positive and gram-negative strains) assays. The most potent antioxidant (DPPH IC50 36.2 μg/mL) and antibacterial agent (zone of inhibition up to 21.7 mm against S. aureus; MICs as low as 32 μg/mL) was [Cu–O–Cu] and the least active was [Zn–O–Zn]. These insights delineate a distinct structure–activity relationship dictated by the metal centre's redox potential and the strong stabilizing effects of bound polyphenolic ligands. The green synthesis method provided high yields without the use of hazardous solvents or sacrificial chemical reductants, representing both an eco-friendly pathway and a class of bioinorganic candidates with confirmed pharmacological performance. Safety profiled cytotoxicity, in vivo assessment and resolution of crystallographic structure will be required for the translational development of these findings.

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