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**RESEARCH ARTICLE**

**BIOLOGICAL SYNTHESIS AND CHARACTERIZATION OF COPPER OXIDE NANOPARTICLES
 USING *ANTIGONON LEPTOPUS* LEAF EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY**

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Abstract

During the last two decades, the biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally sociable technologies in nanoparticle synthesis. The routinely used chemical and physical methods in the synthesis of nanoparticles employ toxic chemicals and non-polar solvents. Therefore, there is a need for the development of eco-friendly process to synthesize nanoparticles through green chemistry using plants and microorganisms. The present study has been taken to synthesize copper oxide nanoparticles (CuO-NP's) using green biosynthetic method by reduction of copper sulphate solution with *Antigonon leptopus* leaf extract which acts as reducing agent and efficient stabilizer at room temperature. Synthesized nanoparticles were characterized using UV-visible spectrophotometer, FT-IR, X-ray diffraction and SEM methods. The morphology of the nanoparticles are crystalline, more or less spherical, cuboid and planar nanoclusters with panoramic view and range from 110-280nm in size. XRD analysis has given a clear picture on the presence crystalline cubic phase of monoclinic Copper oxide nanoparticles. Copper oxide nanoparticles exhibited potent antibacterial activity against gram positive and moderate activity against gram negative bacterial strains tested. The present study successfully demonstrates the convenient utilization of *Antigonon leptopus* leaf extract to get structurally and morphologically interesting and potential antibacterial Copper oxide nanoparticles.

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Introduction:-

The field of nanotechnology is one of the most active research areas in modern material science. Nanoparticles exhibit new and improved properties based on specific characteristics such as size, distribution and morphology. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine and bionanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of

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treatments for many diseases and disorders. During the last two decades, the biosynthesis of nanoparticles (silver, gold, platinum, copper and palladium) has received considerable attention due to the growing need to develop environmentally sociable technologies in material synthesis (Goodsell *et al.*, 2004; Chandran *et al.*, 2006; Song *et al.*, 2010).

Synthesis of nanoparticles can be performed using a number of routinely used chemical and physical methods. However, altogether these methods are energy and capital intensive and they employ toxic chemicals and non-polar solvents in the synthesis procedure and later on synthetic additives or capping agents, thus precluding their applications in clinical and biomedical fields. Therefore, the need for the development of a clean, reliable, biocompatible, benign and eco-friendly process to synthesize nanoparticles leads to turning researchers toward “green” chemistry and bioprocesses (Jain *et al.*, 2011).

Copper nanoparticles have been successfully synthesized by radiolysis, laser irradiation, thermal decomposition, reverse micelles, vapour deposition, sonoelectrochemical, flame spray and chemical reduction. However, these methods suffer from drawbacks such as unsafe reaction conditions, use of expensive chemicals and instruments and longer reaction time. To overcome these problems, some green methods for synthesis of copper nanoparticles are reported using plant leaf extracts such as *Capparis zeylanica* Linn, tamarind, lemon juice, *Ocimum sanctum*, *Magnolia kobus* leaf extract, *Syzygium aromaticum* (cloves) aqueous extract, *Aegle marmelos* and *Nerium oleander* (Sastri *et al.*, 2013; Lee *et al.*, 2013; Kulkarni *et al.*, 2014). Hence there is scope to develop new methods for the synthesis of CuO nanoparticles.

On the other hand, Copper is highly toxic to microorganisms such as bacteria and non-toxic to animal cells, due to which it is considered an effective bactericidal metal. It is also considered safe for human beings for applications such as food package and in water treatment (Devi, 2014). Copper nanoparticles are attractive to many researchers due to their lower cost compared to noble metals such as Ag, Au and Pt. Colloidal copper has been used as an antimicrobial agent for decades. They find applications in heat transfer systems as super strong materials, sensors (Vinod *et al.*, 2013), bactericidal agents used to coat hospital equipments and also as catalysts (Nasirian, 2012).

Water-soluble cupric oxide nanoparticles or CuO nanoparticles are stable over a wide range of pH and temperature. This excellent stability in the form of aqueous colloidal suspension makes the application of the water-soluble CuO nanoparticles easier in aqueous system. Reports on biosynthesis and characterization of nanocrystalline CuO are relatively few and method for the synthesis of CuO nanoparticles is simple, mild, and environmentally friendly.

In the present investigation, the plant *Antigonon leptopus*, belonging to the flowering plant family polygonaceae was selected. Traditionally the leaves of *Antigonon leptopus* are used to reduce swelling and to treat diabetes, hypertension and menstrual pains. In traditional medicine, it is used for the treatment of nephritis, hepatitis and colitis.

Therefore, in the present study, copper oxide nanoparticles were synthesized using leaf extract of *Antigonon leptopus* and their antimicrobial potential against selected pathogenic bacterial strains were studied.

Materials and methods:-

Reagents and chemicals:-

Pure and analytical grade chemicals were used in all experiments including synthesis of copper oxide nanoparticles and media preparation for the growth of bacterial cells. Nutrient Agar, Peptone, Beef extract, Agar-agar, Nutrient broth, Ethanol, Dimethylsulphoxide, Ampicillin (Broad spectrum antibiotic) were purchased from Himedia, Mumbai, India.

Plant material:-

Fresh leaves of *Antigonon leptopus* were collected from Andhra University campus, Visakhapatnam. The collected leaf material was tightly packed in polyethene bag and then transferred to the laboratory. Then the leaves were washed with distilled water twice and stored at room temperature.

Test organisms for Antibacterial studies:-

Bacillus subtilis (MTCC 121), *Bacillus licheniformis* (MTCC 429), *Staphylococcus aureus* (MTCC 96), *Streptococcus pneumoniae* (MTCC 2672), *Escherichia coli* (MTCC 118), *Klebsiella pneumoniae* (MTCC 2405),

Pseudomonas aeruginosa (MTCC 424), *Sphingomonas sanguinis* (MTCC 5495) were collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Preparation of plant leaf extract:-

Twenty five grams of *Antigonon leptopus* leaves were accurately weighed, thoroughly washed under running tap water followed by washing with double deionised water to remove surface impurities. They were crushed using a mortar and pestle and finely macerated. After homogenization, 100ml of double deionized water was added and heated over a water bath maintained at 80°C for 15 minutes. The extract obtained was filtered through muslin cloth and then through Whatmann No. 1 Filter paper (pore size 25µm) and stored in refrigerator for further experiments.

Phytochemical tests:-

The plant extract so obtained was subjected to preliminary phytochemical screening as follows.

Tannins:- To 2 ml of extract, 2 ml of 5% FeCl₃ was added and observed for the formation of yellow brown precipitate.

Alkaloids:- To a few ml of extract, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Saponins:- A few ml of extract was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with few drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Terpenoids:- To 2 ml of extract, 5 ml CHCl₃, 2 ml acetic anhydride, and concentrated H₂SO₄ were added carefully to form layer. Reddish brown coloration of interface was observed to detect the presence of terpenoids.

Flavonoids:- To 2 ml extract, few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings were added. The solution was observed for the appearance of magenta red or pink colour after 3 min.

Phenolics:- To 2 ml of extract, 1 ml of 1% ferric chloride solution was added. Blue or green colour indicates phenols.

Test for Anthraquinones: To 1ml of plant extract, few drops of 10% ammonia solution were added; appearance pink colour precipitate indicates the presence of anthraquinones.

Detection of Phytosterols:- To few ml of extract, 2 ml acetic anhydride, to this, one or two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Test for coumarins:- To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for anthocyanins:- To 2ml of extract, 2ml of 2N HCl was added followed by the addition of NH₃. Pinkish red to bluish violet coloration indicates the presence of anthocyanins.

Synthesis of Copper oxide (CuO) Nanoparticles Using *Antigonon leptopus* leaf extracts:-

Ten ml solution of leaf extract was introduced drop wise into 100 ml of 1mM (0.001M) solution of copper sulphate (CuSO₄.5H₂O) in a 250 ml Erlenmeyer flask under continuous stirring (Abboud *et al.*, 2013). After the complete addition of leaf extract, the flask was then kept stirring for overnight at room temperature. Within a particular time, the green colour solution was changed into straw yellow, which indicates the formation of copper nanoparticles. The CuO nanoparticles solution thus obtained was purified by repeated centrifugation at 12,000 rpm for 15 min followed by re-dispersion of the pellet in deionized water to remove any unwanted biological materials. Then the Cu nanoparticles were dried in oven at 80°C. The obtained products of CuO nanoparticles were stored in air tight container for further analysis.

Characterization of Copper oxide Nanoparticles:-

UV-Spectrophotometer analysis:-The synthesized copper oxide nanoparticles were characterized through Shimadzu UV-1800. The reduction of copper nanoparticles was monitored by UV-spectrophotometer range of absorbance from 250-480nm. The spectra of the intact plant extract were used as a baseline and subtracted from the spectra of a mixture of extracts and synthesized nanoparticles.

FT-IR analysis:- The prepared Copper oxide nanoparticles were then subjected to FT-IR spectroscopy measurements. It was used to identify the possible functional groups of biomolecules responsible for the reduction and capping of the nanoparticles, which are present in the leaf extract. FTIR analysis was carried for the reduction of Copper ions with the spectral range of 400-4000 cm^{-1} using FT-IR Spectrophotometer, Shimadzu, Japan.

Scanning Electron Microscope (SEM-EDX):-

Morphology and mean particle size of the CuO nanoparticles were determined by SEM analysis. The SEM analysis was established by using Scanning Electron Microscope (SEM) Jeol Asia PTE Ltd, Japan with 1nm resolution at 20 kV with 20 mm Oxford, UK, EDS detector. The elemental composition in the reaction mixture was determined by EDX analysis.

X-Ray Diffraction:- The crystalline structure of the copper oxide nanoparticles was determined by X-Ray diffraction analysis using X-Ray Diffraction Unit (XRD) Pan Alytical, X-Pert pro, Netherlands operating at 40 kV with 2 sec time interval at room temperature.

Antimicrobial activity:-

Active cultures were generated by inoculating a loopful of culture in 100 ml nutrient broth and incubating in a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5×10^5 CFU/ml.

Antibacterial activity:-

Antibacterial activity of copper oxide nanoparticles was screened against eight bacterial strains by Agar well diffusion method of Murray *et al.*, (1995) modified by Olurinola (1996).

Nutrient Agar plates were prepared and swabbed using sterile L-shaped glass rod with 100 μl of 24 h mature broth culture of individual bacterial strains. The wells were made by using sterile cork borer (6 mm) wells created into the each petri plate. Varied concentrations of Copper oxide nanoparticles (25 and 50 $\mu\text{g}/\text{well}$) were used to assess the activity of the nanoparticles. The nanoparticles were dispersed in sterile water and the standard antibiotic, Ampicillin (20 $\mu\text{g}/50 \mu\text{l}$) as positive control was tested against the bacterial pathogens. Then the plates were incubated at 37°C for 24 h, the zone of inhibition was measured in millimeter (mm) of the every well and also the values were noted.

Minimum inhibitory concentration (MIC) assay:-

Minimum Inhibitory Concentration (MIC) of Copper oxide nanoparticles was determined according to the method of Elizabeth (2001).

A series of two fold dilution of Copper oxide nanoparticles, ranging from 100-2000 $\mu\text{g}/\text{ml}$, were prepared. After sterilization, the medium was inoculated with the aliquots of culture containing approximately 5×10^5 CFU/ml of each organism of 24 h slant culture in aseptic condition and transferred into sterile 6 inch diameter petri plates and allowed to set at room temperature for about 10 min and then kept in a refrigerator for 30 min. After the media was solidified, wells were made and different concentrations of CuO nanoparticles ranging from 100-2000 $\mu\text{g}/\text{ml}$ were added to the wells of each petri plate. The blank plates were without nanoparticles. Inhibition of the growth of the organism in the plates containing copper oxide nanoparticles was judged by comparison with the growth in the control plates. The MIC was determined as the lowest concentration of the nanoparticle inhibiting visible growth of each organism on the agar plate.

Results:-

Preliminary phytochemical analysis for *Antigonon leptopus* leaf extract was done using standard test procedures to confirm the availability of active phytochemicals in the aqueous leaf extract. Phytochemical analysis showed the presence of tannins, alkaloids, flavonoids, phenols and anthocyanins (Table.1).

Synthesis of Copper oxide-Nanoparticles (Visual Inspection):-

During the biosynthesis, formation of nanoparticles is indicated by the change in colour of the mixture (copper sulphate and leaf extract). After 24 hrs of reaction, the reaction mixture changes its colour from light to dark colour indicating the synthesis of CuO nanoparticles due to the reduction of Cu^+ ions and due to the excitation of Surface plasmon vibration in metal nanoparticles (Fig.1).

Table 1:- Preliminary Phytochemical screening tests of *Antigonon leptopus* leaf extract

Plant Metabolite	<i>Antigonon leptopus</i> Aqueous leaf extract
Tannins	+
Alkaloids	+
Saponins	-
Terpenoids	-
Flavonoids	+
Phenolics	+
Anthraquinones	-
Phytosterols	-
Coumarins	-
Anthocyanins	+

+ Presence - Absence

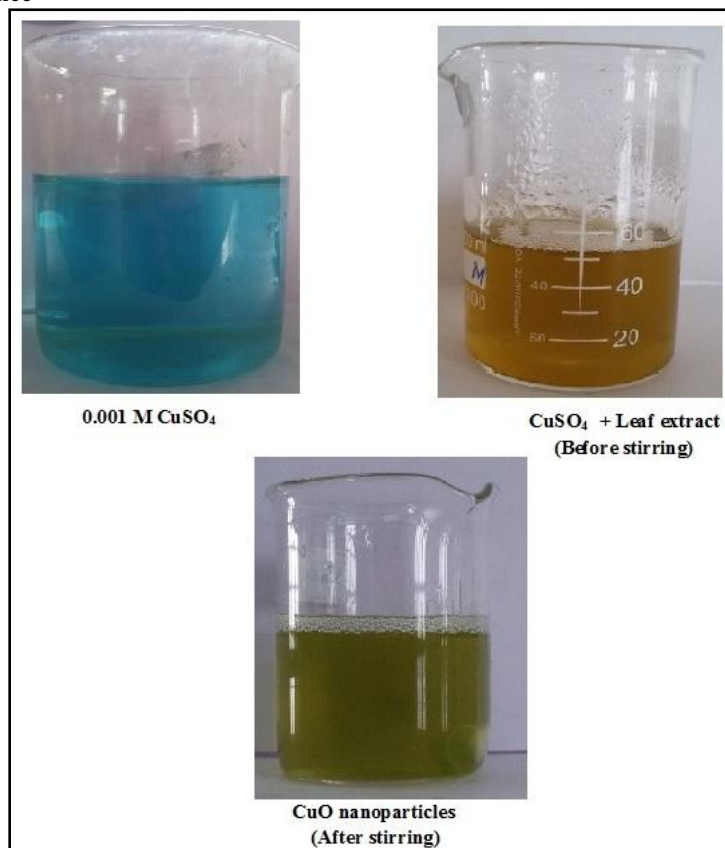


Fig.1:- Digital photograph of visible color changes, after adding the *Antigonon leptopus* leaf extract into 0.001M Copper sulphate solution.

Characterization of Copper oxide Nanoparticles:-**UV-Visible Spectral Analysis:-**

UV-Visible absorption spectrum is the preliminary characterization to know the optical property of synthesized nanoparticles. The result obtained from UV-Visible spectroscopy analysis of the nanoparticles sample is presented in Fig.2 where similar pattern of absorption maxima was obtained when the CuO nanoparticles were synthesized using 5ml, 10ml, 15ml and 20ml of *Antigonon leptopus* leaf extracts. The reduction of pure Cu to Copper oxide nanoparticle was monitored by measuring the UV-Visible spectrum, the most confirmatory tool for the detection of CuO nanoparticles. UV-visible spectra of the formed CuO Nanoparticles dispersed in water exhibited the maximum absorption peaks at about 380 nm.

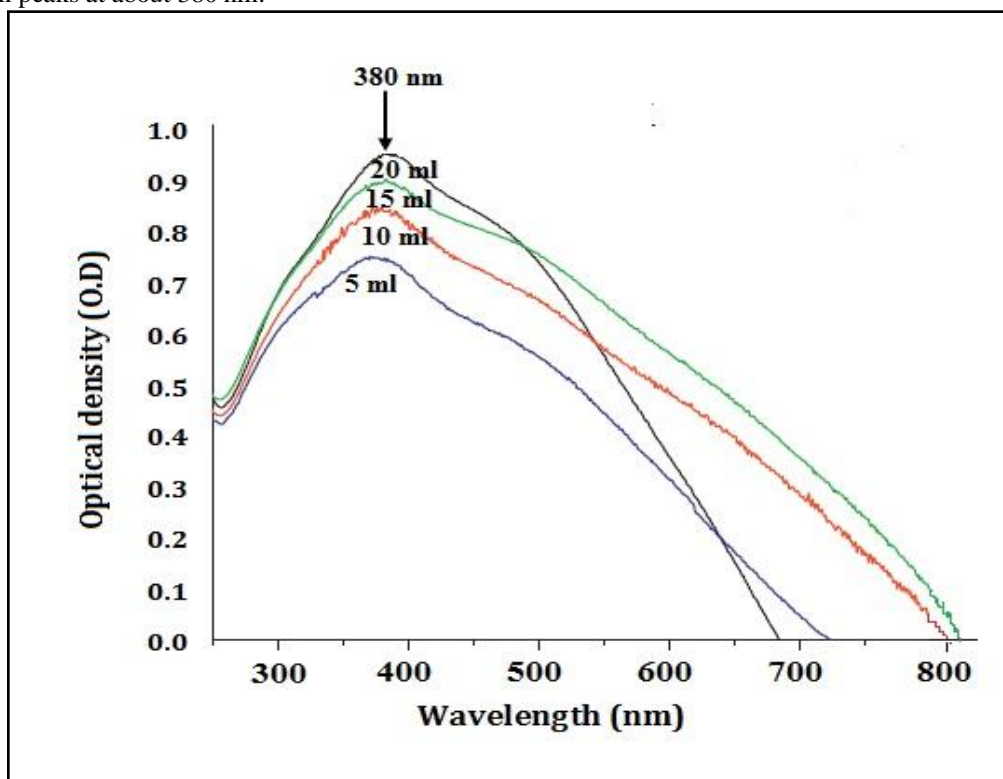


Fig.2:- UV-visible spectra of Copper oxide nanoparticles synthesized using *Antigonon leptopus* plant leaf extract

FTIR analysis:-

FTIR spectra of biosynthesized copper oxide nanoparticles were recorded to identify the capping and efficient stabilization of metal nanoparticles by biomolecules present in *Antigonon leptopus* leaf extract. The FTIR spectrum of synthesized CuO nanoparticles is shown in Fig.3. For copper oxide nanoparticles, peak values at 3901, 3842, 3852, 3465.8 and 1638.5 cm^{-1} were observed. Peak at 1638.5, 3465.8 cm^{-1} corresponds to C=O stretching of amides and O-H stretching of phenolic compound respectively. The other peaks obtained in copper nanoparticle sample are 3852, 3842, 3901 cm^{-1} due to O-H Stretching of hydrogen bonded alcohols and phenols.

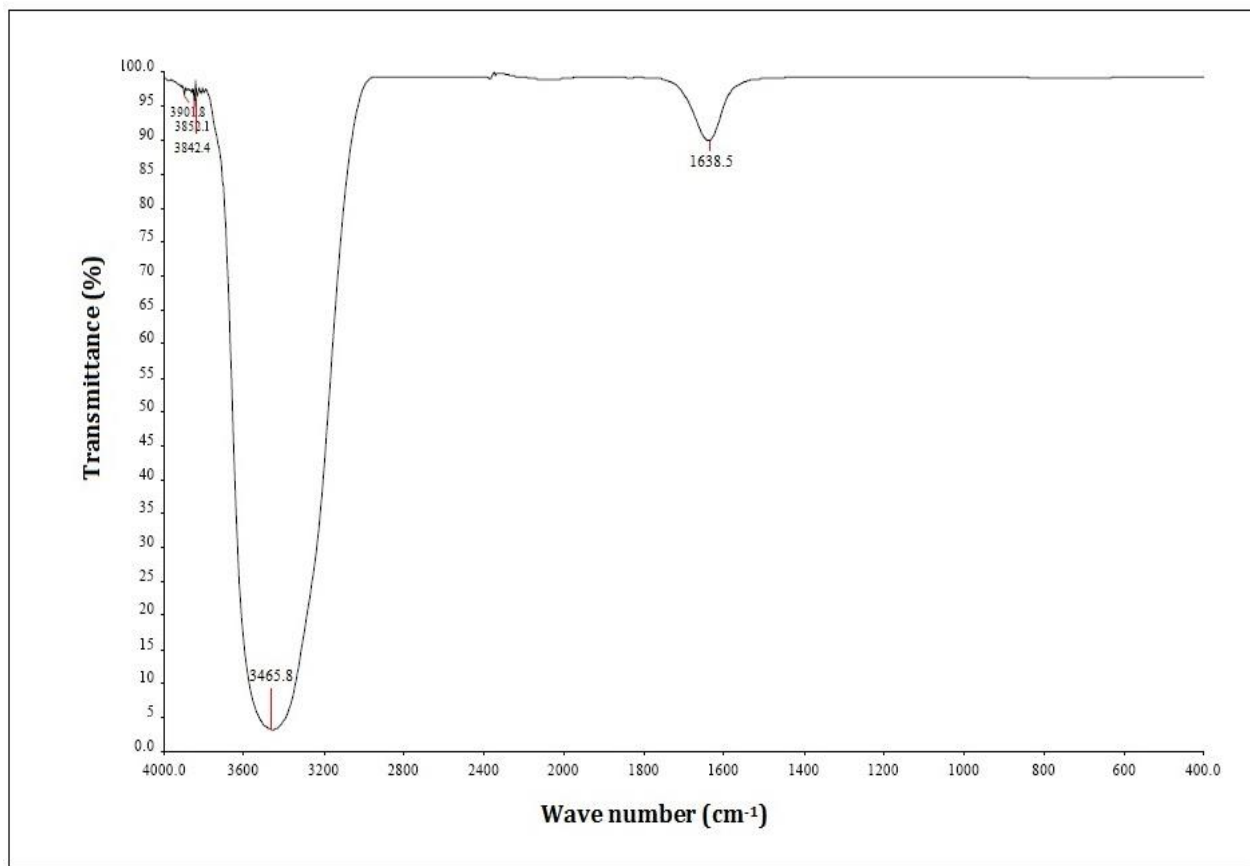


Fig. 3:- FTIR analysis of Copper oxide nanoparticles

Scanning Electron Microscope (SEM) analysis:-

SEM images revealed that the synthesized copper oxide nanoparticles are clustered and the surfaces of the aggregates are rough. The SEM images indicated that the crystalline CuO nanoparticles are cuboid and size of particles is ascertained from the SEM scale ranged between 110-280nm (Fig. 4). Energy Dispersive X-ray Spectroscopy (EDX) analysis showed the presence of elemental copper oxide signal confirmed in the sample (Fig.5). To find out the purity of the metal particles synthesized, EDX spectrum was obtained which showed along with copper, there were other elements viz. Al and Si. The appearance of silica and aluminium in the EDX spectrum is because of the aluminium grid base and silica holder used during spectral sample preparation and these are considered as preparation borne impurities.

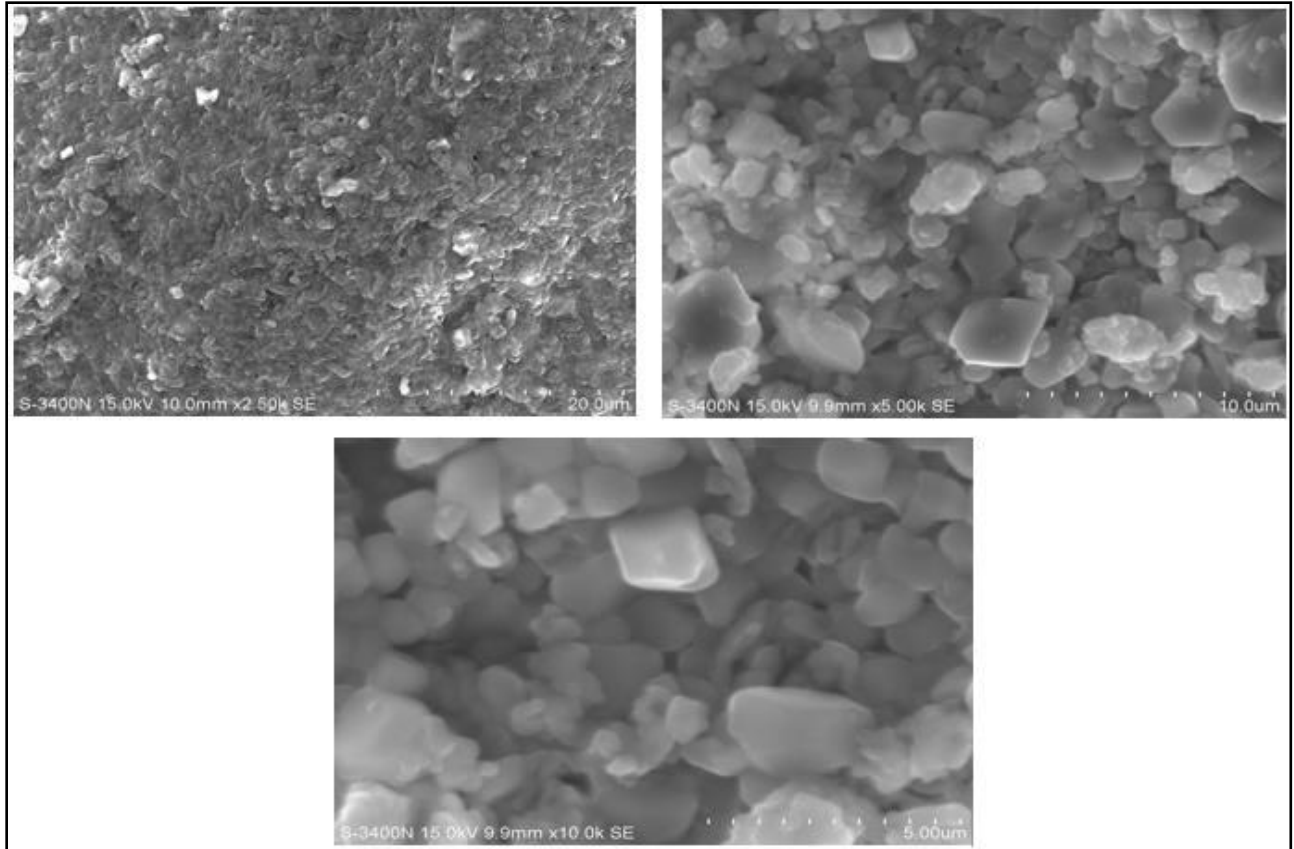


Fig.4:- SEM images of synthesized copper oxide nanoparticles.

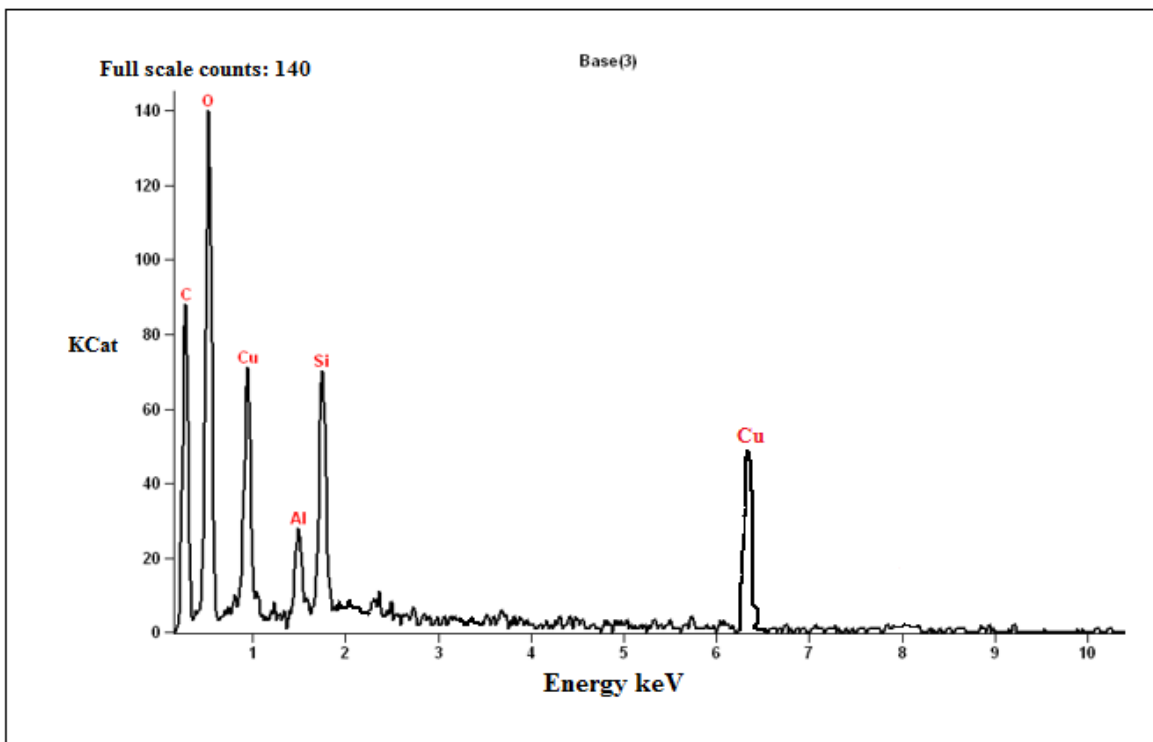


Fig.5:- Dispersive X- ray Spectroscopy (EDX) analysis of elemental copper.

XRD analysis:-

The phase identification and crystalline structure of the nanoparticles were characterized by X-ray diffraction. The X-ray diffraction pattern obtained for the Copper oxide nanoparticles synthesized using *Antigonon leptopus* leaf extract is shown in Fig. 6. The synthesized particles when subjected to XRD analysis, given a clear picture on the presence crystalline cubic phase of monoclinic Copper oxide (CuO) exhibiting 2θ values 28.28, 34.46, 35.98, 38.66, 47.12, 54.80 and 57.98. In addition, the peak observed at 2θ value of 28.31 might be due to the presence of trace amount of hollow CuO nanoparticles. Above all, it is encouraging to note that the 2θ values of the synthesized copper oxide nanoparticles are also matched with Joint Committee for Powder Diffraction Standard (JCPDS).

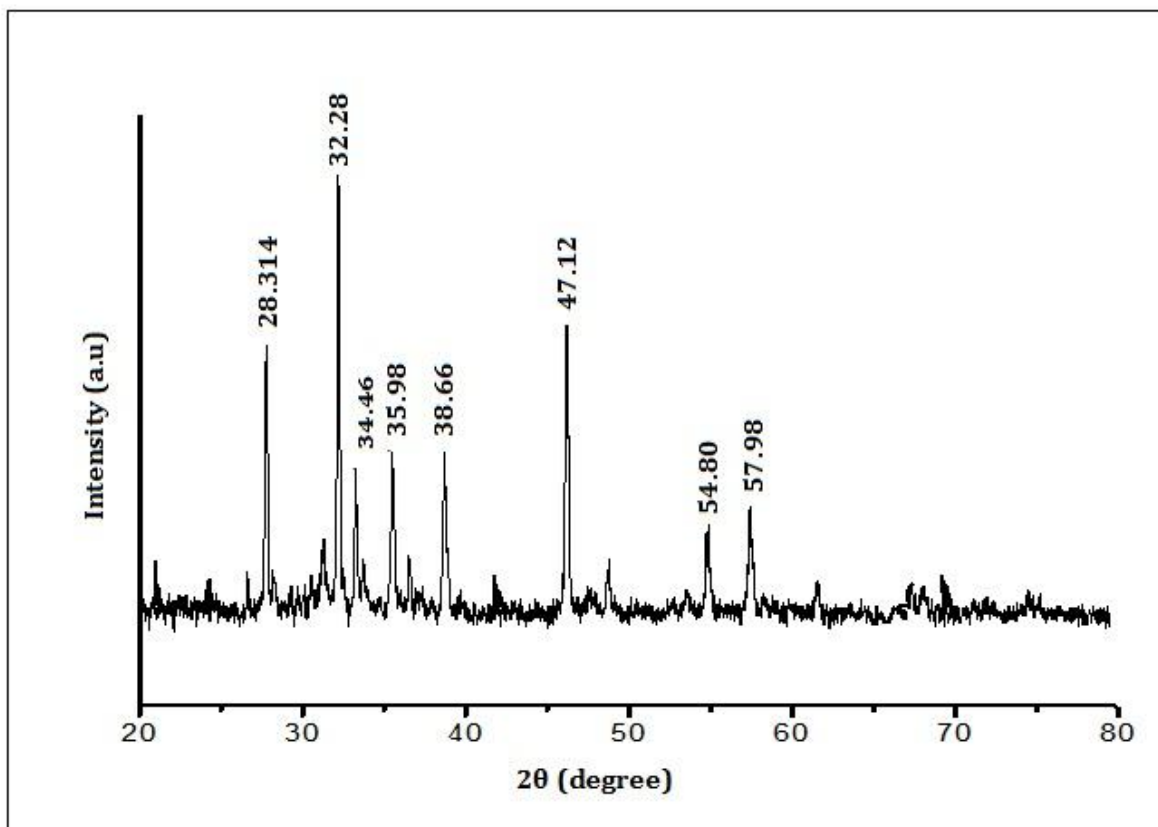


Fig. 6:- XRD spectrum of synthesized copper oxide nanoparticles

Antibacterial activity of Copper oxide nanoparticles:-

The antibacterial properties of the CuO nanoparticles were evaluated against four Gram positive and four Gram negative bacterial strains using agar well diffusion method (Fig.7). Table 2 shows the effect of CuO nanoparticles on the growth of both Gram positive and Gram negative bacteria. CuO nanoparticles showed significant antibacterial activity on Gram positive bacterial strains than Gram negative pathogenic bacterial strains tested.

Of the bacterial strains tested, copper nanoparticles strongly inhibited the growth of Gram positive bacteria - *Bacillus licheniformis* (25 mm), *Streptococcus pneumoniae* (24 mm) *Bacillus subtilis* (23 mm), *Staphylococcus aureus* (23mm) and at a concentration of 50 $\mu\text{g}/50\ \mu\text{l}$. On the other hand, Copper nanoparticles moderately inhibited the growth of Gram negative bacteria- *Escherichia coli* (18mm), *Klebsiella pneumoniae* (16mm) and *Pseudomonas aeruginosa* (15mm) at a concentration of 50 μg . These nanoparticles showed a low inhibitory effect on the growth of *Sphingomonas sanguinis*. Hence, the nanoparticles are less susceptible to *Sphingomonas sanguinis*. Minimum inhibitory concentration of CuO Nanoparticles for the antibacterial activities were presented in Table- 3.

Table.2:- Effect of CuO nanoparticles on the growth of bacteria

Name of the Bacterial strain	Zone of Inhibition (Diameter in mm)		
	Cu Nanoparticles		Ampicillin (20 µg)/ 50 µl
	25 µg/ 50 µl	50 µg/50 µl	
Gram positive bacteria			
<i>Bacillus subtilis</i>	12	23	28
<i>Bacillus licheniformis</i>	13	25	27
<i>Streptococcus pneumoniae</i>	11	24	28
<i>Staphylococcus aureus</i>	12	23	28
Gram negative bacteria			
<i>Escherichia coli</i>	12	18	28
<i>Klebsiella pneumoniae</i>	10	16	27
<i>Pseudomonas aeruginosa</i>	8	15	28
<i>Sphingomonas sanguinis</i>	6	12	26

Bacterial strains were spread on agar plates. Different amounts of CuO nanoparticles (25 µg/50 µl & 50 µg/50 µl) were placed in the wells. Controls contained Ampicillin (20µg/50 µl) in place of CuO nanoparticles. The incubation period was 24 h at 37°C. Zone of inhibition was measured as described in methods.

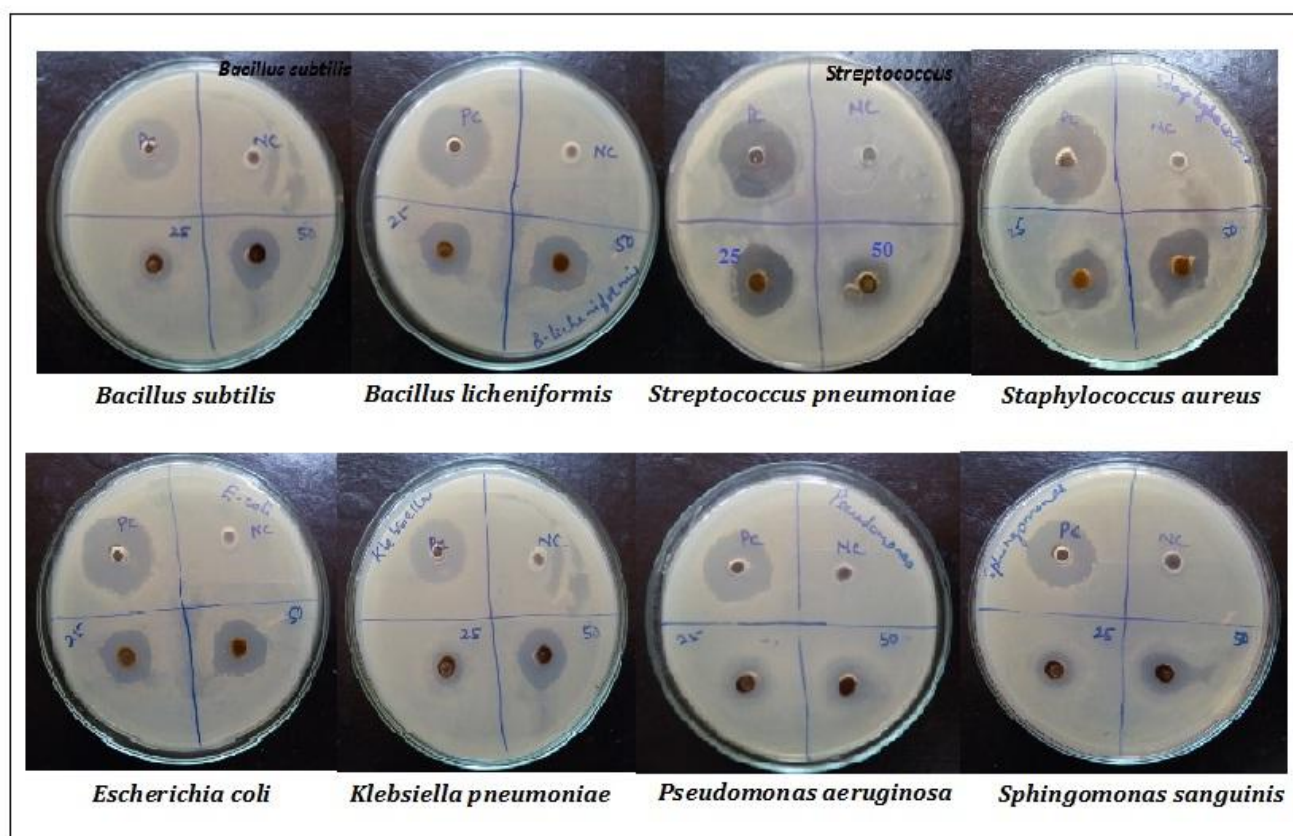
**Fig.7:-** Antibacterial activity of Copper oxide nanoparticles

Table 3:- Minimum Inhibitory concentration (MIC) of CuO nanoparticles on bacterial growth.

Name of the bacterial strain	Minimum Inhibitory Concentration (MIC) of CuO nanoparticles ($\mu\text{g/ml}$)
<i>Bacillus subtilis</i>	200
<i>Bacillus licheniformis</i>	200
<i>Streptococcus pneumoniae</i>	200
<i>Staphylococcus aureus</i>	200
<i>Escherichia coli</i>	250
<i>Klebsiella pneumoniae</i>	300
<i>Pseudomonas aeruginosa</i>	400
<i>Sphingomonas sanguinis</i>	500

Bacterial strains were spread on agar plates. Different concentrations of CuO nanoparticles (0.1-1 mg/ml) were placed in the wells. Control contained Ampicillin (20 $\mu\text{g}/50\ \mu\text{l}$) in the place of nanoparticles. The incubation period was 24 h at 37 $^{\circ}\text{C}$. Zone of inhibition was measured and minimum inhibitory concentration of CuO nanoparticles was determined.

Discussion:-

Nanotechnology and Nanoparticles based product and applications are increased now a days due to the biological effectiveness. Current research in bactericidal nanomaterials has opened a new era in pharmaceutical industries. In the present investigation, the green synthesis method is used to synthesize the Copper oxide nanoparticles using aqueous leaf extract of *Antigonon leptopus* which is cost effective and is eco-friendly approach compared to other methods.

Preliminary phytochemical analysis for *Antigonon leptopus* leaf extract revealed the presence of plant bioactive metabolites like tannins, alkaloids, flavonoids, phenols and anthocyanins. In the present study, the leaf extract acts as reducing agent and capping agent in the synthesis of CuO nanoparticles. However, with regard to the chemical reaction involved in the biosynthesis of CuO nanoparticles between copper sulphate and leaf extract, the phytochemical constituents present in leaf extract might have facilitated the formation of CuO nanoparticles. Thus, the aqueous leaf extract of *Antigonon leptopus* is found to be a potential source bioreductant to reduce metal salts into their nanoparticles.

Recently, several researchers exploited plant extracts, exudates, gums and other parts of plants for the synthesis of Cu nanoparticles (Kavitha *et al.*, 2013). Guajardo-Pacheco *et al.* (2010) reported a method of producing metallic nanoparticles of Cu by using soybeans extract as a chelating agent. *Ocimum sanctum*, a traditional medicinal plant of India has been used as a source of bio-reduction and stabilizer for synthesis of Cu nanoparticles and the constituents such as alkaloids, glycosides, tannins and aromatic compounds may be responsible for the synthesis of nanoparticles (Vasudev and Pramod, 2013).

It has been reported that the amino acids (Beveridge and Murray, 1980), enzymes (Mandal *et al.*, 2006) and abundance of hydroxyl and carboxylate groups present in plants might have facilitated the formation of $\text{Cu}(\text{OH})_2$, which hydrolyzed later into nanocrystalline CuO (Vinod Vellora *et al.*, 2013). Nonetheless, it is presumed that the phytochemical constituents either individually or synergistically could have influenced the bioreduction in such metal oxide nanoparticle synthesis.

The characteristics of the synthesized Copper oxide nanoparticles were studied using UV-Visible spectrophotometer, FT-IR, SEM with EDX and XRD analysis. The reduction of pure Cu to CuO nanoparticle was monitored by measuring the UV-Visible spectrum, the most confirmatory tool for the detection of CuO nanoparticles. UV-visible spectra of the formed CuO Nanoparticles dispersed in water exhibited the maximum absorption peak at about 380 nm. Similar absorption maximum of CuO nanoparticles was reported by Raja *et al.* (2015) where they synthesized CuO nanoparticles using *Gloriosa superba* L. extract as sole source of phytochemicals as reducing and capping agent.

The surface plasmon absorption in the metal oxide nanoparticles is due to the collective oscillation of the free conduction band electrons which are excited by the incident electromagnetic radiation. This type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter. Surface Plasmon absorption band with a maximum at 380 nm indicates the formation of CuO nanoparticles (Dhaneswar *et al.*, 2013).

Copper oxide nanoparticles synthesized using flower extract (aqueous) of *Cassia alata* showed a broad peak at 263 nm in the UV-Vis spectrophotometer indicating the presence of copper oxide (Jayalakshmi & Moorthi, 2014). Lee *et al.* (2013) reported that during the reduction of the copper ion to copper nanoparticles using *Magnolia kobus* leaf extract, the maximum absorbance occurs at 560nm and steadily increases in intensity as a function of reaction time.

The FTIR analysis was used to identify the capping, reducing and stabilizing capacity of the leaf extract in the synthesis of CuO nanoparticles. For copper oxide nanoparticles, peak values at 3901, 3842, 3852, 3465.8 and 1638.5 cm^{-1} were observed. Peak at 1638.5, 3465.8 cm^{-1} corresponds to C=O stretching of amides and O-H stretching of phenolic compound respectively. The other peaks obtained in copper nanoparticle sample are 3852, 3842, 3901 cm^{-1} due to O-H stretching of hydrogen bonded alcohols and phenols. The FTIR analysis of CuO nanoparticles suggested that they might be surrounded by the any of these organic molecules such as polyphenols, flavonoids, alkaloids and terpenoids.

Similar type of results were reported by Kalainila *et al.*, (2014) that the chemical constituents present in plant leaf extract such as flavonoids, alkaloids and fatty acids are responsible for the reduction of copper ions to copper nanoparticles due to their capping and reducing capacity.

Kulakarni *et al.* (2015) reported that synthesized copper nanoparticles using *Eucalyptus* sp. leaf extract were surrounded by proteins and metabolites such as phenolic acid, carboxylic acid and flavonoids and from the analysis of FTIR studies it was confirmed that phenolic compounds have stronger ability to bind metal indicating that phenols could possibly form metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that biological molecules could possibly perform dual functions of formation and stabilization of CuO nanoparticles in aqueous medium.

The SEM images indicated that the crystalline CuO are cuboid and size of particles ranged between 110-280nm. Energy Dispersive X-ray Spectroscopy (EDX) analysis was performed to find out the purity of the nanoparticles synthesized and EDX spectrum showed along with copper, there were other elements viz .Al and Si. Copper oxide nanoparticles synthesized by the plant extract of *Capparis zeylanica* showed that the synthesized nanoparticles are spherical and relatively uniform shape, confirmed in the range of 60-100nm (Saranyadevi *et al.*, 2014). Kulkarni *et al.* (2015) reported that the typical SEM image revealed that the product mainly consists of particle-like Cu nano crowded together with biomolecules of *Eucalyptus* sp. leaf extract. However, further observations with higher magnification reveal that these crowded Cu nanoparticles are groups of smaller nanoparticles which exhibit good uniformity.

The synthesized particles when subjected to XRD analysis, showed the presence crystalline cubic phase of monoclinic Copper oxide (CuO) exhibiting 2θ values 32.28, 34.46, 35.98, 38.66, 47.12, 54.80 and 57.98 which are closely matched with the values of monoclinic phase CuO reported by Amrut *et al.*, (2010), Vinod *et al.*, (2013) and Abboud *et al.*, (2013). Above all, it is hopeful to note that the 2θ values of the synthesized copper oxide nanoparticles are also matched with Joint Committee for Powder Diffraction Standard (JCPDS).

XRD pattern of synthesized Cu nanoparticles using *Eucalyptus* sp. leaf extract demonstrated a high crystalline level with diffraction angles corresponding to the characteristic of face-centered cubic of copper lines (Kulkarni *et al.*, 2015). Raja *et al.* (2015) reported that X-ray diffraction studies of copper oxide nanoparticles using *Gloriosa superba* L. plant extract showed that the particles are monoclinic in nature. XRD pattern of synthesized Cu nanoparticles using a leaf extract of *Ocimum sanctum* showed a high crystallinity with characteristic face-centered cubic (FCC) of copper (Kulkarni and Kulkarni, 2013).

In the present study, CuO nanoparticles showed significant antibacterial activity against Gram positive bacterial strains than Gram negative bacterial strains tested. Hassan *et al.*, (2012) and Vinod *et al.*, (2013) reported that Copper oxide (CuO) nanoparticles synthesized from leaf extracts acts as potential antimicrobial agent against

infectious organisms such as *E. coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphillis typhus*, and *Staphylococcus aureus*.

The copper nanoparticles synthesized using *Nerium oleander* leaf aqueous extract also exhibited good bactericidal activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis* (Gopinath *et al.*, 2014).

Caroling *et al.*, (2015) reported a convenient and environment friendly method for the synthesis of copper nanoparticles by biologically reducing CuSO₄ with aqueous extract of Goose Berry (*Phyllanthus embilica*) under optimum conditions and the particles showed potent antimicrobial activity against *Staphylococcus aureus* and *Escheria coli*.

Raja *et al.*, (2015) demonstrated convenient utilization of *Gloriosa superba* L. extract as a fuel for the efficient synthesis of CuO nanoparticles through green synthesis method and their potent antibacterial activity against *Klebsiella aerogenes*, *Pseudomonas desmolyticum*, *Escherichia coli* and *Staphylococcus aureus*.

The presence of an inhibition zone clearly indicates the mechanism of the bactericidal action of nanoparticles involves disrupting the membrane. Extent of inhibition depends on the concentration of nanoparticle as well as on the initial bacterial concentration. The reason could be that the smaller size of the particles which leads to tightly adsorbed on the surface of the bacterial cells so as to disrupting the membrane which would lead to the leakage of intracellular component, thus killing the bacterial cells (Sathish *et al.*, 2009). The association of copper with oxygen and its reaction with sulfhydryl (-S-H) groups on the cell wall forms R-S-S-R bonds, thereby blocks respiration and cause cell death.

Copper ions released subsequently may bind with DNA molecules and lead to disordering of the helical structure by cross-linking within and between the nucleic acid strands and also disrupt the biochemical processes and protein denaturation and cause cell death (Kim *et al.*, 2000).

Conclusion:-

In the present study, the biological approach of synthesis of Copper oxide nanoparticles using *Antigonon leptopus* leaf extract, the reducing, stabilizing and capping agent appears to be ecofriendly and provides cost effective, easy and proficient way for synthesis nanoparticles alternative to conventional chemical and physical methods. The characteristics of the synthesized Copper oxide nanoparticles were studied using UV-Visible spectrophotometer, FTIR, SEM with EDX and XRD. Copper oxide nanoparticles exhibited significant antibacterial activity against Gram positive bacteria when compared to Gram negative bacteria. Thus, *Antigonon leptopus* leaf extract may be effectively used for the synthesis of copper oxide nanoparticles through green route synthesis and as the biosynthesized copper nanoparticles showed excellent antimicrobial activity, CuO nanoparticles could be used in addition with antibiotic medicines to combat diseases caused by human pathogenic microorganisms.

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