SYNTHESIS OF BETA VULGARIS BASED COPPER OXIDE NANOPARTICLES AND THEIR PHARMACEUTICAL APPLICATIONS

Abstract

Based on the optimization results the bulk quantities of copper oxide nanoparticles is successfully synthesized using the *Beta vulgaris* aqueous extract. The optimised system is characterised based on UV -Visible, FT-IR, XRD, SEM, TEM, EDX (EDAX), and particle size analyser. The green synthesised BV metal Oxide nanoparticles are subjected for various pharmacological applications. The biologically synthesized copper oxide nanoparticles is in the average sizes of 32 - 82nm. Regarding Photocatalytic activity, BV-CuO nanoparticles revealed the highest degradation capacity. On, Anti-bacterial activity BV-CuO nanoparticles showed the better inhibitory effects against five bacterial strains. In In-vitro anti-cancer activity copper oxide nanoparticles exhibits anti-cancer effects on MCF-7 cell line. Beta Vulgaris mediated copper oxide (BV-CuO) nanoparticles showed greatest anti-diabetic activity on alpha-amylase assay. Similar, Beta Vulgaris mediated Copper oxide (BV-CuO) nanoparticles showed greatest anti-diabetic activity on alpha-glucosidase assay. The photocatalysts may provide a promising application for the degradation of dyes from industrial effluents.

Keywords: Copper oxide nanoparticles, Beta Vulgaris, Pharmaceutical applications, Optimization, Characterization.

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I. INTRODUCTION

Nanotechnology has become one of the most promising technology in all areas of science .Metal nanoparticles produced by nanotechnology have received global attention due to their extensive applications in the field of biomedical and physiochemical fields .Recently green synthesizing metal nanoparticles using microorganisms and plants has been extensively studied and has been recognized as a green and efficient way in the synthesis of metal nanoparticles. Here, we explore the potential uses of various biological sources of nanoparticles , synthesis and the application of bio synthesised metal nanoparticles. Green synthesis of nanoparticles and their applications have been an interesting area in the field of research and technology. Basically, green synthesis of nanoparticles gaining more attention because of minimization of waste, reduction of derivatives, pollution control as well as renewable feed stock. Green synthesis of metallic nanoparticles has been adopted to accommodate various biological methods. Among this green synthesis of metal oxide nanoparticles using plant extracts is rather a simple and easy process to synthesise nanoparticles. The parameters of nanoparticles are modulated by varying the concentration of the precursor, temperature, pH, additive concentration and incubation time. These parameters are optimised by UV- Visible Spectrum. To study and examine the qualitative analysis and quantitative estimation of the aqueous extract of *Beta vulgaris* by standard procedure. To optimize the synthesized metal oxide nanoparticles such as Copper oxide. The photocatalytic properties of *Beta vulgaris* mediated Copper oxide nanoparticles on methyl orange dye. To test and compare the In-vitro anti-microbial activities of *Beta vulgaris* mediated Copper oxide nanoparticles against six bacterial strains. To study and compare the In-vitro anti-breast cancer activities of *Beta vulgaris* mediated Copper oxide nanoparticles by MTT assay. To analyse and compare the In-vitro anti-diabetic activities of *Beta vulgaris* mediated Copper oxide by alpha- amylase and alpha- glucosidase assays.

II. MATERIALS AND METHODS

1. **Preparation of *Beta vulgaris* extract:** In the present study, Nano particles were synthesized by green method. Copper oxide is sysnthesized in the presence of Beta vulgaris extract. The vegetable *Beta vulgaris* was authenticated as *Beta vulgaris* L.(Amaranthaceae)the speciemen was accessed at the Department of Botany, St. Joseph's College, Trichy. Beet root was washed several times with water to remove the dust particles present in the root. After washing 250gms of *beta vulgaris* was weighed and chopped chopped in to fine pieces. The finely chopped beta vulgaris was then transferred in to a beaker .The *beta vulgaris* was washed with distilled water repeatedly. The root blended finely by using a mixer grinder and made in to a smooth paste. The grinded paste was filtered by using normal filter paper. Second filtration was carried out using Whatman filter paper. The filtration set up was left for 2-3hrs to allow the sediments to settle down at the bottom of the beaker. Again the sediments were removed by using filter papers. The filtered extract was allowed to cool at room temperature .The extract was further stored in the refrigerator and it was utilized for further quantitative, qualitative analysis and nanoparticle synthesis

2. Nanoparticle Synthesis

- **Optimization:** The chemical reduction has become very important in order to maintain eco -friendly research work .The alternative way of finding the synthesis of nanoparticles is green method. However, its toxicity capacity depends on many variables like Concentration, pH, temperature, incubation time and additive concentration .These multiples variants may disturb in the ability to inhibit the growth of microorganisms. Thus ,optimizing parameters will refined the synthesis of metal oxide nanoparticle synthesis and also increase its anti microbial capacity .pH of the reaction will inhibit the growth of microorganisms .The average size of the metal nanoparticles are with the greatest intensity in the size distribution .The polydispersity of nanoparticles were characterised and the yield obtained are high. These factors were significantly studied for variable responses by the optimization process made more effective and to improve its pharmacological applications.

- **Optimization of Copper Oxide Nanoparticles:** Copper nanoparticles have attracted considerable attention due to its optical property ,mechanical and electrical properties .Moreover it is cheaply available ,easy affordable ,with normal room temperature the yield obtained will be more .It is also has wide range of applications in many pharmacological applications . An optimization was carried out with the purpose to fix the system for the bulk synthesis of copper oxide nanoparticles. Copper sulphate used as a precursor in the synthesis of copper oxide nanoparticles .Copper sulphate concentration varies from 1M, 0.1M, 0.01M, 0.001M and 0.0001M concentration .The size distribution of nanoparticles can be observed by changing different range of pH value .pH value varies from 2,3,4,5,6,7,8,9,10,11,12 .The temperature variation from 30˚C,40˚C,50˚C,60˚C,70˚C,80˚C,90˚Cand 100˚C influence the growth of nanoparticles . Additive -precursor ratio 1:1, 1:4, 2:3, 3:2, 4:1 variation was carried out with five levels .The rate of incubation vary from 0 Mins, 30Mins, 1 hour, 2hour, 3hour.By the strong intense peak of UV-Visible spectrum the system was fixed . After the optimization ,the bulk synthesis of copper oxide was carried out .The best combination of the system was found to be *betavulgaris* mediated copper oxide particles was measured in 1:4 ratio ,the suitable precursor concentration is 0.001 M ,with the normal pH condition at the temperature of 30˚C.The lower the temperature growth of nanoparticles will be more. Bulk synthesis of copper oxide nanoparticles from the optimized condition each system was prepared in a bulk medium .The optimized system was used for further synthesis. In the synthesis of copper oxide nanoparticles .The freshly prepared *beta vulgaris* extract of 200 ml was taken in a 500ml beaker .The precursor copper sulphate was prepared in the concentration of 0. 001M .Now the precursor was taken in a beaker kept in a stirrer with the room temperature .The pH of the solution was maintained as 7 neutral medium .The reaction started with the constant stirring and after every 1 hour the freshly prepared beta vulgaris was added at regular inteval of time .Slowly 10ml of the extract was added about 10ml each for every addition with the continuous stirring .The colour of the solution was blue in colour and after the completion of the reaction it has become blueish green that is after 3 hours of interval of time .This colour change confirms the formation of copper oxide nanoparticles .Finally the reaction mixture was allowed to cool at room temperature for complete reaction to occur .Then the solution was centrifuged with the high speed at 6000rpm .The particles settled

down .The nanoparticles are then washed for several time with distilled water.The collected particles then allowed to dry heating in a hot air furnace at 200ºC .The nanoparticles are dried and the dried fine powder of *Beta vulgaris* copper oxide nanoparticles were collected and preserved in a air tight container for further studies.

3. Applications of Synthesised Copper Oxide Nanoparticles

- **Photocatalytic Degradation of Methyl Orange Dye:** Under the low pressure of 125 W UV lamps the investigation done in a Heber Multi lamp (254 nm) under continuous stirring .The synthesised *Beta vulgaris* mediated metal oxide nanoparticles reduce the methyl orange dye .For every 30 minutes of regular interval of time 3ml of sample was tested (methylene orange of 10 ppm).It was treated with 0.1 gms of respective nanomaterial which was placed over the light source halogen .The progress of the reaction was recorded by using UV visible spectrophotometer .With the standard calibration curve which was obtained by the absorbance of the untreated Methylene orange the percentage of the removal was calculated using the formula.

$$\%\ of\ Methylene\ Blue\ Degraded = \frac{I.C - F.C}{I.C} \times 100$$

Where,
I.C- Initial Absorbance of Methylene blue (Blank)
F.C- Sample Absorbance

- **Antibacterial Activity of Synthesised Copper Oxide Nanoparticles:** Inoculum preparation involves obtaining the organisms in an optimal state .It is very compatible with inoculation in to the tissue culture ,cell culture and fermenters .The prime objective is to achieve a high level biomass .The purpose of inoculation was defined as the process of adding effective bacteria to the host .The nutrient broth was prepared, then specific bacterial colonies were inoculated into the broth culture and are used for antimicrobial activity.

- **Inhibitory Assay o f Alpha Amylase:** The alpha inhibitory assay was carried out by using the Green syntheised metal oxide nanoparticles . 250µL of nanoparticles was (20-100 µg/ml) placed in a tube and 250µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was pre-incubated at 25˚C for 10 min, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at regular time intervals and then further incubated at for 25˚C for 10min. The reaction gets stopped by addition of 500µL of dinitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled at room temperature. The reaction mixture was diluted by adding 5ml distilled water and the absorbance was measured at 540nm using spectrophotometer. A control was prepared during the same procedure by replacement of the extract with distilled water. The α-amylase inhibitory activity was calculated in terms percentage inhibition.[5]

%Inhibition = [(Abs control – Abs aqueous extract) / Abs control] x 100

- **Alpha-Glucosidase Inhibitory Assay:** The activity of the aqueous extract on α - glucosidase determined by α-glucosidase from saccharomyces cerevisiae .P-nitro phenyl glucopyranoside (p-NPG) was prepared in 20mM phosphate buffer as a substrate solution and pH 6.9. 100µL of α- glucosidase (1.0 U/mL) was pre- incubated with 50µL of the different concentrations (20-100µg/ml) of the Metal oxide nanoparticle for 10min. Then 50µL of 3.0mM (pNPG) substrate was dissolved in 20mM phosphate buffer (pH 6.9) were added to start the reaction. The reaction mixture was incubated at 37˚C for 20min and stopped by adding 2ml of 0.1M Na_2CO_3.The α-glucosidase activity was determined by measuring the yellow- colored p-nitro phenol released from pNPG at 405nm. The results were expressed in percentage of the blank control. The α-glucosidase inhibitory activity was calculated by percentage inhibition.[6]

  %Inhibition = [(Abs control – Abs aqueous) / Abs control] x 100

- **Anti Cancer Activity of Synthesised Copper OxideNanoparticles**

  - **Characterisation:** Characterisation is used in different fields of research. This is broadly classified with its structure and properties .The scale of the structures of characterization ranges from angstroms, the individual atoms and chemical bonds were measured in centimeters.

    Many techniques have been practised such as basic optical microscopy .In particular the electron microscope shows the image and analysing the structure composition in smaller scales .Which helps in the understanding of the synthesised nanoparticles and its behavior .[7]The biologically synthesised Metal oxide nanoparticles were subjected for its structural ,optical and morphological techniques like UV-Visible ,FT-IR ,SEM ,TEM, XRD , EDAX ,Zeta potential .

III. RESULT AND DISCUSSION

1. **Extraction of aqueous extract of *Beta Vulgaris*:** Extraction of plants is an essential and crucial process in the preliminary analysis of any plant materials. In this process the active phyto-compounds such as terpenes, steroids, alkaloids, glycosides, saponins, and flavonoids were separated from the plant material via standard protocol with the appropriate solvent system. Several extraction process/methods include digestion, infusion, decoction, maceration, soxhlet extraction, microwave and ultra sound – assisted process. In additionally, the extraction solvents used in the extraction process also gained the equal importance. The relative polarity of the organic solvents determines the type of phytocompounds extracted (solubility) from the plant materials. It is found that on extraction process around 68% of yield (6.8gms/10gms). Phytochemical analysis of aqueous extract of *Beta Vulgaris* is screened and its results were given in figure-2 and table-1.

Figure 1: Extraction and Filtration Images of aqueous extract of *Beta Vulgaris*

2. **Phytochemical Screening Analysis of Aqueous Extract of *Beta Vulgaris:*** The screening results strongly confirms the presence of anthocyanins (major), betacyanin in the *Beta Vulgaris* extract along with that other plant constituents like saponin, terpenoid, poly phenolic, and flavonoid, also the results supports the minimal of alkaloids, tannin, steroids, antroquinone, glycosides, and coumarins. Results of phytochemical screening is given in the figure-2 and table-1. Likewise the quantitative results of root of *Beta Vulgaris* is shown in table-2. From the result it was found that the flavonoid (6.170gms/10gms) is rich in *Beta Vulgaris* followed by the saponin (5.130gms/10gms), terpenoids (4.020gms/10gms), carotenoids (1.320gms/10gms), alkaloids (0.270gms/10gms), and lycopenes (0.187gms/10gms) are found in *Beta Vulgaris*.

Table 1: Phytochemical Screening Results of *Beta Vulgaris*

S. No	Phytochemicals	Aqueous extract
1	Tannin	+
2	Saponin	++
3	Flavonoids	++
4	Steroids	+
5	Terpenoids	++
6	Triterpenoids	+
7	Alkaloids	+
8	Antroquinone	+
9	Polyphenol	++
10	Glycoside	+
11	Coumarins	+
12	Betacyanins	+++
13	Anthocyanins	+++

Table 2: Quantitative Analysis Results of Root of *Beta Vulgaris*

Phytochemicals	Results (g/10gm)
Flavonoids	6.170
Terpenoids	4.020
Saponin	5.130
Lycopenes	0.187
Alkaloids	0.270
Carotenoids	1.320

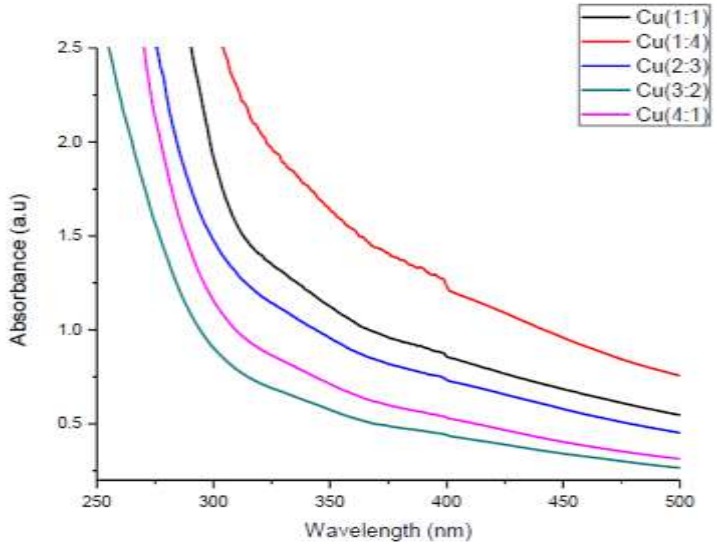
Anthocyanin and betacyanin are the secondary metabolites which are major plant pigments and readily soluble in water. They all the actual reason for the colour of the plant/plant parts like flowers, fruits, leaves, bark and etc. Anthocyanins and betacyanins are belonging to the family of flavonoid which was mostly found as glycosylate form. These colored compounds also possess the pharmaceutical potent which gives the numerous health benefits. Scientific reports on cell line studies (In-vitro), animal studies (In-vivo) and clinical trials on human evidenced that these compounds hold the biological effects of anti-microbial, anti-oxidant, anti-cancer and also improves the neurological health and protects from the non-communicable diseases.

IV. OPTIMIZATION, BULK SYNTHESIS AND CHARACTERISATION OF THE COPPER OXIDE NANOPARTICLES

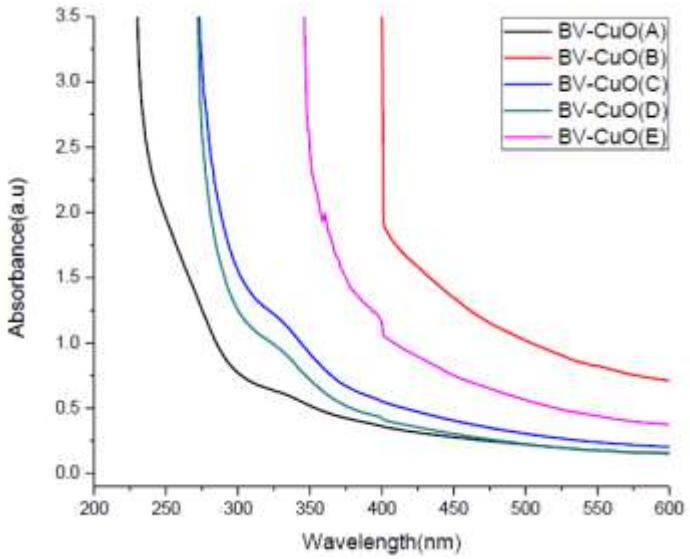
1. Optimization Results Of Copper Oxide Nanoparticles

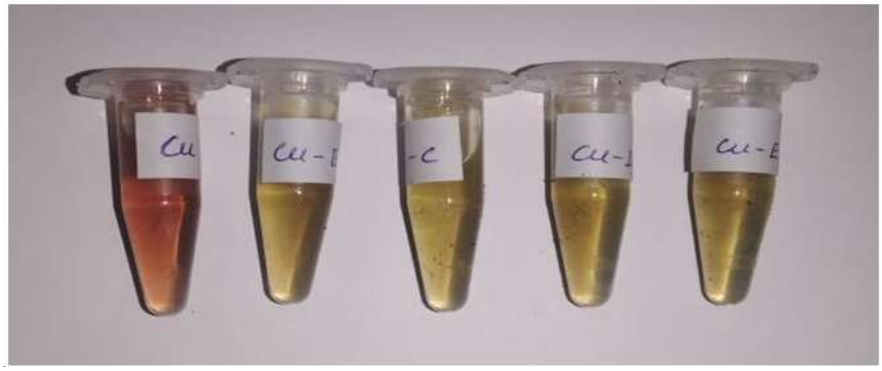
- **Additive-Precursor Combination:** In the optimization process initially the additive (*Beta Vulgaris* extract) and precursor (copper sulphate solution) combinations of 1:1, 1:4, 2:3, 3:2, and 4:1 were performed and their images were shown in figure. While adding the beta vulgaris extract (dark red colour) to the precursor (blue colour) the reaction mixture turns into greenish brown with the brown precipitate of copper nanoparticles. Addition of excess additive increase the formation of copper nanoparticles. Visibly the combination 1:4 bottle has the high deposits of brown precipitate than the others.

When the ratio of plant extract is increased from 1 to 4 the colour of the reaction mixture gets more intensed (dark brown). After 48 hours one milliliters of each combinations are collected then taken for UV-Visible spectral analysis to confirm the nanoparticle formations. The combined absorption spectral results of five combinations were given in the figure. From the spectral report it is understood that all the combinations shows the absorbance peaks at 395nm the combination 1:4 exhibits highest absorbance peak compare to other combinations.



- **Concentration of the Precursorl:** In this study five different concentration of precursor copper sulphate are used such as 0.0001M, 0.001M, 0.01M, 0.1M and 1M. The 1:4 combination mixture are prepared in 5 bottles by using the different concentrations of precursor and are named as A, B, C, D and E respectively. From the visual appearance the precursor concentration changes from 0.0001M to 1M (A to E) the colour of the reaction mixture changed from reddish pink to yellow. After 48 hours, one milliliters of each combinations are collected then taken for UV-Visible spectral analysis to confirm the nanoparticle formations. The combined absorption spectral results of 5 precursor concentration combinations were given in the figure. From the spectral report it is understood that the change of precursor concentration shifts the maximum absorption spectra from 250 to 395nm. Sample B (0.001M) revealed the highest absorption maxima compared to A, C, D and E. This is due to insufficient of reducing agents. In this process the additive ratio is fixed only the concentration of the precursor is changed. At 0.001M concentration reducing reduce maximum copper element and further increase of concentration of precursor lowers the absorption spectra. From that, the concentration precursor is fixed as 0.001M and it is maintained in throughout the optimization process.



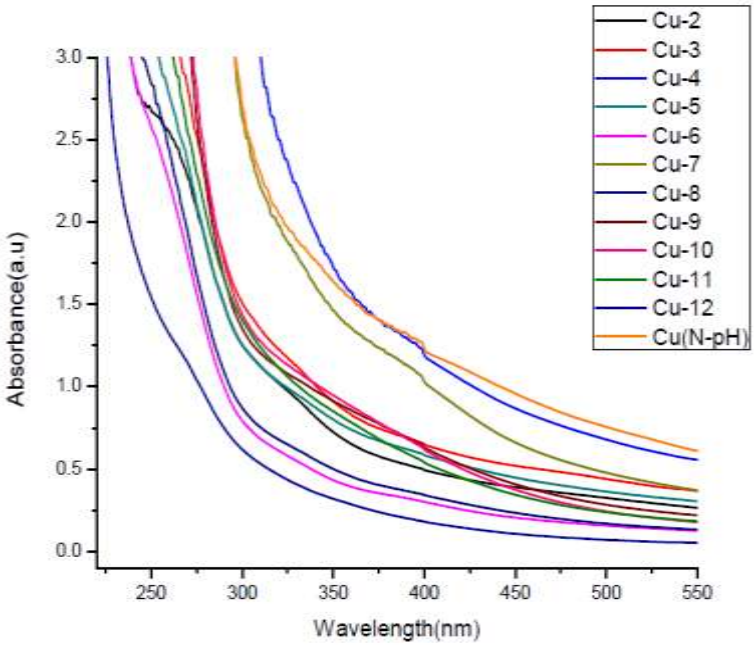


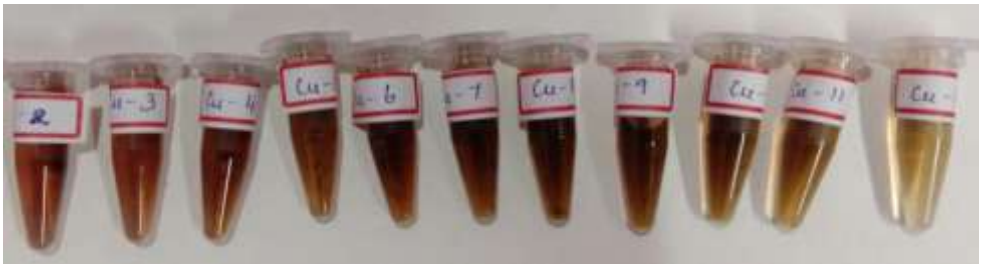
- **Ph of the Reaction Mixture:** Since, 1:4 combination favours the copper nanoparticle formation it is fixed for further optimization process. The 1:4 additive-precursor combination mixture are prepared in 11 bottles. The pH of the normal reaction mixture is found to be 5.4 to this dilute hydrochloric acid is added to reduce (for 2-4pH) the pH of reaction mixture similarly, to increase the pH dilute ammonium hydroxide is added (for 8-12pH). In the pH optimization the range of pH as follows 2, 3, 4, 5, 6, 7, 8, 9, 10, 11and 12. From the visible observation, it is found that at neutral pH (7) the reaction mixture turns into dark brown liquid. When reduce the pH to 6, 5, 4, 3, and 2 (acidic medium) the colour of the reaction mixture turns to greenish brown to dark pink. At basic pH 8, 9, 10, 11 and 12 the reaction mixture's colour changes from greenish brown to yellowish green.

  This because beta vulgaris extract is rich sources of anthocyanin and usually, anthocyanins turns to reddish pink in the acidic medium i.e. from 1 to 6 pH and at neutral pH reddish purple color furthermore at basic pH anthocyanins turns to green colour. After 48 hours, one milliliters of each combinations are collected then taken for UV-Visible spectral analysis to confirm the nanoparticle formations. The combined absorption spectral results of 11 pH combinations were given in the figure. The change of pH directly affects the stability of the nanoparticles. From the spectral report it is understood that the change of pH from 2 to 12 shifts the absorption spectra

from 360 to 395nm. The pH from 6 to 2 there is a decrease in wavelengths which is due to the reduction of size of the nanoparticles (the acidic medium favors). Similarly, at above neutral pH i.e. 8 to 12 the wavelength are shifted to higher and are due to increase of size of the nanoparticles (basic medium).

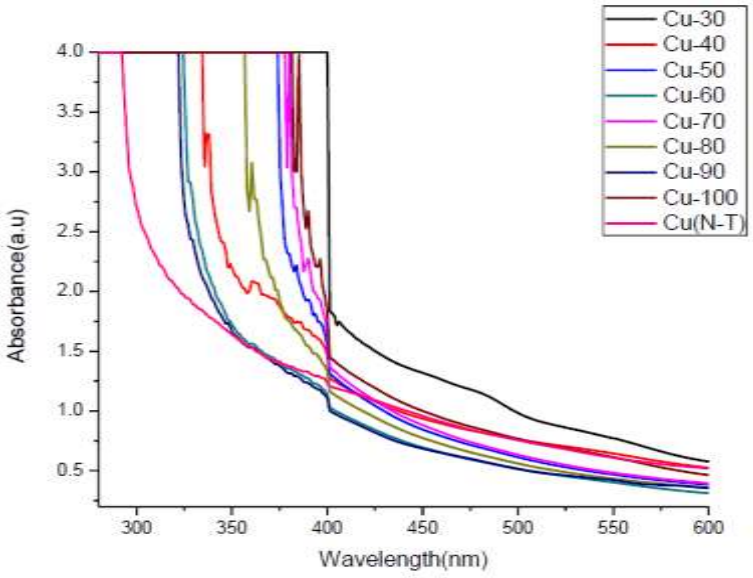
The summarized results shows that, at normal pH (original pH without adding acid or base) increase the yield of the nanoparticle than the others.

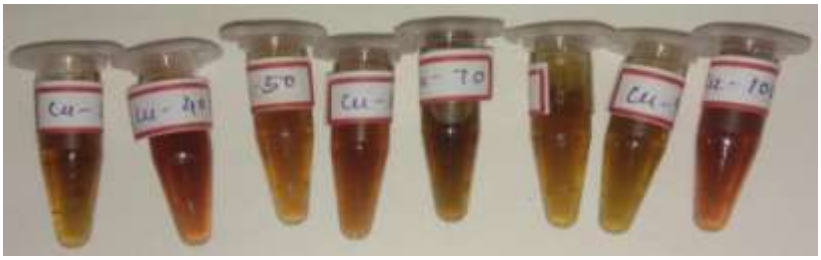




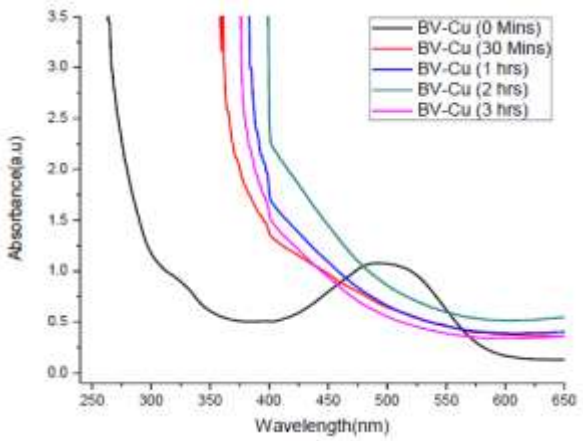
- **Temperature of the Reaction Mixture:** Since, 1:4 combination favours the copper nanoparticle formation it is fixed for further optimization process. The 1:4 additive-precursor combination mixture are prepared in 9 bottles. In the temperature optimization process each bottles are maintained in different temperature as follows 30, 40, 50, 60, 70, 80, 90, and 100. After 48 hours, one milliliters of each combinations are collected then taken for UV-Visible spectral analysis to confirm the nanoparticle formations. The combined absorption spectral results of 9 temperature combinations were given in the figure. The temperature is the one of the factor affecting the shape, size, and property of the nanoparticles. The temperature changes from 30 to 100 shows impact on its colour, visually the colour changes from green to dark reddish brown colour (30 to 100^{o}C). Moreover, the increase in temperature may degrade the anthocyanin and the low or room temperature increase the stability of

anthocyanins. The change of pH directly affects the stability of the nanoparticles. From the spectral report it is understood that the change of temperature shifts the absorption spectra from 300 to 398nm. The temperature at 30^{o}C, the absorbance is found to be maximum while increase of temperature not favors the synthesis of copper oxide nanoparticles.

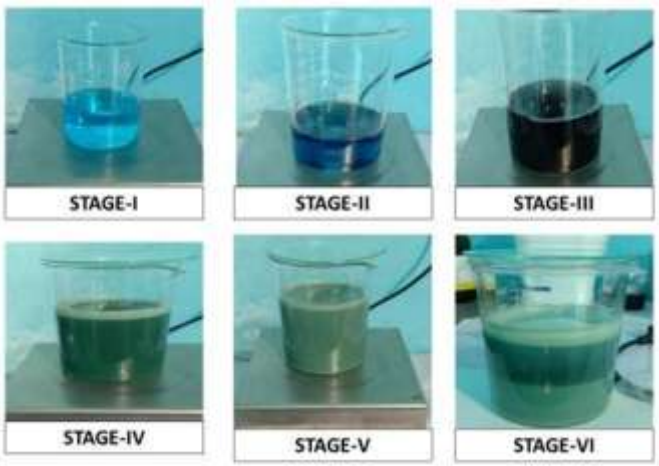




- **Reaction time:** The double the amount of 1:4 additive-precursor combination mixture prepared in 100ml beaker. In the time optimization process once additive added to the precursor immediately one ml of sample is taken out from the mixture and analyzed the UV-Visible spectrum. Similarly at the time intervals of 30minutes, 1hour, 2hours and 3 hours the samples were taken out from the reaction mixture and tested its absorption properties. The colour changes is observed during the time interval it is found that when time extended from 0 to 3hours the reaction mixture colour changes from light green to dark brown with colloidal form. At '0' minutes no reaction is observed and after 30mins the reduction of copper is taken place and it shows corresponding peak at 375nm and the wavelength increased to 410nm while time increased. The growth and stability of nanoparticles was depended on time. When time increased the quality and the quality of synthesized nanoparticle is found to high.



2. **Bulk Synthesis of Beta Vulgaris Mediated Copper Oxide Nanoparticles:** Based on the optimization results, the bulk synthesis of copper oxide nanoparticles were carried out. From the above results, it concluded that the best combination for the beta vulgaris mediated copper oxide particles is 1:4, the suitable precursor concentration is 0.001M, with the normal pH condition at the temperature of 30^{o}C. In the bulk synthesis 100ml of 0.001M of copper sulphate solution is initially taken in the 500ml beaker and kept on the magnetic stirrer with stirring. To this initially 50ml of beta vulgaris extract is added in drop wise and set the temperature 30^{o}C then allowed to react for 1 hour on stirrer with continuous stirring. Likewise every one hour time interval 50ml of beta vulgaris extract is added. After complete addition of 400ml of extract the whole reaction mixture is allowed to stirring on magnetic stirrer for 5 hours. Once the reaction is over the whole reaction mixture (stage V) is stored in reaction chamber for 48 hours. Then, visibly can understand that the copper nanoparticles were settled in the bottom of the beaker and the unreacted copper sulphate solution and extract was move to the liquid layer. Finally, the copper nanoparticles were collected by centrifuging at 6000rpm and collected particles were again washed with double distilled water followed by the methanol to remove the unreacted additive and precursor. In the obtained particles (precipitate) copper is in the form of hydroxide and to make it into oxide the precipitate is further calcinated at 200^{o}C at muffle furnace. The increase of additive concentration and reaction time the intensity colour changes is observed and are shown as stage-I (light blue) stage-II (dark blue) stage-III (blue-black), stage-IV (dark green), stage-V (greenish white) and stage-VI (blueish green) in the figure.



- **Characterization of Beta Vulgaris Mediated Copper Oxide Nanoparticles (BV-CuONPs)**

  - **UV-Visible Observation of BV and BV-CuONPs:** The UV-visible spectra of aqueous extract of *beta vulgaris* extract showed the three absorption maxima at 212 nm, 220 nm, and 330 nm which are due to presence of unsaturated double bond or hetero atoms (C=C, S, N, O) in the primary and secondary metabolites of the beta vulgaris extract. Mostly the metabolites such as anthocyanins, flavonoids, phenolics, terpenoids and alkaloids are present in the BV extract which are having the above functional and hetero grops. UV-Visible spectroscopy measure the surface plasmon resonance of the nanoparticles in solutions. From the literature it is evidenced that, the copper oxide nanoparticles shows the plasmon resonance between 200-350nm. The obtained BV-CuONPs exhibits the two peaks at 331nm (due to small size nanoparticles) and 498nm (due to large size nanoparticles) which confirms the copper oxide plasmon resonance and are close agreement with the previous studies. The UV-Visible spectrum of BV and BV-CuONps are shown in figure-.

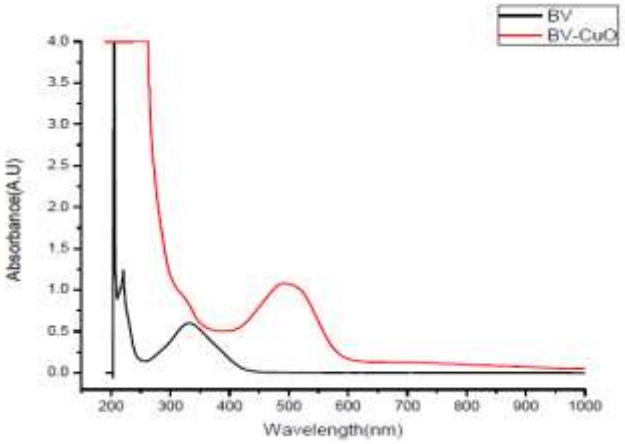


Figure 1: UV Spectrum of *Beta Vulgaris* Aqueous Extract (BV) and its Mediated Copper Oxide Nanoparticles (BV-Cuonps)

V. FT-IR OF BV AND BV-CUONPS

FT-IR spectrum useful to identify the functionality associate with the synthesized nanoparticles. The resulted FT-IR spectra of BV and BV-CuONPs were shown in figure-. From the resulted FT-IR data of BV extract reveals the following functionalities of intermolecular bonding of O-H stretching of alcohol (3409cm^{-1}), C-H stretching of alkanes (2948cm^{-1}, 2864cm^{-1} & 2840cm^{-1}), S-H stretching of thiol(2522cm^{-1}),C=C stretching of alkene (1648cm^{-1}) , C-H bending of alkane (1453cm^{-1}), S=O stretching of sulfonyl chloride(1412cm^{-1}), C-O stretching of aliphatic ether (1111cm^{-1}), C-O stretching of secondary alcohol (1053cm^{-1}), CO-O-CO stretching (1017cm^{-1}), and C-halogen stretching (655cm^{-1}). Likewise synthesized BV-CuONPs showed the few absorption peak on FT-IR analysis and the observed frequencies are 3348 cm^{-1}, 2098 cm^{-1}, 1622 cm^{-1}, 1383 cm^{-1}, 1197 cm^{-1}, 1158 cm^{-1}, 994 cm^{-1} , 961 cm^{-1} and 659 cm^{-1} which are due to O-H stretching of intra molecular bonding of alcohol, C=C=C stretching of alkene, C=C stretching of alkene, C-H bending of

alkane, C-O stretching of ester, C-O stretching of alcohol, C=C bending of alkene and C-X stretchings. Similarly the bands at 602 cm^{-1}, and 518cm^{-1} (below 650 cm^{-1}) responsible for M-O stretching of metal-oxygen bonds. The combined spectral results of FT-IR reveals that, after forming the nanoparticles the O-H, C-O, and C=C frequencies are gets suppressed and the range below 600 cm^{-1} (M-O bond) the peaks were elongated which confirms the Cu-O metal bonds in the beta vulgaris mediated copper oxide nanoparticles.

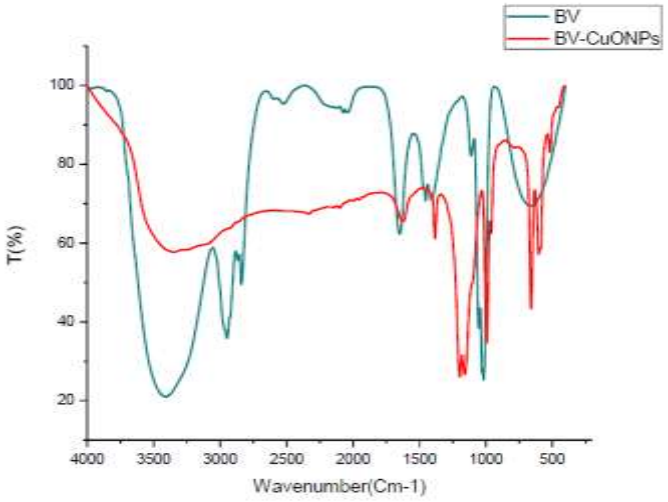


Figure 2: FT IR Spectrum of BV and BV-CuONPs XRD of BV-CuONPs

XRD spectral analysis provides the structural and crystalline nature of the synthesized BV- CuONPs. XRD peaks of obtained BV-CuONPs showed the 2Ɵ values of 31.06^0, 33.81^0, 36.05^0, 36.8^0, 41.74^0, 47.98^0, and 53.11^0 which are well agreement with the plane of (110), (111), (202), (020), (113), (311) and (220). The reported XRD peak data with the plane are matches closely with the standard copper oxide diffraction card JCPDS file number of 48-1548. In addition the average sizes of the BV- CuONps was estimated by the Debye-Scherer formula and shapes of the BV-CuONps were confirmed as face center cubic. Beside that with the assigned peaks some of the unassigned peaks were noticed in the XRD data and are due to the phytocompounds of aqueous extract of beta vulgaris and which is displayed in the figure-3. The average crystalline size of the BV-CuONPs is found to be 43.96nm.

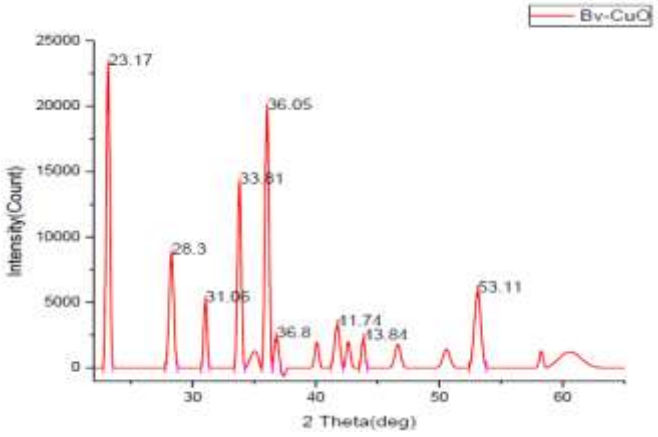


Figure 3: XRD Pattern of Synthesized BV-CuONPs

1. **SEM images of BV-CuONPs:** The structural and the morphological characterizations of biogenic synthesized BV-CuONPs nanoparticles were examined with the Scanning Electron Microscope. The accelerating voltage used in the analysis is 15.00kV. The magnifications images of synthesized BV-CuONPs are shown in the figure. It is found that the obtained copper oxide nanomaterials are in polydispered form with spherical and few rectangular shaped morphologies along with the accumulations.

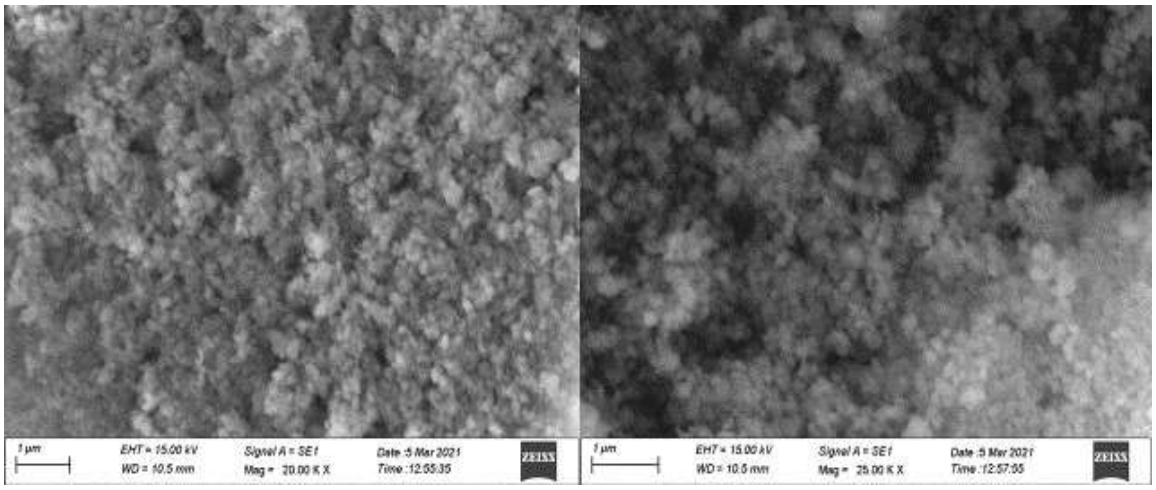


Figure 4: SEM images of Synthesized BV-CuONPs EDX of BV-CuONPs

In qualitatively and quantitatively the elemental composition of the synthesized BV-CuONPs is screened by the EDX. The resulted EDX images are shown in the figure and can identified that the prominently the copper peak is reported with the weight percentage of 73.46% followed by oxygen at 26.54%. The obtained narrow and the strong diffraction peaks of the BV-CuONPs revealed the high crystalline nature of that. Along with the existed copper and oxygen peaks some of the impurities peaks also found in the results and are due to the plant pytoconstituents present in the aqueous extract (involved in the M-O complexation).

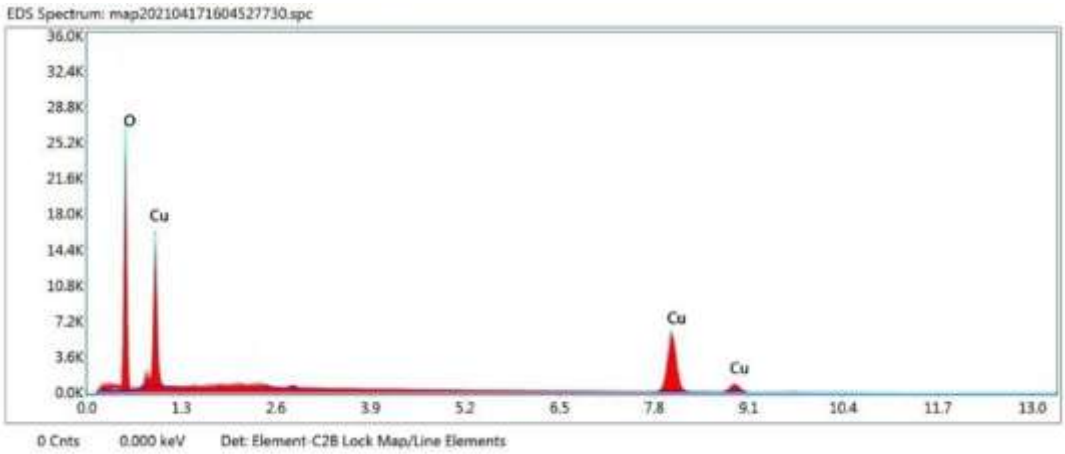


Figure 5: EDX of Synthesized BV-CuONPs TEM images of BV-CuONPs

The TEM analysis is the best electron microscope on morphologic analysis and SAED analysis provide the size and crystalline nature of the synthesized BV-CuONPs. The figure shows the TEM and SAED analysis images of BV-CuONPS. The results, strongly suggested that the synthesized nanoparticles were in agglomerated form with the spherical shapes which are resulted by interconnecting each other. This spherical shape with agglomeration also supported by the images of SEM analysis. The SAED images shows that the nanoparticles BV-CuONPs are polycrystalline nature which exhibits the concentric circles. Formed nanomaterials showed smaller grains and high porous size with the hallow structures in TEM analysis also have the lattice fringes of ~0.286nm. This smaller lattice fringes attributes the smaller grains in the formed BV-CuONPs.

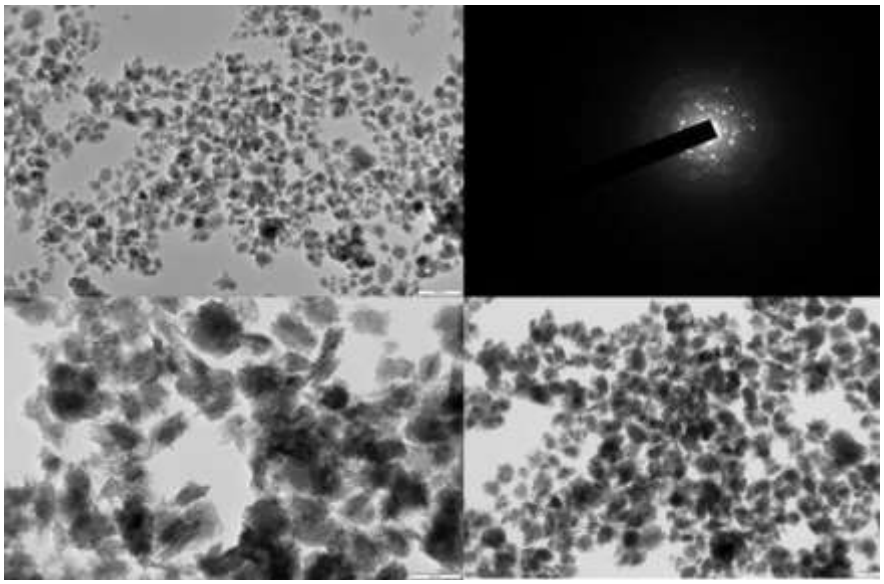


Figure 6: TEM Images of Synthesized BV-CuONPs Particle Size and Zeta Potential of the BV-CuONPs

The particle size analyzer determines the average size of the nanomaterial BV-CuONPs and its results are shown in figure. The particle size analyzer reveals that BV-CuONps has the three categories (peak) of size distributions. First peak observed at the size of 2.859nm with intensity and width of 58.3% and 0.3626 d.n. Second peak is obtained with the size of 47.09nm, intensity of 36.1% and width of 7.665d.n. Similarly, the third distribution is found with the size of 5356nm, intensity of 5.6% and width of 338.1d.n. Particle size analyzer found that the 94.4% of BV-CuONps have the particle size of below 50nm. Correspondingly, the zeta potential measure the charge on the synthesized BV-CuONps. The higher value of zeta potential either positive or negative will stabilize the colloidal dispersion. The zeta potential image of BV-CuONPs are given in the figure-. The result reveals that the zeta potential, zeta deviation and the conductivity of beta vulgaris mediated copper oxide nanoparticle is -4.09mV, 5.87mV and 1.62mS/cm respectively.

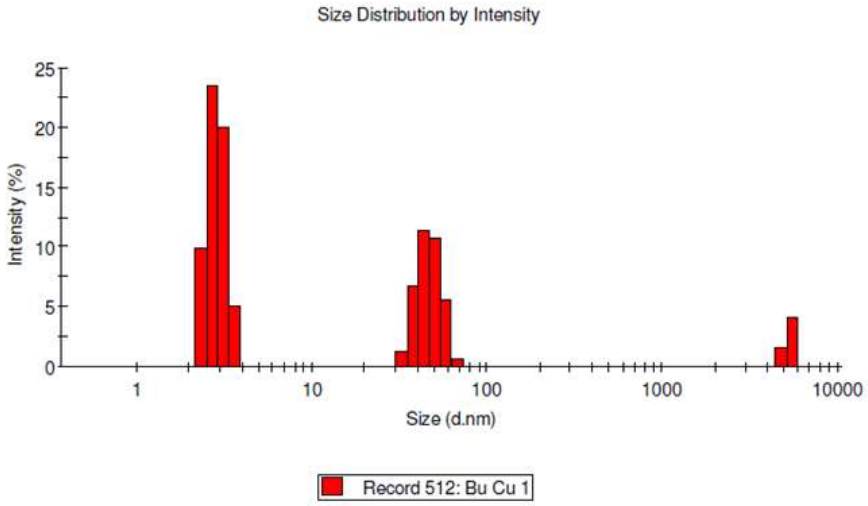


Figure- : Particle Size Analyzer of Synthesized BV-CuONPs

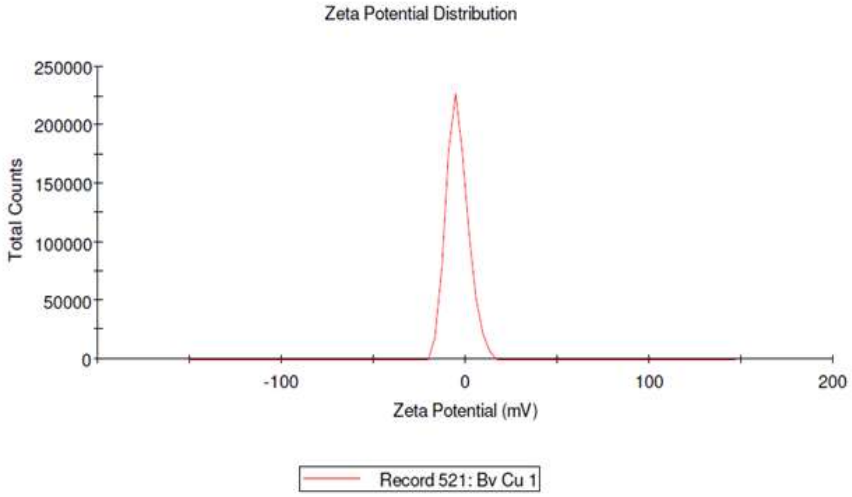
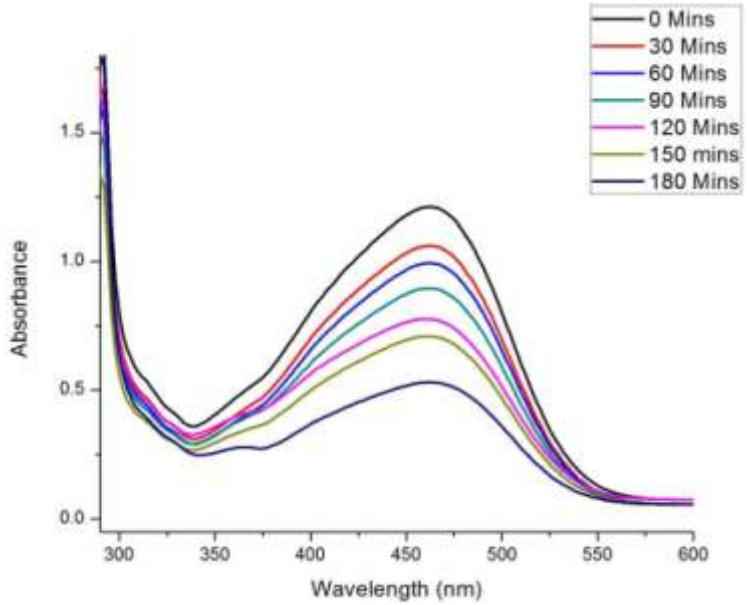


Figure- : Zeta Potential of Synthesized BV-Cuonps Photocatalytic Activity Results of BV-Cuonps

Initially the absorbance of methyl orange dye is noted as 1.212 after that around 10mg/5ml of synthesized BV-CuONPs were added to the methyl orange solution then the absorbance of reaction is observed. Every 30 minutes time intervals the absorbance of the reaction mixture is noted. The photocatalytic activity results of beta vulgaris mediated copper oxide nanoparticles (UV-light irradiated) on methyl orange is studied and their results are given in the table. The combined UV- visible absorption spectra was summarized in the figure. The report observed the time depended activity on irradiation. When the irradiation time extended the percentage of methyl orange degraded is increased. Generally, the methyl orange dye shows the absorption peak at 464nm on UV-Visible. All the time intervals the positions of absorption peaks were found at the 464nm only the intensities of peaks were varied on irradiation. When the irradiation time increases the intensities of absorption peak decreased from 1.212 to 0.531. The table evidenced that 57.18% of methyl orange dye degraded by the BV-CuONPs at 180 minutes time duration.

S.No	Time (Minutes)	Initial Absorbance (Blank)	Sample Absorbance	Percentage of Degradation
1	0	1.212	1.212	0
2	30	1.212	1.0611	12.45049505
3	60	1.212	0.9937	18.01155116
4	90	1.212	0.8952	26.13861386
5	120	1.212	0.7767	35.91584158
6	150	1.212	0.7092	41.48514851
7	180	1.212	0.53111	56.1790429

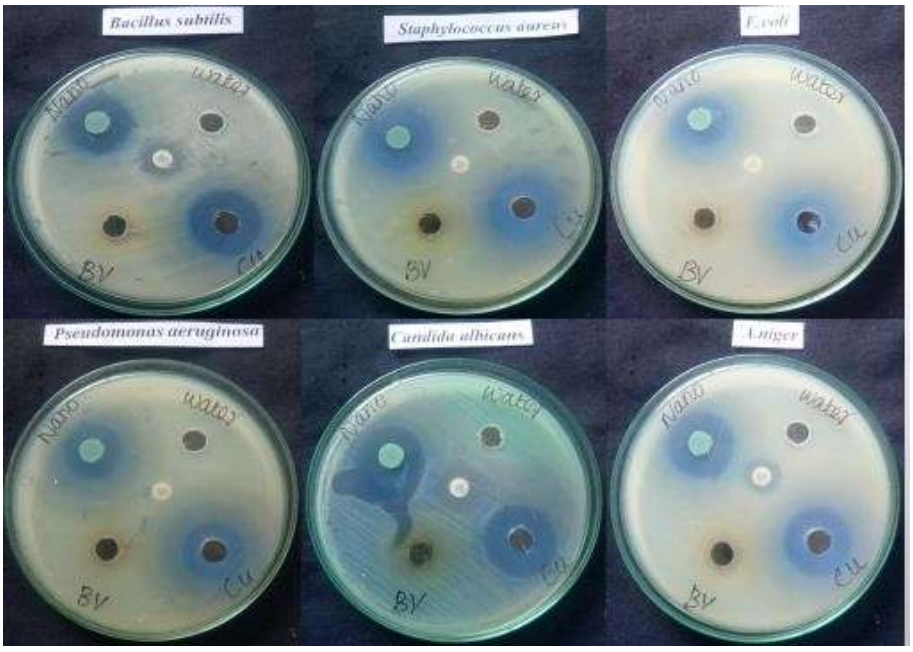


2. **Mechanism of Photocatalytic Activity:** The mechanism of photocatalytic activities as follows:

- The metal oxide semiconductor illuminates the greater energy which removes the electron from the valence band and moved to the conduction band .This vacancy created a hole in the valence band and electrons present in the conduction band.The holes will interact with the surrounding water molecules and produces hydroxyl groups whereas the electrons reacts with oxygen and produce superoxide radicals .Now these two radicals will reacts with water soluble organic compounds .These hydroxyl groups will react with the organic compound and reduce in to water and carbon di oxide .The super oxides react with water and produces hydrogen peroxide .This will be further react with the electrons and produce Hydroxyl ions .This hydroxyl ions will reduce organic compounds in to water and carbon di oxide .The above reaction will give better result in the basic medium and It produces more hydroxyl ions .But in acidic medium it reduces the resulting ion and leads to the formation of water molecules. Methyl orange is one of the very common azo dye. It is mainly used in the several industries including the textile ,paper ,printing and food industries .Methyl orange shows red colour and has sharp end point .

3. **Antibacterial Activities of BV-CuONPs:** The antibacterial efficacies of beta vulgaris extract, precursor copper solution, beta vulgaris mediated copper oxide nanoparticles and the antibiotic amoxicillin were screened comparatively. The summarized results of four samples against six bacterial strains were shown in table and their images are displayed in figure. While comparing the results, the beta vulgaris mediated copper oxide nanoparticles showed the highest zone of inhibition (i.e highest activity) on six bacterial strains compare to beta vulgaris extract, precursor copper solution, and the antibiotic amoxicillin. In addition the nanoparticle BV-CuONPs showed the greater inhibitory effect (35mm/ml) on the Bacillus subtilis and least effect (30mm/ml) on the pseudomonas.

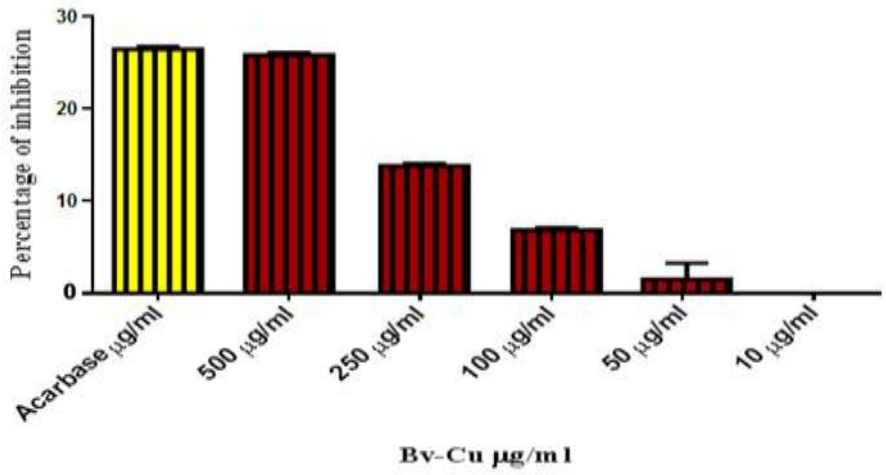
SAMPLE	WATER Extract 50 µl added and Zone of inhibition (mm/ml)				
	BV-CuONPs	Copper precursor	BV Extract	Positive Control	Water
Bacillus subtilis	35	30	18	20	-
Staphylococcus aureus	33	30	18	20	-
E.coli	32	30	18	20	-
Pseudomonas	30	30	18	20	-
Candida albicans	32	28	18	20	-
A.niger	26	22	18	20	-

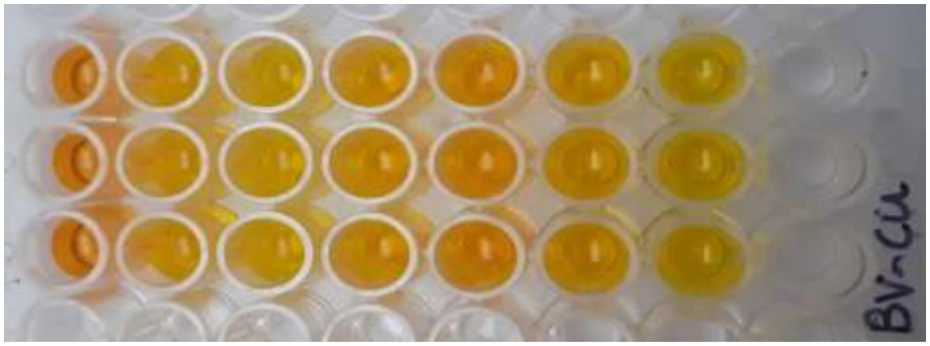


4. **Anti-Diabetic Activities of BV-CuONPs by Alpha-Amylase Assay:** The anti-diabetic activities of beta vulgaris mediated copper oxide nanoparticles by alpha- amylase assay

were screened and their results are shown in the table and their images are given in the figure. The table revealed that the at 10 µg/ml concentration the BV-CuONPs does not showed any inhibitory effect on alpha amylase enzyme. After increasing the concentrations of 50, 100, 250, and 500 µg/ml the copper nanoparticles revealed the inhibition. At higher concentration i.e. 500 µg/ml the BV-CuONPs exhibits the inhibition effect. The drug acarbose is used as a standard in the analysis when comparing the results with standard the synthesized BV-CuONPs exhibits less activity than the acarbose. However at the 500 µg/ml the copper nanoparticles showed the same inhibitory effect of standard acarbose (22.90%).

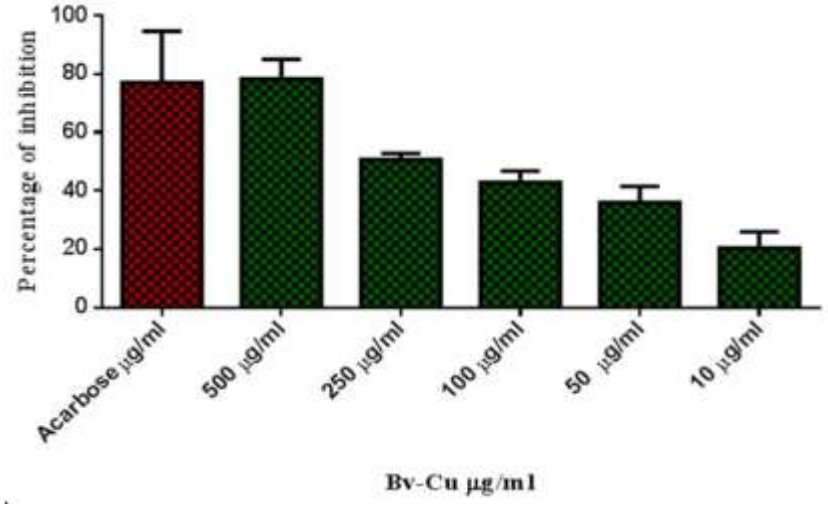
S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Acarbose	23.07	23.2	23.38	23.22
2.	500 µg/ml	22.97	22.76	22.97	22.90
3.	250 µg/ml	13.55	13.58	13.45	13.53
4.	100 µg/ml	8.61	7.27	8.01	7.96
5.	50 µg/ml	2.53	2.71	2.33	2.52
6.	10 µg/ml	0	0	0	0





5. **Anti-Diabetic Activities of CuONPs by Alpha-Glucosidase Assay:** The anti-diabetic activities of beta vulgaris mediated copper oxide nanoparticles by alpha- glucosidase assay were screened and their results are shown in the table and their images are given in the figure. The table revealed that the when the concentration of BV-CuONPs increased from 10 to 500 µg/ml the BV-CuONPs showed the concentration depended activity on alpha-glucosidase assay. At higher concentration i.e. 500 µg/ml the BV-CuONPs exhibits the highest inhibition of 78.72%. The drug acarbose is used as a standard in the analysis when comparing the results with standard thesynthesized BV-CuONPs exhibits less activity than the acarbose. However at the 500 µg/ml the copper nanoparticles showed the same inhibitory effect of standard acarbose (78.72%).

S. No	Tested sample concentration (µ/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Acarbose	81.64	92.05	57.80	77.16
2.	500 µg/ml	74.02	80.87	77.26	78.72
3.	250 µg/ml	51.78	52.05	48.49	50.77
4.	100 µg/ml	47.39	40.82	40.27	42.83
5.	50 µg/ml	39.72	38.63	29.58	35.98
6.	10 µg/ml	25.75	20.82	14.52	20.36

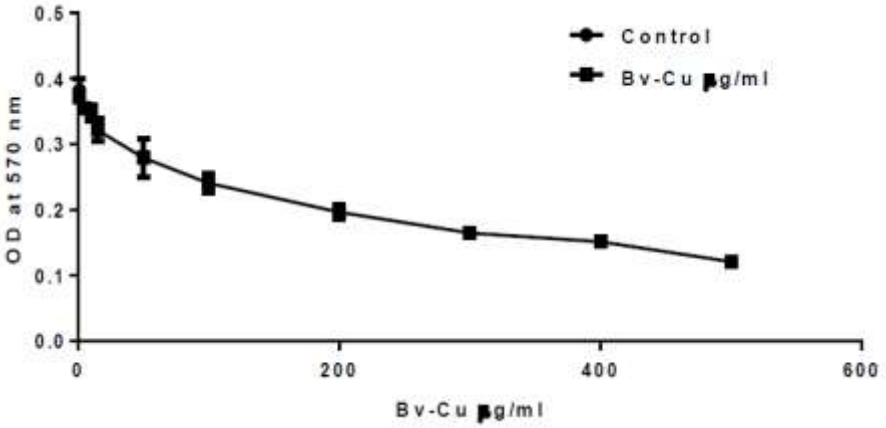


6. **Mechanism of Anti diabetic Activity:** The α-amylase and glucosidase decreases the reabsorption of glucose in the intestine .α-amylase present in the salivary glands and pancreas .This involves in the hydrolysis of complex carbohydrates in to a mixtures of oligosaccharides and disaccharides further converted in to monosaccharides by α-glucosidase .Converts starch in to glucose.

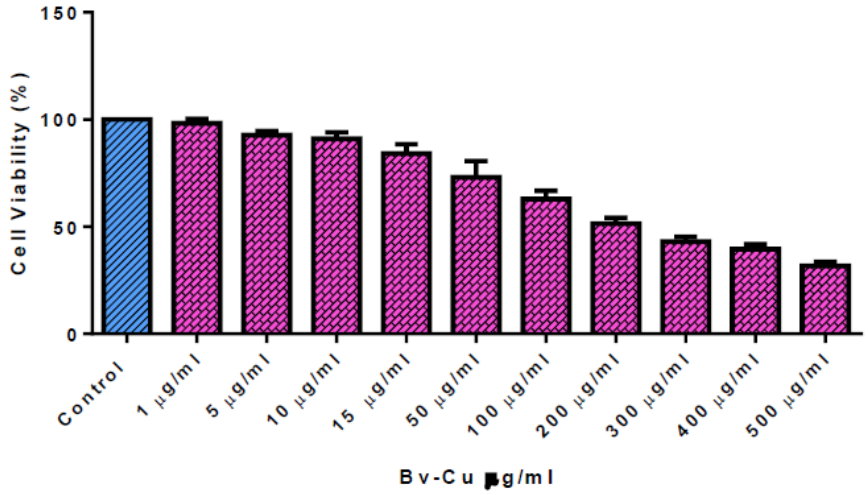
   The α-glucosidase will slow down the digestion by blocking enzyme in the small intestine that breaks the carbohydrate .So the digestion of carbohydrate become slow .The glucose from the blood enters the blood stream more slowly thus reducing the rise in the blood glucose level.

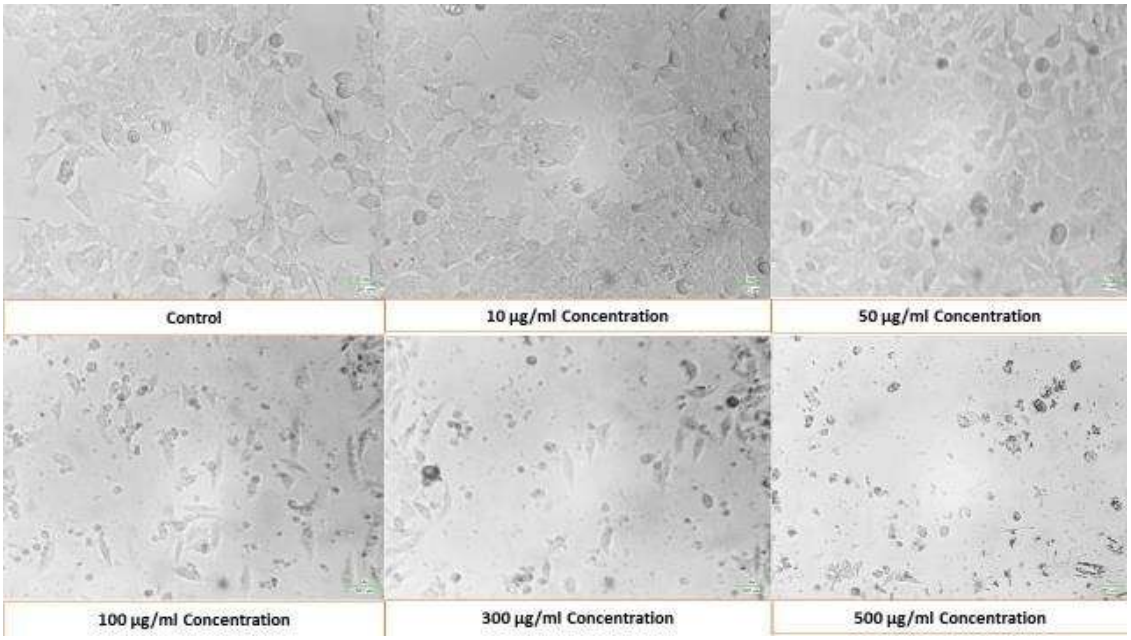
7. **Anticancer Activity of BV-CuONPs:** The in-vitro anti-cancer activity on breast cancer cell line is screened by standard MTT protocol. This assay measures the absorbance of the living cells from that the cell viability percentage is calculated. The percentage of cell viability measured by the quantity of formazan produced in the MCF7 cells with MTT. Only the viable i.e. living MCF7 cells with active metabolism can perform the conversion of MTT into formazan. When cancer cells dies it will lose this conversion ability thus the color changes helped to measure the number living MCF7 (breast cancer) cells. The absorbance readings of the control and the sample were shown in the table and the figure. From the absorbance table it is understood that the control has the maximum absorbance value of when the concentration of BV-CuONPs increased the absorbance value decreased it means while increasing the sample concentration the number of living MCF7 cell decreased. The absorbance value decreased from 0.328 to 0.121. From that readings the cell viability of the control and sample is calculated which are given in the table and figure. The table and the figure clearly show that while increasing the concentration of BV-CuONPs the cell viability percentage decreased from 100% to 31.67%. The IC_{50} value is calculated from the concentration verses cell viability graph. The IC_{50} value referred as 50% of cancer cell destroyed by the sample lower IC_{50} value means higher breast cancer activity. The IC_{50} of the sample BV-CuONPs is found to be 216.567 µg/ml.

S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm (in triplicates)			Average
1.	Control	0.395	0.362	0.390	0.382
2.	1 µg/ml	0.384	0.374	0.368	0.375
3.	5 µg/ml	0.350	0.363	0.350	0.354
4.	10 µg/ml	0.335	0.359	0.349	0.348
5.	15 µg/ml	0.311	0.341	0.313	0.322
6.	50 µg/ml	0.285	0.305	0.248	0.279
7.	100 µg/ml	0.250	0.248	0.224	0.241
8.	200 µg/ml	0.208	0.187	0.194	0.196
9.	300 µg/ml	0.156	0.167	0.171	0.165
10.	400 µg/ml	0.151	0.143	0.160	0.151
11.	500 µg/ml	0.128	0.114	0.121	0.121



S. No	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	1 µg/ml	100.52	97.90	96.33	98.25
3.	5 µg/ml	91.62	95.02	91.62	92.75
4.	10 µg/ml	87.89	93.97	91.36	91.00
5.	15 µg/ml	81.41	89.26	81.93	84.2
6.	50 µg/ml	74.60	79.84	64.92	73.12
7.	100 µg/ml	65.44	64.92	58.63	62.99
8.	200 µg/ml	54.45	48.95	50.78	51.39
9.	300 µg/ml	40.83	43.71	44.76	43.1
10.	400 µg/ml	39.52	37.43	41.88	39.61
11.	500 µg/ml	33.50	29.84	31.67	31.67





8. **Mechanism of Anti Cancer Activity:** The Folkman's hypothesis says that the formation of new blood vessels promotes the growth of tumors in the human body by the supply of oxygen and nutrients .This green synthesised metal oxide nanoparticles inhibit the growth of the vascular endothelial cells act as a protective barrier for the blood vessels and stops the formation of blood vessels called angiogenesis property .This anti-angiogenesis drugs plays the important role in cancer treatments and these biogenic properties of nanoparticles are acts as a angiogenesis inhibitors.

VI. CONCLUSION

Preliminary analysis confirms the occurrences of active phytochemicals in the aqueous extract of Beta Vugaris root both in qualitatively and quantitatively. The rapid biological synthesis of copper oxide nanoparticles using *Beta vulgaris* aqueous extract provides the environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were also characterized with the spectral methods such as UV-Vis, FT-IR, SEM, TEM, XRD and EDX. The important outcome of the study will be the development of value added product from *Beta vulgaris* for nanotechnology based industries. All these techniques proved that the concentration of Beta Vulgaris extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The biologically synthesized copper oxide nanoparticle is a source of antidiabetic, antimicrobial and anticancer activities that can be important in disease prevention.

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