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RESEARCH ARTICLE

GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING AQUEOUS WILAYATI *THULSI (HYPTIS SUVEOLENS)* EXTRACT- CHARACTERIZATION AND STUDY OF ANTIBACTERIAL EFFECTS

Mekala, J. and *Rajan, M. R.

Department of Biology, The Ganthigram Rural Institute - Deemed University, Gandhigram- 624 302
Dindigul District, Tamil Nadu, India

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ABSTRACT

The present study deals with the green synthesis and characterization of copper oxide nanoparticles using aqueous leaf extract and their antimicrobial effects on pathogens. The plant extracts plays a vital role in the field of nanotechnology as it is environment friendly and does not involve any harmful chemicals. The current study focused on morphology and size of copper oxide nanoparticles synthesized using *Hyptis suaveolens* aqueous leaf extract. The synthesized copper oxide nanoparticles were characterized by using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM), Energy dispersive X-ray analysis (EDAX) and X-ray diffraction (XRD). Further, as-formed CuO Nps exhibit significant antibacterial activity against pathogenic bacterial strains namely Gram ve *Escherichia coli and* Gram +ve bacteria *Staphylococcus aureus*, *Candida albicans*. The green synthesized CuO NPs are cost-effective, biogenic molecules with the capability to serve as antimicrobial agents against fish bacterial pathogens.

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INTRODUCTION

Nanotechnology is an entirely new scientific technology that has been born from the combination of nanotechnology and biotechnology. The first relation between human life and nanoscales were developed naturally in Ayurveda, the old Indian system of medicine (Nima and Ganesan, 2013). In recent years, bio synthesis of nanoparticles is an important approach in nanotechnology. Nanotechnology has appealed many researchers from several fields like biotechnology, physics, chemistry, material science, engineering and medicine (Sundaramurthy and Parthiban, 2015). Nano size materials exhibit unique electronic, magnetic, optical, catalytic and medicinal properties as compared with the traditional and commercial bulk materials (Henan Sylvia Devi and Thiyam David Singh, 2014). Chemical synthesis leads to the presence of some toxic chemicals absorbed on the surface that may cause adverse effects in medical applications. Recently green synthesis of different nanoparticles carried out by using plants (Nima and Ganesan, 2013). Biological methods for nanoparticles synthesis using plant parts have advantages over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticles synthesis. Among the oxides of transition metals, copper oxide nanoparticles are of special because of their efficiency as nanofluids in heat transfer application. Water-soluble cupric oxide nanoparticles are stable over a wide range of ph and temperature. This excellent stability in the form of aqueous colloidal suspensions makes the application of the water-soluble cuo nanoparticles easier in aqueous systems (Jayalakshmi and Yogamoorthi, 2014). *Hyptis suaveolens* (L) Poit commonly known as *Wilayati tulsi* belongs to the family Lamiaceae and is an ethno botanically important medicinal plant. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics. Almost all parts of this plant are being used in traditional medicine to treat various diseases. The leaves of *H. Suaveolens* have been utilized as stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases (Chitra Shenoy *et al*., 2009). Crude leaf extract is also used as a relief to colic and stomach-ache. Leaves and twigs are considered to be antispasmodic and used in antrheumatic and antisuporific baths, an anti-inflammatory, antfertility agents (Venkateshappa and Sreenath, 2013). All used as antiseptic agent for burns, wounds and various skin complaints (Mandal *et al*., 2016). Phytochemical are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence

Corresponding author: Rajan, M. R.
Department of Biology, The Ganthigram Rural Institute - Deemed University, Gandhigram- 624 302, Dindigul District, Tamil Nadu, India, Tamil Nadu, India.

mechanism and protect from various diseases. Therefore, in the present study, copper oxide nanoparticles are synthesized using aqueous leaf extract of *Hyptis suveolens*, belonging to family Lamiaceae profusely growing in the wild. Use of copper oxide nanoparticles and their release into aquatic environment lead to unexpected hazards to aquatic organisms including fishes. In addition, CuO-Nps could be accumulated in aquatic organisms and transferred to higher tropic levels, representing a health hazard to animals and human beings. The study related to the green synthesis and characterization of copper oxide nanoparticles by using Wilayati Tulsi is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Collection and preparation of Wilayati tulsi leaf extract

The *Hyptis Suaveolens* leaf samples of were collected from Gandhigram Rural Institute, Dindigul district, Tamil Nadu, India. Then it was cleaned and subsequently dried under shade to remove moisture completely. Then powdered and stored**.** 10 g of powdered leaves were taken in a beaker along with 100 ml of deionized water and it is allowed to boil at 60°C for 30 min under reflux condition, cooled down to room temperature. The prepared solution was initially filtered through normal filter paper there by powdered leafy materials and filtered out. The filtrate was again filtered through whatman No.1 filter paper to get clear solution. The filtrate was stored at 4°C for future work (Anuj Bhasker *et al*., 2014).

Synthesis and characterization of copper nanoparticles:

For the synthesis of copper oxide nanoparticles, 1.0 mM $CuSO_4$ solution was added to the heated plant extract at 1:1 ratio[5] and stirred with magnetic bead for 2 min at 30 c. The synthesized CuO nanoparticles were indicated by turning of light brown colour to pale green. The change in the colour of the reactants indicates the formation of copper oxide nanoparticles. The content was washed with double distilled water thrice by repeated centrifugation at 3500 rpm for 10 min and dry it room temperature (Anuj Bhasker *et al*., 2014).

Characterization

The synthesized CuO nanoparticles were preliminarily characterized by using UV visible spectrophotometer to confirm its presence. UV spectral measurement was done in a range of wavelength between 200-800 nm (Rohit Guin *et al*., 2014). The FT-IR spectra of plant leaf extract and synthesized Copper oxide nanoparticles were recorded by KBr pellet method using FT-IR spectrometer (range 4000–400 cm-1). The morphology of the synthesized CuO NPs was observed by Field emission scanning electron microscopy (Sundaramurthy, and Parthiban, 2015). X-ray diffraction (XRD) can be used to estimate the size of the particles. XRD gives a diffraction pattern of the sample and this was compared to the reference peak/pattern (JCPDS) (Rohit Guin *et al*., 2014). The presence of elemental copper was confirmed through EDAX. Energy dispersive analysis x-ray spectrometer takes advantage of the photon nature of the light. In the x-ray range the energy of a single photon is just sufficient to produce a measurable pulse x-ray[10]. Further, antibacterial activity against various bacterial pathogens viz. both Gram-positive (*C. albicans* and *S. aureus*) and Gram negative (*E.coli)* (Acharyulu *et al*., 2014) were carried out.

RESULTS AND DISCUSSION

1. Phytochemical analysis

Phytochemical studies for identification and elucidation of active constituent in plant material. Phytochemical are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have Terphenoids, alkaloids and phenolic compounds (Abdul Wadood *et al*., 2013). In the present work phytochemical screening of *Hyptis suaveolens*, aqueous extract was studied. The extract showed potent radical scavenging ability and the percentage inhibition was found directly proportional to the increase in concentration or percentage of the plant extract. Results indicate presence of carbohydrates and reducing sugars, proteins, tannins, saponins, flavinoids, Terphenoids, steroids, phenols, alkaloids and glycosides in leaves (Table 1).

Table 1. Qualitative Phytochemical screening of *Hyptis suaveolens L.*

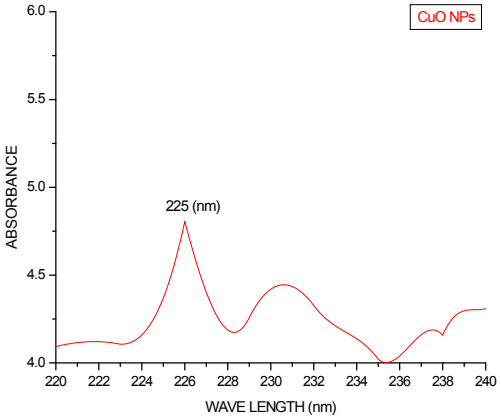
S.No	Phytochemical	Results
1.	Tannins	+
2.	Saponins	-
3.	Flavinoids	+
4.	Terphenoids	+
5.	Steroids	+
6.	Phenols	+
7.	Alkaloids	+
8.	Glycosides	+
9.	Carbohydrates and reducing sugar	+
10.	Proteins and amino acids	-

The extract contain Phytochemical that cause inhibition mainly phenols and flavinoids. This property of plant may be important in preventing oxidative stress related diseases. The studies are in conformity with the Antioxidant activity and phytochemical analysis of *Hyptis Suaveolens* (L.) Poit (Kumkum agarwal and RanjanaVarma, 2013). Alkaloids are used as anaesthetic agents and are found in medicinal plants. This study is in conformity with the Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan (Abdul Wadood *et al*., 2013). Presence of Terphenoids in *Hyptis suaveolens* either as single constituents or in combination is responsible against bacterial infections, digestive problems and also as an appetizer. This study is in conformity with the Phytochemical Studies of *Hyptis Suaveolens* (L.) Poit (Rose Mary *et al.*, 2014) and Phytochemical Screening of aqueous extracts from *Hyptis suaveolens* LAM for Fungal Growth Inhibition (Mbatchou *et al*., 2010).

UV- Visible Spectroscopy analysis

The appearance of green color evident that the formation copper oxide nanoparticles in the reaction mixture and the efficient reduction of the copper iron to copper oxide, the formed color solution allowed to measure the absorbance against distinct wave length to conform the formation of Copper oxide nanoparticles. UV-vis scanning of reaction product showed SPR absorption band and peaks (Fig. 1) Reaction mixture 1:1 ratio, in which reduction of Cu ions just to occur and SPR band intensities was less and peak is broad which suggest partial reduction of CuO ion and formation of larger CuO NPs with SPR at 225 nm. The studies are conformity with the Green synthesis of copper oxide nanoparticles using aqueous extract of flowers of *Cassia alata*

and particles characterization (Jayalakshmi and Yogamoorthi, 2014) and Green synthesis of copper oxide nanoparticles using *Aloe Vera* leaf extract and its antibacterial activity against fish bacterial pathogens (Vijay Kumar *et al*., 2015).

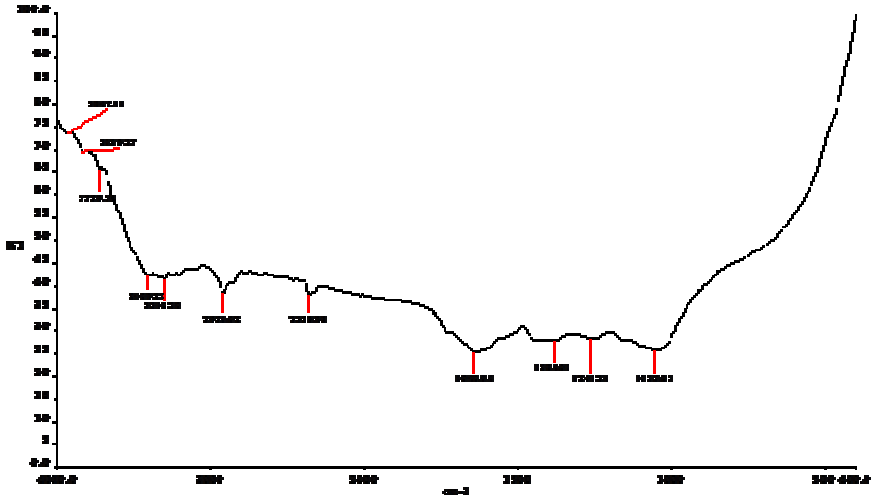


UV-Vis spectrum of CuO NPs

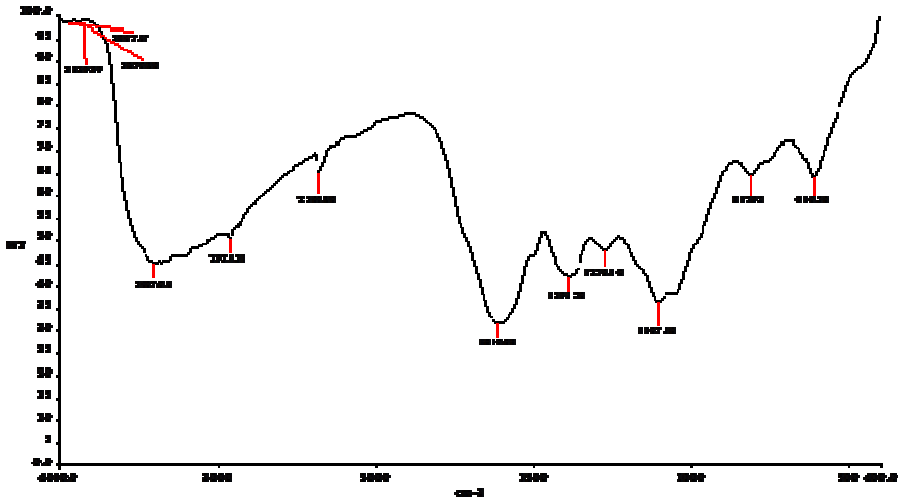
Figure 1. UV-visible absorption spectroscopy analyses (A) leaf extract (B) copper Nanoparticles

Fourier transforms infra red Spectroscopy (FT-IR) analysis

FT-IR spectroscopy analysis was carried out to find the functional groups of bio molecules that were bound specifically along the copper oxide nanoparticles surface. FT-IR measurements of both the *Hyptis Suveolens* aqueous extract and the synthesized dried copper Oxide nanoparticles were carried out to identify the possible bio-molecules responsible for the reduction, capping of and efficient stabilization of the bio-reduced CuO Nps. The FT-IR spectra of the *Hyptis Suveolens* aqueous extract and the synthesized CuO Nps are shown in Fig. 2 (a) and (b). The Fig. 2 (a) displays a number of adsorption peaks at 3942.11, 3409.27, 2923.02, 1640.44, 1055.63 cm^{-1}, like as N – H stretch of amines O-H stretch of Carboxylic group, N-H bending of amines, C-N stretch of aliphatic amines as given in the Table 2. The fig 2 (b) synthesis copper oxide nanoparticles, peak values at 3947.47, 3407.04, 2924.51, 1610.08, 1276.10, 1107.36, 817.95, and 616.56 cm^{-} was observed. Peak at 616.56 cm^{-1}, 3947.47cm^{-1} corresponds to O-H stretch phenolic compounds, N-H stretch of amines, O-H stretch of Carboxylic group, N-H bending of amines, C-H stretch of aliphatic amines, C-Cl stretch of alkaloids, C-H bending of alkanes as given in Table 3.



(A)



(B)

Fig. 2. FT-IR analysis of (A) plant extracts and (B) Copper Oxide nanoparticles

Table 2. FT-IR analysis of leaf extract

S.No	Frequency	Bond	Functional group
1.	3409.27	N-H Stretch	Amines
2.	3310.20	N-H Stretch	Amines
3.	2923.02	O- H Stretch	Carboxylic group
4.	2358. 98	CH – Stretch	Alcohols and Phenols
5.	1640.44	N-H bend	1 ^{o} amines
6.	1261.21	C- H Stretch	Alcohols and phenols
7.	1055.63	C –N Stretch	Aliphatic amines
8.	2358.98	C-H Stretch	Alcohols and phenols

Table 3. FT-IR analysis of copper Oxide nanoparticles

S.No	Frequency	Bond	Functional group
1.	3407.04	N-H Stretch	Amines
2.	2924.51	O–H Stretch	Carboxylic group
3.	2360.80	C–H Stretch	Aldehydes
4.	1610.08	N – H bend	1^{o} amines
5.	1276.10	C – H Stretch	Aromatic aimes
6.	1107.36	C – N Stretch	Aliphatic amines
7.	817.95	C –Cl Stretch	Alkaloids
8.	616.56	C – H bend	Alkanes

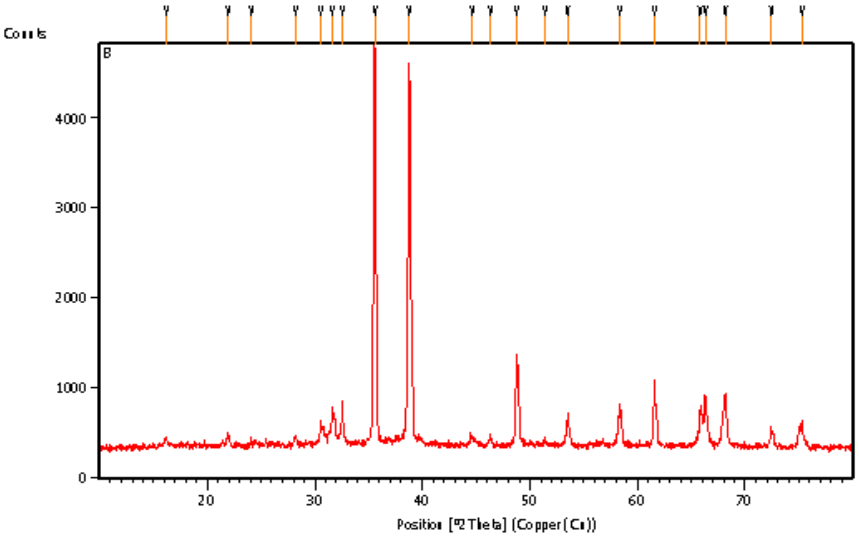
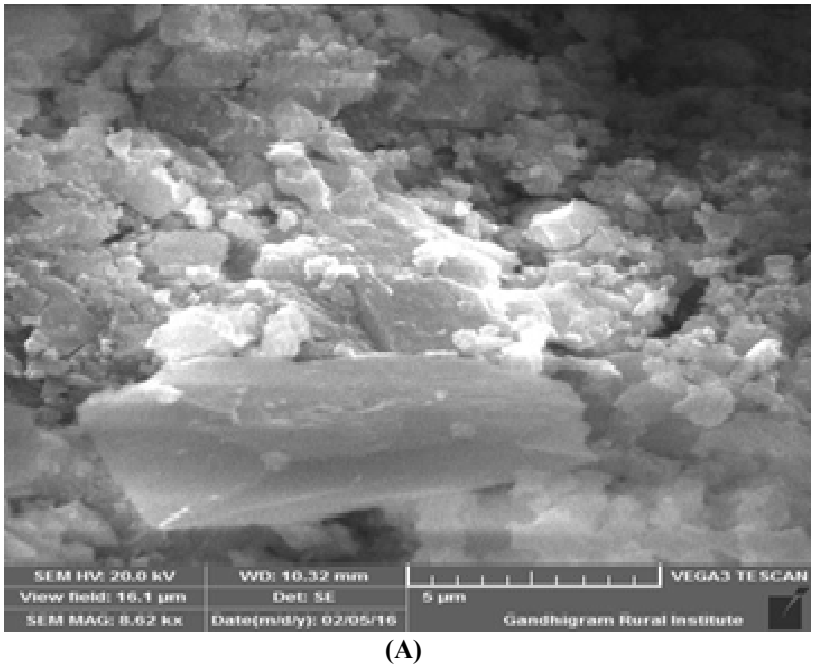
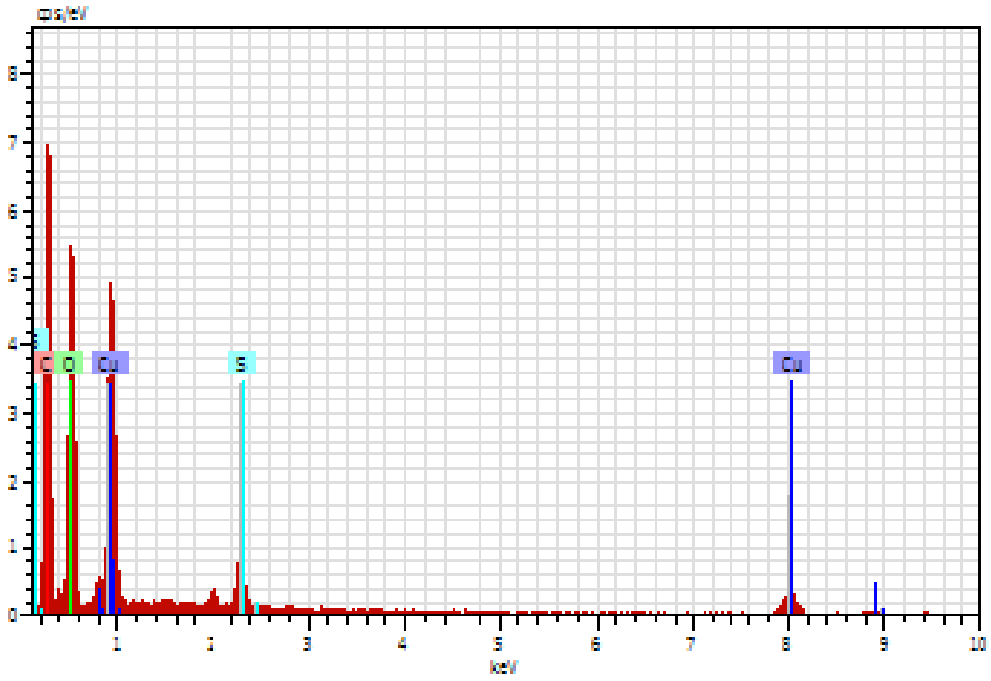


Fig. 3. XRD pattern of the synthesized copper oxide nanoparticles



(A)



(B)

Fig. 4. (A) SEM Micrograph of CuNPs and (B) EDX analysis of CuNPs

Table 4. Effect of copper sulphate and Copper Oxide Nanoparticles on Antibacterial Activity

S.No.	Bacterial Species	Zone diameter (mm sample of inhibition		
		Antibiotic	Copper oxide	Copper sulphate
1	*Staphylococcus aureus*	21	23	15
2	*Candida albicans*	16	20	10
3	*Escherichia coli*	15	21	8

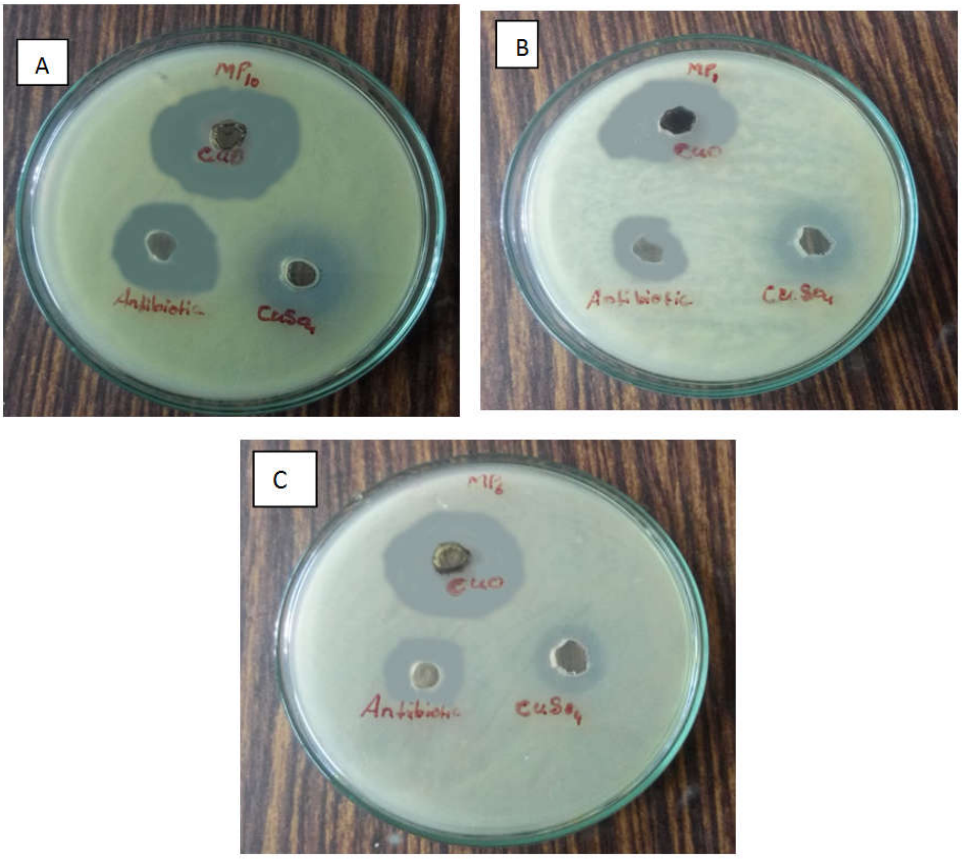


Fig. 5. Zone of inhibition of (A) *Staphylococcus aureus* (B) *Candida albicans* (C) *Escherichia coli*

Studies are in conformity with the biosynthesis of copper oxide nanoparticles using *pyrus pyrifolia* leaf extract and evolve the catalytic activity (Sundaramurthy and Parthiban, 2015) and biosynthesis of copper nanoparticles using aqueous guava extract characterisation and study of antibacterial effects (Caroling *et al*., 2015) and synthesis of copper oxide nanoparticles using D*esmodium gangeticum* aqueous root extract. This functional group plays a very important role in the synthesis copper oxide nanoparticles (Rohit Guin *et al*., 2014).

X-ray diffraction analysis

The XRD pattern of synthesized CuO nanoparticles from *Hyptis Suveolens* leaf extract of is shown in Fig 3. XRD analysis showed a series of diffraction peaks at 2θ of 32.55, 35.56^o, 38.75^o, 48.75^o, 53.48^o, 58.34^o, 61.57^o, 65.43^o, 66.27^o, 72.40^o, 75. 25^o. which were assigned to (110), (111), (200), (202), (020), (202), (113), (-311), (022), (311) and (004) planes respectively. Which are in good agreement with those of powder CuO obtained from the International Centre of Diffraction Data card (JCPDS-80-1916). This study conformity with the Green Synthesis of CuO Nanoparticles using Phyllanthus Amarus Leaf Extract and their Antibacterial Activity against Multidrug Resistance Bacteria (Acharyulu *et al*., 2014) and antibacterial activity of synthesized Copper Oxide Nanoparticles using *Malva sylvestris* Leaf Extract (Abdul Wadood *et al*., 2013).

SEM and EDAX analysis

The surface morphology and micro structure of CuO Nps is investigated by using SEM analysis as shown in Fig 4(A). It clearly shows that the particles are small and uniform size, almost spherical in nature which is free from agglomeration are in conformity with the Green synthesis of copper oxide nanoparticles using aqueous extract of flowers of *Cassia alata* and particles characterisation (Jayalakshmi *et al.,* 2014) and Synthesis of Copper Oxide Nanoparticles by a Novel Method and its Application in the Degradation of Methyl Orange (Acharyulu *et al*., 2014). The EDAX pattern clearly shows that copper oxide nanoparticles formed by the reduction of copper ions using fresh aqueous *Hyptis suveolens* extract are crystalline in nature Fig. 4 (B). The EDAX spectrum was recorded in the spot-profile mode. The optical absorption peak is observed at 8keV, which is typical for the absorption metallic copper Oxide nanoparticles. Strong signals from the copper oxide atoms are observed, while weaker signals for C, Si, and atoms were also recorded. From the EDS signals, it is clear that copper oxide nanoparticles reduced by aqueous extract *Hyptis Suveolens* have the weight percentage of elemental copper oxide as 92%.This study conforms with the Green synthesis of CuO nanoparticles using *Gloriosa superba* leaf extract and their antibacterial activity (Raja Naikaa *et al*., 2015) and Green Synthesis of Copper Oxide Nanoparticles Using Aloe Vera Leaf Extract and its Antibacterial Activity against Fish Bacterial Pathogens (Vijay Kumar *et al*., 2015)

Antibacterial activity

The antibacterial activities of biosynthesized Copper Oxide nanoparticles against two gram-positive bacteria *Staphylococcus aureus, Candida albicans* and gram-negative bacteria *E.coli.* using agar well diffusion method. In agar well diffusion method the CuO Nps showed significant antibacterial activity on all the three bacterial strains. Biosynthesized copper nanoparticles showed clear zone of inhibition as indicated in the Table-4 against *E.coli, staphylococcus aureus* and *Candida albicans*. It is reported that CuO nanoparticles attach to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and release CuO ions. Amaxlin 25µg/ml was used as control. The studies are in conformity with the Green synthesis of copper oxide nanoparticles using natural reducer and stabilizer and an evaluation of antimicrobial activity (Jayandran *et al*., 2015) and Antibacterial activity of synthesized copper oxide nanoparticles using *Hyptis suaveolens* leaf extract (Awwad *et al*., 2015).

Conclusion

In summary, first report an eco-friendly and simple method for the green synthesis of CuO NPs by using *Hyptis suveolens* leaf extract singly without the involvement of any other chemical reagent. The biosynthesized nanoparticles have been characterized by UV-VIS spectroscopy, FT-IR, XRD, SEM and EDAX. CuO Nps exhibit significant antibacterial activity against all the three bacterial strains, i.e., Gram ve E.coli and Gram +ve bacteria. *Staphylococcus aureus, Candida albicans* and show significant zone of inhibition to CuO Nps compared to the positive control Amoxil. The study successfully demonstrates the convenient utilization of h*yptis suveolens* extract to get structurally and morphologically interesting and potentially antibacterial CuO nanoparticles.

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