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"Green synthesis of CuO nanoparticles using *Crinum latifolium* leaf extract and *in vitro* investigation of antimicrobial and antioxidant activities"

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Abstract: *Crinum latifolium* is an ornamental and medicinal plant widely grown in various Indian regions and worldwide. In aurveda, this plant having a significant medicinal importance. In present study, this plant was used in green synthesis of CuO nanoparticles using Copper chloride. However before the green synthesis of CuO nanoperticles, Plant leaves were extracted in double distilled water and methanol. Qualitative phytochemical screening for presence of bioactive compounds and antimicrobial activity against gram negative bacteria (*E.coli and Salmonella*), gram positive bacteria (*K. pneumoniae*) and fungi (*Fusarium oxysporum*) were analyzed. After green synthesis of CuO nanoparticles, were characterized using different available techniques UV visible, FTIR and XRD. Moreover about this the synthesized CuO nanoparticles were investigated for antimicrobial potentiality and antioxidant activity.

***Index Terms* -** Crinum latifolium, Phytochemicals, Antioxidants, CuO nano particles and Antimicrobial activity.

I. Introduction

In ancient India conventional medicinal system was based on the herbal plants which make this significant. These ancient conventional healing states the vast knowledge about the plants and importance of plant's constituents against specific diseases. Some of this vast indigenous knowledge is documented in manuscript while a most of the knowledge is not documented. Such valuable and undocumented knowledge is localized to some indigenous communities and verbally transferred from one generation to the other only. The conventional medicinal system has been documented in manuscripts as Ayurveda, Yoga, Naturopathy, Homeopathy, Siddha and Unani; abbreviated as AYUSH.

With the recent updated idea of 'evidence-based validated medicine', scientific evaluation of claims mentioned in ancient texts is now an advanced area of research. These old claim was taken as new approach which contributed significantly to the scientific support of the traditional claims.

Amaryllidaceae is a family of perennial, herbaceous, and bulbous plants identified as therapeutic potential in tradional medicine. The *Crinum latifolium* belongs to genus *Crinum* fall under the Amaryllidaceae family, is widely used as traditional medicine and many new research works has been carried out to investigate its pharmacological potentiality (Ghosal et al, 1985). However, previous literature states that no significant pharmacological breakthrough has been established for *Crinum latifolium*. The pharmacological studies revealed that pharmacognostic parameters are very crucial to establish the authenticity of a plant as an herbal drug. This is the basis of the present study.

Crinum is generally known as "Sudarshan" in Hindi and "Madhuparnika" in Sanskrit. This plant is significantly aplied as a tonic, in the treatment of allergic disorders, inflammation and tumor diseases. The leaf juice consists of remarkable medicinal property and applied as topically to cure skin diseases and on piles to reduce pain and inflammation (Sainkhediya et al, 2014). *Crinum* is also a potential analgesic, immune stimulating, antineoplastic, antiviral and antimicrobial herbal drug, as a remedy for blood pressure, rheumatism and in weakness (Kirktikar et al, 1987) . *Crinum latifolium* is an Ayurvedic medicine used in different ayurvedic formulations such as Mahasudarshan churna which is conventionally used as antiviral, anti-malarial and antipyretic (Tambekar et al., 2011), thus have vast industrial interest.

Extensive attentions are being paid on natural healing drugs from plants, are easy to get and find safe with low chances of side effect against serious illness. Mostly medicinal plants are less expensive and easily available to the large population especially in economically weaker countries. Availability, low cost, less side effects and efficacy are the factors that popularize the herbal medicaments and signifies its therapeutic values (Ivanova et al., 2005). Several researchers have investigated the therapeutic benefits of some plants (Mandal et al., 2007; Mishra, 2009). The Crucial basis of pioneering medicines and healthcare natural materials is medicinal plants (Joshi et al., 2019). The extraction and characterization of various bio metabolites from plants is the main process has resulted in the development of a number of medicines with high activity profiles (Pradhan et al., 2019). Emerging market demands of such natural drugs among a large population has pushed the therapeutic plants towards the category of endangered plant species which also cases the genetic variability (Parkhe et al., 2018). On large scale use of antimicrobial agents causes drug resistance among pathogenic microorganism. Thus the continued persistence of drug-resistant organisms, as well as increasing evolutionary adaptations among pathogenic organisms' due to routinely used antimicrobials, have reduced the efficiency of presently used antimicrobial agents. Moreover the antibiotics result the side effects, which necessitate the search for alternative medications from unconventional sources such as plants. Studies has suggested that more than 80% of the world's population have faith on plants to accomplish their basic health care needs (Fazel et al., 2008). Plants are still a key source of commercially used medications. Many synthetic medications have been shown to be effective. Currently, natural products as well as active plant extracts demands are being increasing every day due to new medication investigations and evaluations (Bauer et al., 1966) [13]. However, no wide investigation on the antibacterial activity of *C. latifolium* leaves has been made, the current study aims to explore the antimicrobial activity *C. latifolium* leaves against bacterial and fungal species. These extracts are also subjected to preliminary phytochemical analyses in order to identify bioactive chemicals with antibacterial activity. Spectrophotometric analysis was used to evaluate the total phenol, flavonoid, and alkaloids content.

II. RESEARCH METHODOLOGY

2.1. Collection and processing of plant material

The plant *Crinum latifolium* was collected from Gwalior district (M.P.), India. The Plants leaves were washed under running tap water, air dried in the shade and ground to a fine powder and stored in the airtight bottle.

2.2 Preparation of Leaf extract

Leaf powder (10 g) of the *Crinum latifolium* were extracted in 100 methanol and double distilled water separately water using the soxhalet apparatus soaked. The filtrates were concentrated on a rotary evaporator and then stored at 4°C till further use (**Swaminathan, *et al.*, 2017**).

2.3. Phytochemicals screening

The qualitative phytochemicals screening of crude leaf extract was carried out using standard phytochemical methods with slight modifications (Harborne, 1998; Evans, 1989).

2.4. Bacterial strains

Klebsiella pneumoniae, *Salmonella sps*, *Fusaium oxusporum*, *Candida albicans* and *E.coli* used in the present study were procured from the MTCC, Chandigarh, India.

2.5. **Determination of antimicrobial activity**

The antimicrobial activities of *Crinum latifolium* leaves extracts were determined using the agar-well diffusion method of Akpata and Akinrimisi (1977) with slight modification. Crude leaves extracts in different concentrations 25 µg/µl, 50 µg/µl, 75µg/µl and 100µg/µl (10 mg/ml).

2.6. Characterization of nanoparticles

Synthesized CuO were characterized by using Systronic 2702 UV-vis spectrophotometer at wavelengths ranging from 200 to 700 nm for UV-vis spectroscopy. Shimadzu Fourier-transform infrared in the range of 4500–400 cm^{-1} was applied for the IR-spectroscopy.

III. RESULTS AND DISCUSSION

Present study was carried out on plant leaves of *Crinum latifolium* collected from Botany department premise of Jiwaji University Gwalior (MP) India. Qualitative Phytochemicals screening and antimicrobial study was done on aqueous and Methanol crude extracts.

In the present study phytochemicals test were qualitatively tested to find the presence of bioactive compounds as shown in table 1.Saponins, Steroids and Glycosides were found absent in aqueous extract. Similarly in methanol extract Saponin and steroids were also found absent. However Glycosides was detected in methanol extract.

Furthermore, terpenes, flavonoids, Phenolics, Carbohydrate, Alkaloids and Anthraquinones were found present in both crude extracts, when qualitatively analyzed. Tannins and Proteins were found absent in both extract of *Crinum latifolium.* Presence of Phytochemicals in plant imparts the antimicrobial and antioxidant activity. From this point of view alkaloids plays a significant role.

Table 3.1. Qualitative Phytochemical screening of *Crinum latifolium leaves*

Phytochemicals	Methanol Extract	Aqueous Extract
Saponin	ND	ND
Steroid	ND	ND
Glycosides	+	ND
Terpenes	+	+
Flavonoids	+	+
Phenol	+	+
Tannin	+	+
Alkaloids	+	+
Anthroquinines	+	+
Proteins	ND	ND
Carbohydrates	+	+

Abbreviations: ND: Not detected; (+) present

Thus the presence of bioactive molecules enhances the manifold significance of this plant against the reactive oxygen species. Reactive oxygen species are the group of free radicals, produces oxidative stress within the cells.

Qualitatively evaluated phytochemicals in the *Crinum latifolium* were flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, Gums and mucilage's, Fats and fixed oils, Except Naphthoquinones, Phytosterols and proteins. Phytosterols, saponins and Fats and fixed oils all given phytochemicals were present in ethanolic extract of leaves..

3.2 Antioxidant Activity

3.2.1 Total phenolic content of *Crinum latifolium* and CuO-CL nano particles

In the present study, the total phenolic content of *Crinum latifolium* leaf extracts and CuO–CL nanoparticles was determined and expressed as **gallic acid equivalents (GAE mg/g)**,are presented in **graph**. the methanolic extract of *Crinum latifolium* leaves exhibited a total phenolic content of **36.1 ± 0.43 mg GAE/g**, whereas the aqueous extract showed a comparatively lower value of **29.8 ± 0.3 mg GAE/g**. The higher phenolic content observed in the methanolic extract indicates that **methanol is a more efficient solvent for extracting phenolic compounds** due to its suitable polarity and ability to dissolve a wide range of phenolic constituents.

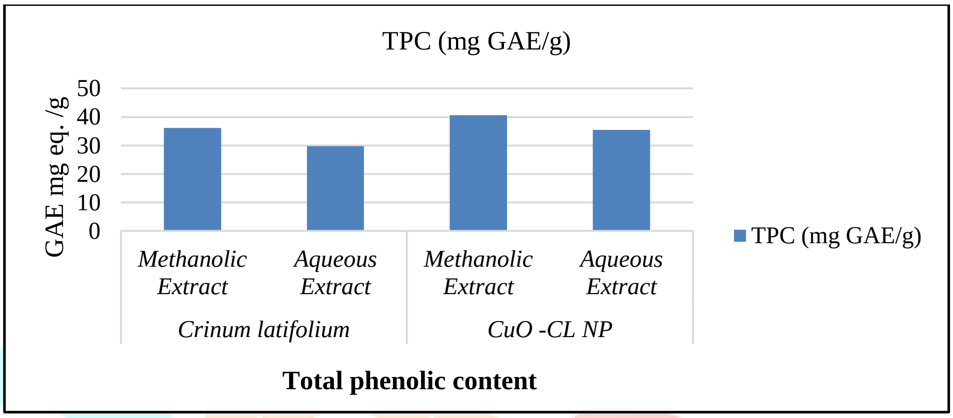


Fig. 3.2. Total phenolic content of *Crinum latifolium* and CuO-CL nano particles

The synthesized **CuO–CL nanoparticles** showed relatively higher phenolic content comparatively to crude plant extracts., the methanolic extract of CuO–CL nanoparticles showed a total phenolic content of **40.6 ± 0.45 mg GAE/g**, while the aqueous extract exhibited **35.5 ± 0.5 mg GAE/g**. Furthermore, *Crinum latifolium* has been reported to contain several biologically active compounds including **phenolic compounds, flavonoids, and Amaryllidaceae alkaloids**, which contribute to its medicinal properties such as antioxidant and antimicrobial activities (Bisyan, 2023).

3.2.2 Total Flavonoid Content of *Crinum latifolium*and CuO-CL nano particles

In the present study, the total flavonoid content of *Crinum latifolium* leaf extracts and CuO–CL nanoparticles was determined and expressed as **mg quercetin equivalent per gram (mg QE/g)**.

The methanolic extract of *Crinum latifolium* leaves exhibited a flavonoid content of **45.8 ± 0.6 mg QE/g**, whereas the aqueous extract showed a slightly lower value of **40.3 ± 0.9 mg QE/g**.

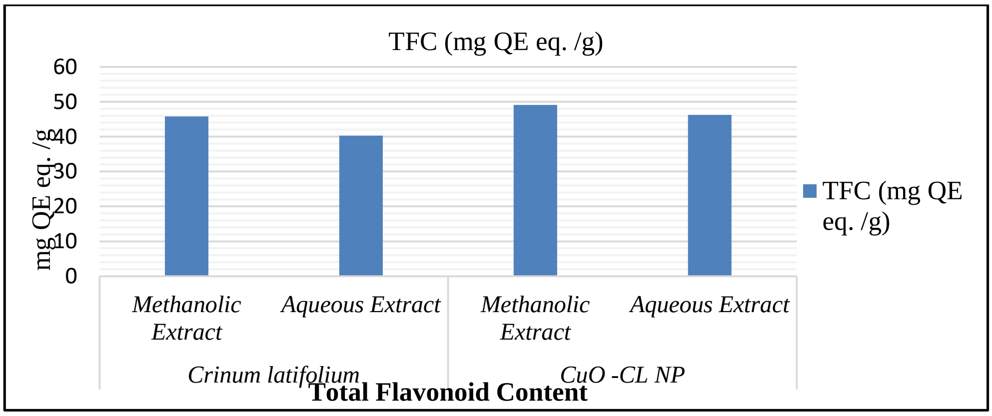
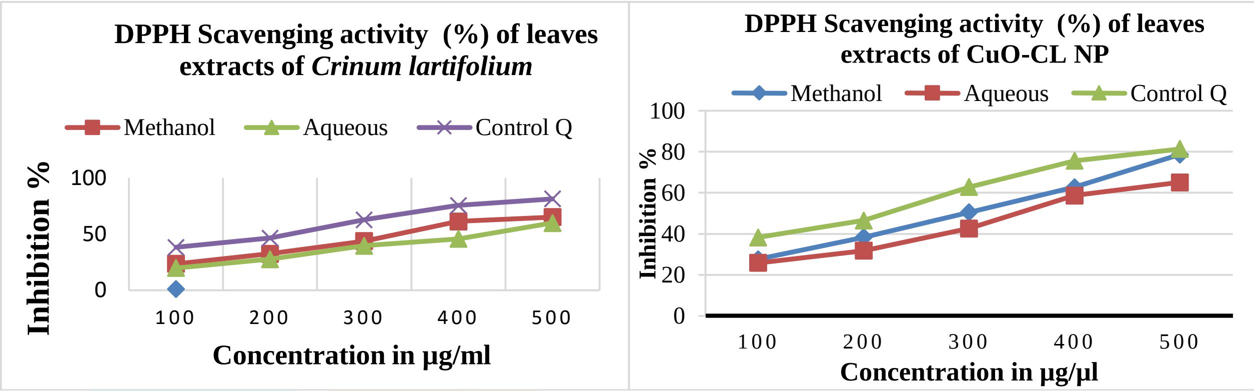


Fig.:3.3 Total Flavonoid Content of *Crinum latifolium* and CuO-CL nano particles

Similarly, the synthesized **CuO–CL nanoparticles** showed comparatively higher flavonoid content than the crude plant extracts. CuO–CL nanoparticles synthesized from the methanolic extract showed a flavonoid content of **49.1 ± 0.7 mg QE/g**, while the nanoparticles from aqueous extract exhibited **46.2 ± 0.8 mg QE/g**.

3.2.3 DPPH Scavenging activity (%) of *Crinum latifolium* and CuO-CL NP

The antioxidant potential of the methanolic and aqueous extracts of *Crinum latifolium* leaves was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. At a concentration of **100 mg/ml**, the methanolic extract showed **23.55% inhibition**, whereas the aqueous extract exhibited **19.90% inhibition**. As the concentration increased to **200 mg/ml**, the scavenging activity also increased to **32.50%** for the methanolic extract and **27.77%** for the aqueous extract.



(a) (b)

Fig. 3.4 a, DPPH Scavenging activity (%) of *Crinum latifolium* b. DPPH Scavenging activity (%) of CuO-CL Nano materials

The highest activity was observed at **500 mg/ml**, where the methanolic extract showed **65.22% inhibition**, while the aqueous extract recorded **59.95% inhibition**.
The standard antioxidant **quercetin (control)** showed higher scavenging activity compared to the plant extracts, ranging from **38.28% at 100 mg/ml to 81.42% at 500 mg/ml**

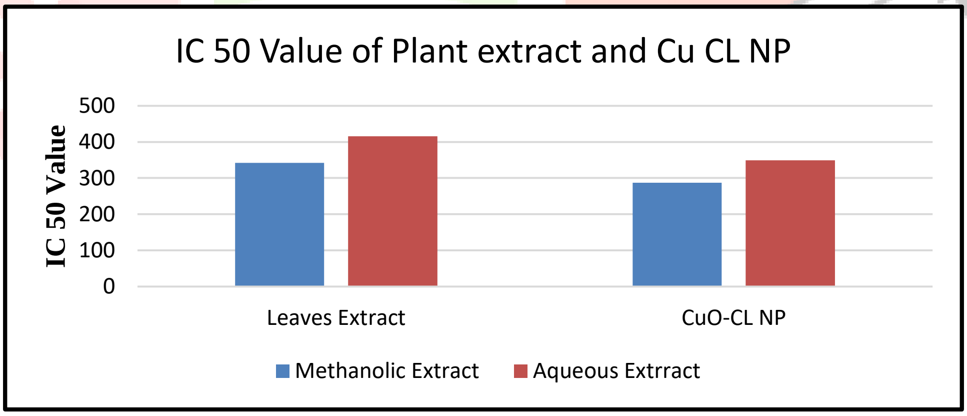


Fig.3.5 Comparative IC 50 values of *Crinum latifolium* Leaf Extract and CuO-CL Nanoparticles.

Overall, the findings suggested that nanoparticle synthesis modifies the chemical composition of the plant extract, CuO-CL nanoparticles retain considerable DPPH scavenging activity with lowest IC 50 value. The results support the potential of *Crinum latifolium* as a suitable biological source for the eco-friendly synthesis of functional CuO-CL nanomaterials.

3.3 Characterization of CuO-CL nano materials

3.3.1 UV absorbance Spectrum

The UV–Visible absorption spectra of CuO-CL nanomaterials synthesized using *Crinum latifolium*leaf extracts showed characteristic absorption bands in the UV region (Fig. A–D). All samples exhibited strong absorption peaks between approximately **350–400 nm**, which is typical for CuO nanoparticles.
Sample A: Sharp peak near ~380–390 nm with rapid decline beyond 450 nm

Sample B: Similar peak position with slightly broader tail toward visible region
Sample C: Broad and intense absorption band extending from ~300 to 500 nm
Sample D: Strong peak near ~370–380 nm with extended absorption tail into visible region

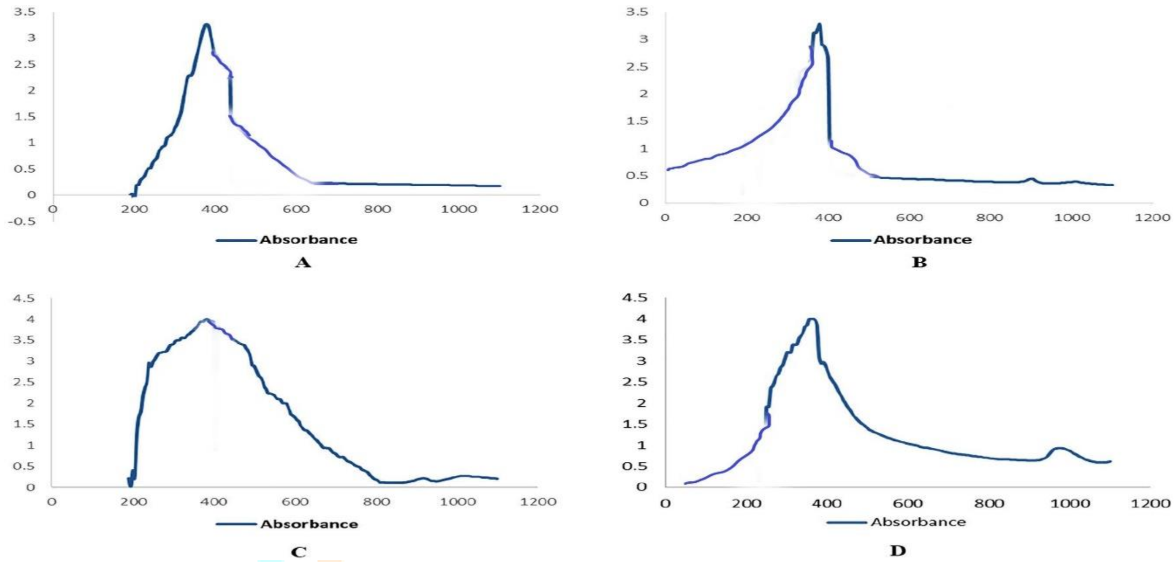


Fig. 3.6 UV-Visible spectrum at different solvents (A. *Crinum latifolium* Methanolic extract: Aqueous Extract; C mechanic CuO-CL -, D Aqueous CuO-CL nanomaterial)

The relatively sharp peaks in Samples A and D suggest smaller and more uniform nanoparticles, while the broader absorption observed in Sample C indicates a wider particle size distribution or possible aggregation. Broadening of the absorption band is commonly associated with polydispersity and surface defects
The extended absorption tail into the visible region observed particularly in Samples C and D may be attributed to defect states, oxygen vacancies, or organic capping molecules derived from the plant extract. Overall, the UV–Vis spectra confirm successful green synthesis of CuO-CL nanoparticles using *Crinum latifolium* extract.

3.3.2 FT-IR spectra of synthesized CuO-CL Nanomaterial

The **Fourier Transform Infrared (FT-IR) spectrum** of Cu-CL nanoparticles synthesized using the aqueous leaf extract of *Crinum latifolium* was recorded to identify the functional groups involved in the reduction and stabilization of copper ions during nanoparticle formation. A broad absorption band observed around **3400 cm^{-1}** corresponds to **O–H stretching vibrations** of hydroxyl groups. These hydroxyl groups are generally associated with phenolic compounds and flavonoids present in plant extracts. Another absorption band appearing near **2900 cm^{-1}** corresponds to **C–H stretching vibrations** of aliphatic hydrocarbons. .A prominent peak detected near **1600–1650 cm^{-1}** corresponds to **C=O stretching vibrations** of carbonyl groups or **amide bonds** present in proteins and enzymes within the plant extract. The absorption band observed around **1400–1500 cm^{-1}** can be attributed to **C=C stretching vibrations of aromatic rings**, indicating the presence of phenolic compounds and flavonoids. These phytochemicals are known to possess strong antioxidant and antimicrobial properties and may contribute to the biological activity of the synthesized nanoparticles. A peak around **1000–1100 cm^{-1}** corresponds to **C–O stretching vibrations** of alcohols, ethers, or carbohydrate derivatives present in the plant extract. In the lower wavenumber region, characteristic peaks around **500–600 cm^{-1}** correspond to **Cu–O stretching vibrations**, confirming the successful formation of copper oxide nanoparticles.

The FT-IR analysis therefore confirms that phytochemicals present in *Crinum latifolium* leaf extract play a crucial role in the **reduction, stabilization, and capping of Cu-CL nanoparticles**.

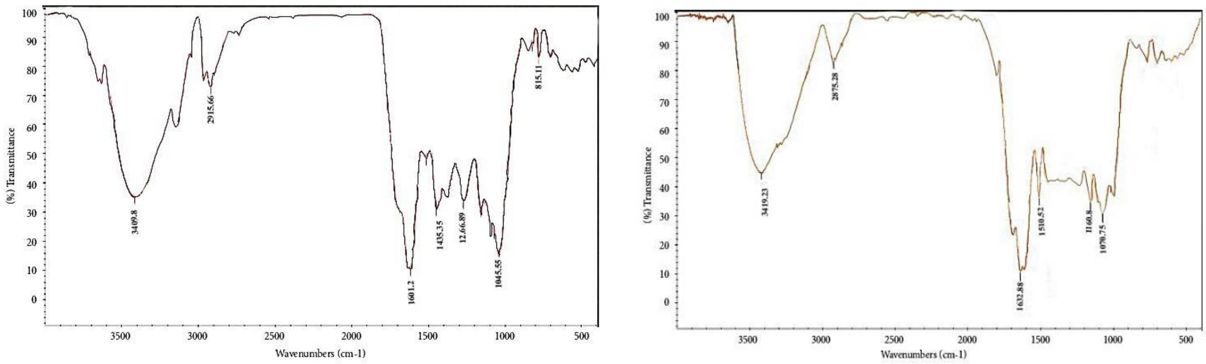


Fig.3.7 FT-IR spectra of synthesized CuO-CL Nanomaterial from aqueous Leaves Extract & from Methanolic Leaves Extract.

A broad and intense absorption band observed at **3419.23 cm⁻¹** corresponds to **O–H stretching vibrations** of hydroxyl groups present in phenolic compounds and flavonoids and play an important role as **reducing agents in nanoparticle synthesis**. Absorption peak at **2875.28 cm⁻¹** corresponds to **C–H stretching vibrations** of aliphatic hydrocarbons. A strong absorption band observed at **1632.88 cm⁻¹** corresponds to **C=O stretching vibrations** of carbonyl groups or **amide linkages.** Bind to the nanoparticle surface and stabilize the synthesized CuO nanostructures.

The peak at **1510.52 cm⁻¹** corresponds to **C=C stretching vibrations of aromatic rings**, indicating the presence of phenolic compounds and flavonoids within the plant extract. Absorption band observed at **1160.8 cm⁻¹** corresponds to **C–O stretching vibrations** of alcohols and esters, while the peak at **1070.75 cm⁻¹** represents **C–O–C stretching vibrations** typically associated with ethers and polysaccharides. In the lower wavenumber region, characteristic absorption peaks corresponding to **Cu–O stretching vibrations (approximately 500–600 cm⁻¹)** confirm the successful formation of copper oxide nanoparticles.

3.3.3 X Ray Diffraction

The **X-ray diffraction (XRD) pattern** of CuO-CL nanomaterial synthesized using *Crinum latifolium* leaf extract is presented in **Fig. 3.8**. The diffraction pattern shows several prominent peaks indicating the **crystalline nature of the synthesized copper oxide nanoparticles**.

The XRD pattern exhibited characteristic diffraction peaks at approximately **2θ = 29°, 35°, 38°, 48°, 53°, and 61°**, which correspond to the crystallographic planes **(021), (110), (111), (131), (113), and (200)** respectively. These diffraction peaks are consistent with the **monoclinic crystalline structure of CuO nanoparticles**, confirming the successful formation of copper oxide nanomaterials through plant-mediated synthesis.

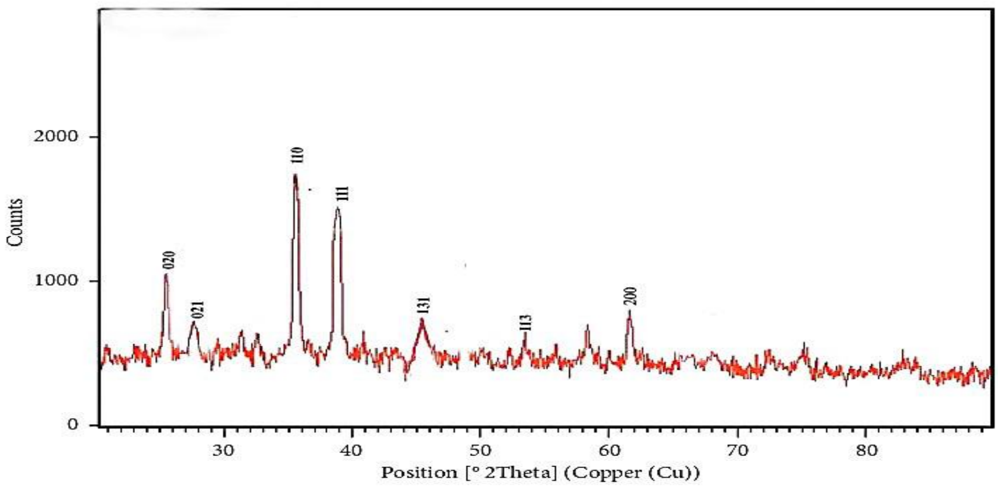


Fig. 3.8. XRD analysis from Methanolic Leaves Extract

Furthermore, *Crinum latifolium* have d the presence of several bioactive phytochemicals such as alkaloids, phenolics, and flavonoids that may act as **reducing and stabilizing agents during nanoparticle synthesis** (Goyal et al., 2023). These compounds may influence nanoparticle nucleation and growth, resulting in stable crystalline CuO nanostructures.

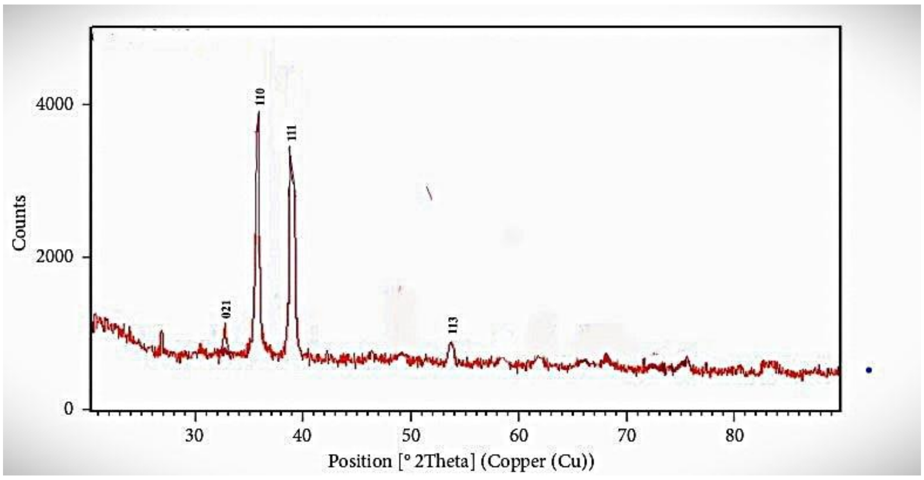


Fig. 3.9. XRD analysis from Methanolic Leaves Extract

The **X-ray diffraction (XRD) analysis** was performed to determine the crystalline structure and phase composition of Cu-CL nanoparticles synthesized using the aqueous leaf extract of *Crinum latifolium*\The XRD spectrum shows several characteristic diffraction peaks at approximately **2θ ≈ 29°, 35°, 38°, 53°, and 61°**, which correspond to the crystallographic planes **(021), (110), (111), (113), and (200)** respectively. These diffraction peaks match well with the standard diffraction pattern of **monoclinic copper oxide (CuO)**, confirming the successful formation of CuO nanoparticles.

Furthermore, phytochemical studies of *Crinum latifolium* have demonstrated the presence of several bioactive compounds including **alkaloids, phenolic compounds, and flavonoids**, which may function as reducing and stabilizing agents during nanoparticle synthesis (Goyal et al., 2023). These compounds help regulate nucleation and growth of nanoparticles, resulting in stable crystalline CuO nanostructures.

3.4 Antimicrobial Activity

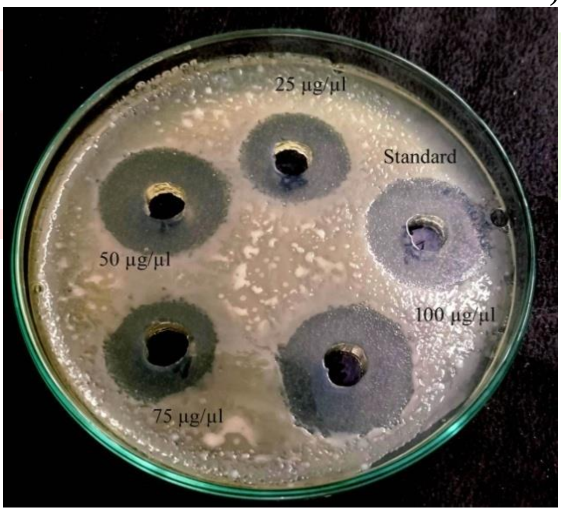
The present study demonstrated the effectively of aqueous and methanol extracts of *Crinum latifolium* leaves against bacteria and fungi as shown in table 2 & 3. In table 1, *Klebisiella pneumonia* gave high antibacterial activity for aqueous extract as ranged from 14.0±0.25 mm to 24.5±1.2 mm. Similarly *Klebisiella pneumonia, Salmonella and Fusarium* also gave high antibacterial and antifungal act tivity for aqueous extract. The inhibition zones were ranged from 12.0±0.3 mm to 21.0±1.1 mm and 13±0.1 mm to 22.0 ± 0.85 mm respectively. Contrary to aqueous extract, estimated antibacterial activity of *E. coli* and antifungal activity of *Candida albicans* were found quite different in methanol extract as shown in Fig. 3.10 (D) & (E).

Kumar et al., (2021) observations stated that methanolic extract of leaves parts of *Crinum latifolium* showed the antibacterial activity against *Streptococcus mutans* with inhibition zones 20.0±0.47 mm at 25mg/ml, 22.0±0.47 mm at 50 mg/ml and 25.0±0.94 mm at 100 mg/ml. Same extract also revealed the inhibition zones 12.0±0.47 mm at 25mg/ml, 15.0±0.47 mm at 50 mg/ml and 16.0±0.47 mm at 100 mg/ml against *Salmonella bongori* [15].

(A)

(B)

C)



(D)

(E)

Figure 3.10. Antimicrobial activity of *Crinum latifolium* leaves

Note: (A) *Klebisiella pneumonae;*(B) *E.coli;* (C) *Salmonella typhimurium* ; (D) *Fusarium oxysporum and* (E) *Candida albicans.*

3.5 Conclusion

Present study on crude, extracts of *C. latifolium* revealed the wide range of antimicrobial activity against a bacteria and fungi. The aqueous and methanolic extract of *Crinum latifolium* leaves showed antimicrobial activities, which may be due the presence of bio metabolites. Subsequently, the bioactive compounds from *Crinum latifolium* can be used to produce antimicrobial medicines for the treatment of microbial infections. Identification, Isolation, biochemical characterization, antimicrobial potentiality and cytoxical evaluation of present phytoconstituents is the base of development of natural pharmacological formulations and becoming the new areas of future research.

The present study confirms that *Crinum latifolium* leaves are a rich source of bioactive phytochemicals, including flavonoids, phenolics, alkaloids, and terpenes, which contribute significantly to their antioxidant and antimicrobial properties. Methanolic extracts consistently demonstrated higher phenolic and flavonoid content, along with superior DPPH scavenging activity, indicating better extraction efficiency and bioactivity compared to aqueous extracts.

The green synthesis of CuO-CL nanoparticles further enhanced these properties, showing increased phenolic and flavonoid content and improved antioxidant potential with lower IC_{50} values. Spectroscopic and diffraction analyses (UV–Vis, FT-IR, and XRD) confirmed the successful formation of stable, crystalline CuO nanoparticles, with plant phytochemicals acting as reducing and stabilizing agents.

Additionally, both crude extracts and synthesized nanoparticles exhibited notable antimicrobial activity against bacterial and fungal strains, supporting their potential as natural therapeutic agents.

IV. Acknowledgment

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