IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Greensynthesis Of Copper Nanoparticles Using Grape Seed Extract

Tharani Priya A and Priya R Iyer

PG and Research Department of Biotechnology Women's Christian College, Chennai-600006

(An Autonomous Institution Affiliated to the University of Madras)

Abstract:

Nanoparticle synthesis has been a very important research area because of the wide use of nanoparticles in many fields. Green synthesis is one step ahead of other synthesis methods due to both cost reduction in production and environmentally friendly approach. Ultrafine particles are the same as nanoparticles and are between 1 and 100 nanometres in size, fine particles are sized between 100 and 2,500 nanometres, and coarse particles cover a range between 2,500 and 10,000 nanometres. Nanoparticle research (mostly metals) is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical. The wide variety of core materials available, coupled with tuneable surface properties, make nanoparticles an excellent platform for a broad range of biological and biomedical applications. Biosynthesis of copper nanoparticles using Grape seed extract was prepared successfully. Phytochemical Analysis of Grape Seed Extract as done for both the Qualitative determination and Quantitative determination were done. The characterization studies such as UV-VIS spectra, FTIR, XRD, SEM were done with the green synthesis copper nanoparticles. The nanoparticles' anti -diabetic efficacy was tested. Antimicrobial activity was studied in bacteria and fungi, zones of inhibition were observed. Antioxidant activity was also performed. Anticancer and Cytotoxicity tests were done to understand the toxicity of the nanoparticles. The application of nanoparticles was done on bean seed, seed coating method.

Keywords: grape seed, copper nanoparticles

Introduction:

The widespread application of nanoparticles in several industries has made nanoparticle synthesis an essential subject of research nowadays. Because of its ecologically benign approach and lower manufacturing costs than conventional synthesis processes, green synthesis is a step ahead. Nowadays, nanoparticle synthesis has been a very important research area because of the wide use of nanoparticles in many fields. Green synthesis is one step ahead of other synthesis methods due to both cost reduction in production and environmentally friendly approach(Demet Gultekin et al., 2017) .

Nanotechnology is the manipulation of matter with at least one dimension sized from 1 to 100 nanometres. Nanoparticles made up of natural or artificial polymer which plays an important role in most of the biological applications. The wide variety of core materials available, coupled with tuneable surface properties, make

nanoparticles an excellent platform for a broad range of biological and biomedical applications (De et al., 2008) .

Copper nanoparticles play an important role in environment applications. Copper nanoparticles have special qualities that set them apart from bulk copper material because of their small size and high surface area-to-volume ratio. Copper nanoparticles are investigated for various biomedical applications, including drug delivery, cancer therapy, imaging, and tissue engineering. Their small size allows for targeted delivery of therapeutic agents and imaging contrast ants to specific sites within the body. Some other applications of copper nanoparticles are used in Agriculture - copper nanoparticles can be incorporated into fertilizers or applied directly to soil to improve plant growth and health.

Materials and Methods:

Materials: Grape seeds, Deionized water, copper sulphate, Whatman filter paper.

Equipment: weighing balance, Magnetic stirrer.

Glassware: Beaker, conical flask, and filter funnel

Methodology

Collection and extraction of Grape seed extract

Grape seeds were procured and washed by deionized water. 2g of grape seed powder was taken in a beaker along with 20 ml of deionized water and heated over a water bath and boiled for 15 minutes. This extract was then filtered and purified using Whatman No.1 filter paper.

PHYTOCHEMICAL ANAYLYSIS FOR GRAPE SEED EXTRACT

QUALITATIVE DETERMINATION OF PHYTOCHEMICALS

- Test for tannins done by ferric chloride test
- Test for flavonoids done by lead acetate test
- Test for alkaloids by Hager’s test
- Test for Saponins by foam test
- Test for Phytosterol by Salkowski test
- Test for Phenol by Ferric chloride test
- Test for Carbohydrates by Molisch's test
- Test for Protein by Biuret test
- Test for amino acids by Ninhydrin test
- Test for Terpenoid by Salkowski test

QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS

- Determination of Total Phenol Content
- Determination of Alkaloids
- Determination of carbohydrates by Anthrone method
- Determination of Tannin
- Estimation of Sterols
- Estimation of proteins by Lowry's Method
- Estimation of Amino acids by Ninhydrin method

SYNTHESIS OF NANOPARTICLES

10 ml of grape seed extract was added to 0.1M of copper sulphate (CuSo4) solution. This mixture was then put in a beaker and placed over the magnetic stirrer for 3 hours. The obtained copper nanoparticles were poured in a petri plate and leave in room temperature for 3-4 days.

CHARACTERIZATION OF NANOPARTICLES

UV- VIS ABSORPTION SPECTROSCOPY

UV-VIS absorption Spectroscopy from the college central instrumentation laboratory, (Shimadzu UV probe 1800, Japan) was used to measure the absorption was used to measure the absorption in ultraviolet and visible range of electromagnetic spectrum. (Asif et al., 2022)

FTIR ANALYSIS

Fourier transformed infrared spectra (FTIR) from the college central instrumentation laboratory (Shimadzu corp (00504)), QATR-S singe reflection ATR accessory was used to study the presence of potential biomolecule and functional groups. (Khan et al., 2017)

X-RAY DIFFRACTION (XRD)

X-Ray diffraction from SAIF IIT Madras in Chennai. XRD was used to study the crystalline structure of nanoparticles. 2 different angles 10 to 80 were performed.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

SEM studies were studied using SEM (JSM-6480 LV) microscopy. The morphology of the nanoparticles was recorded.

Antimicrobial Activities

Antibacterial activity

Using the Agar Well Diffusion method, copper nanoparticles antibacterial qualities were studied.

Antifungal activity

Using Potato Dextrose agar, the antifungal properties of green produced copper nanoparticles were studied.

Cytotoxicity

Cells (1×10^5 / well) (VERO) were plated in 24 well plates and incubated at 37℃ with 5% CO_2 condition. The % cell viability was calculated using the following formula

%Cell viability = A570 of treated cells / A570 of control cells ×100

Graphs are plotted using the % of cell viability at Y axis and concentration of the sample in X axis. Cell control and sample control is included in each assay to compare the cell-cell viability assessments.

Anticancer activity

Cells (1×10^5 / well) (MCF-7) were plated in 24 well plates and incubated at 37℃ with 5% CO_2 condition. The % cell viability was calculated using the following formula

%Cell viability = A570 of treated cells / A570 of control cells ×100

Graphs are plotted using the % of cell viability at Y axis and concentration of the sample in X axis. Cell control and sample control is included in each assay to compare the cell-cell viability assessments.

ANTI-DIABETIC STUDY – α AMYLASE INHIBITORY ASSAY:

The effect of lead oxide nanoparticles on a α-amylase was determined by making various concentrations of lead oxide nanoparticles and standard. i.e., Metformin.

ANTIOXIDANT ASSAY - DPPH FREE RADICAL SCAVENGING ACTIVITY OF BIOSYNTHESIZED COPPER NANOPARTICLES:

0.1ml of plant extract copper nanoparticle suspension was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min incubation in dark. The % inhibition activity was calculated from,

$$100 \times (A0 - A)/A0$$

Where A0 is the absorbance of the control and A is the absorption of the extract.

The inhibition curves were prepared and IC50 values were obtained.

APPLICATION ON SEED COATING

Copper nanoparticles aqueous suspension was made using deionized water in a petri plate. In that paste, bean seeds were coated and leave it for dried. After a few hours the nanoparticle coated seeds were seeded on the soil for germination. Growth was observed(Zhao et al., 2021).

RESULT AND DISCUSSION

COLLECTION OF SAMPLE AND PREPARATION OF SEED EXTRACT:

Grape seed powder was taken with deionized water in the beaker. kept in the boiling water bath and then filtered using Whatman No:1 filter paper. This extract was collected and stored in an airtight container and used in a green synthesis reaction (Demet Gultekin et al., 2017)

Figure 1. Grape seed extract sample

Phytochemical Analysis of Grape Seed Extract:

Qualitative analysis

The results showed positive for Tannins, Flavonoids, Phenol, Alkaloids, Phytosterol, Carbohydrate, Protein, Amino acid and negative for Saponins and Terpenoids. Alkaloids presence was confirmed by Hagers method, phytosterols was confirmed by salkowski method, phenol was confirmed by ferric chloride test, carbohydrate by benedict's test , protein was confirmed by Biuret's test, amino acids was confirmed by Ninhydrin's test, terpenoids was confirmed by Salkowski test, saponin was confirmed by Foam test, tannin was confirmed by ferric chloride test and flavonoids was confirmed by lead acetate test.

PHYTOCHEMICALS	POSITIVE/ NEGATIVE
Tannins	+
Flavonoids	+
Phenol	+
Alkaloids	+
Saponins	-
Phytosterol	+
Carbohydrate	+

Protein	+
Amino acid	+
Terpenoid	-

Table1: Qualitative analysis result

QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS

The quantitative of confirmed phytochemicals were done. Protein presences were confirmed by Lowry's method, Amino acid presence was confirmed by Ninhydrin method, Carbohydrates presence were confirmed by Anthrone test (Kitsiou et al., 2023) .

PHYTOCHEMICAL	AVERAGE	STANDARD DEVIATION	AMOUNT PRESENT
Protein			**12.30mg/100ml**
Amino acid			**19.8 mg/100ml**

Table 2: Quantitative analysis test

SYNTHESIS OF COPPER NANOPARTICLE FROM GRAPE SEED EXTRACT

The copper nanoparticles were synthesized by using grape seed extract with copper sulphate, the presence of which was confirmed by the colour change. Dried nanoparticles were stored in an airtight container (Demet Gultekin et al., 2017).

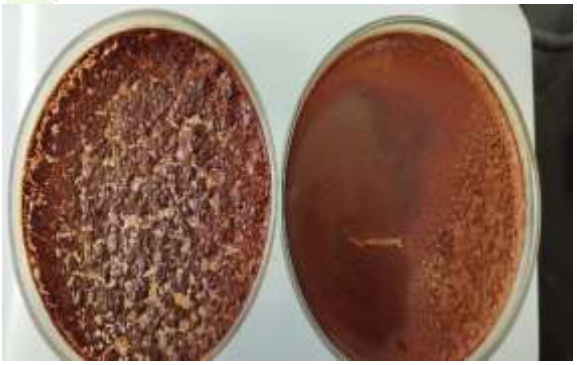

Figure 2. $CuSo_4$ nanoparticle

CHARACTERIZATION OF COPPER NANOPARTICLES

UV-VIS ANALYSIS RESULT:

The characterization of copper nanoparticles was carried out using Shimadzu UV probe 1800, in the wavelength of 200-600. The UV-Vis spectra of synthesized copper nanoparticles were recorded by scanning ranging from 200-600 nm and the absorption peak was shown at 350 nm. The peak at 350 nm confirms the presence of copper nanoparticles from the grape seed extract(Vardhana et al., 2022).

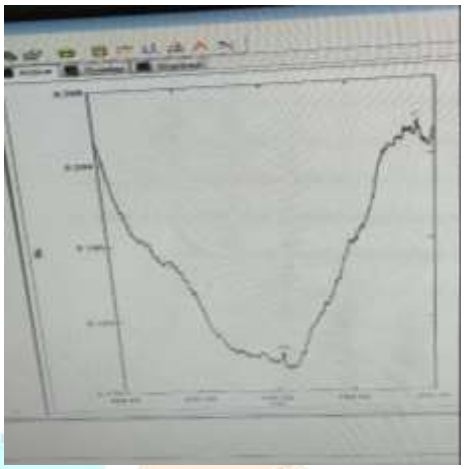

Figure 3. UV-Vis spectra of $CuSo_4$ nanoparticle

FTIR ANALYSIS RESULT:

The FTIR spectra of copper nanoparticles from aqueous seed extract showed the presence of important chemical bonds. The FTIR spectra were performed for copper nanoparticles synthesis extract and the peaks were observed. The peaks at 483 and 437, showed the presence of the carbonyl group. The peaks at 417 and 422, confirms the conjugation in the sample. The intense peaks at 538 and 528 showed the existence of OH group. (Vardhana et al., 2022)

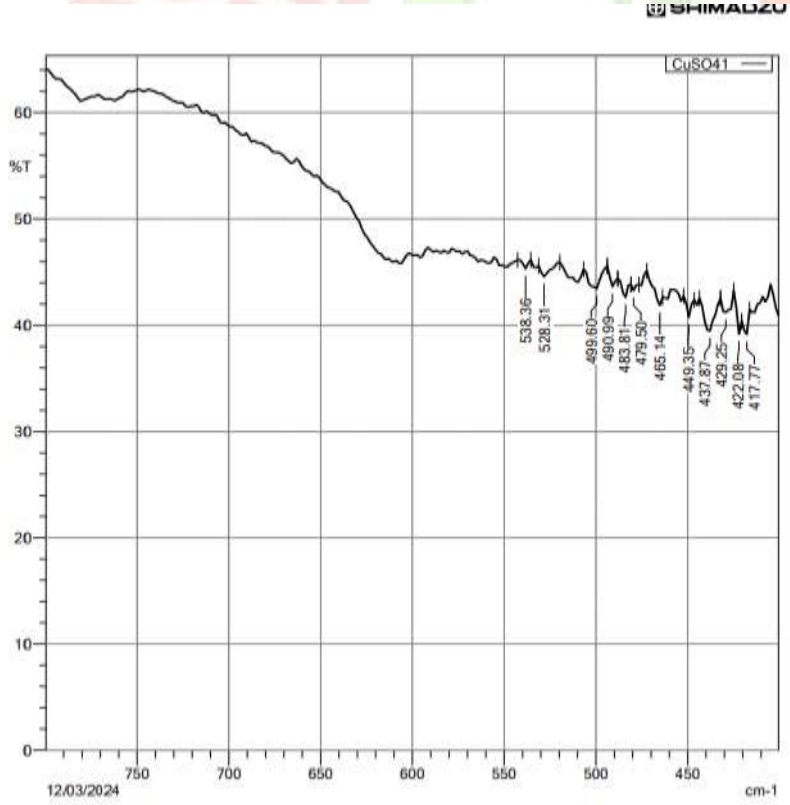

Figure 4. FTIR spectrums of CuSo4 nanoparticle

X-Ray Diffraction (XRD)

XRD characterization was used to identify the crystalline structure of nanoparticles, XRD spectra were seen in the range from 10 to 80 of 2 different angles. The samples were given for the characterization. (Vardhana et al., 2022)

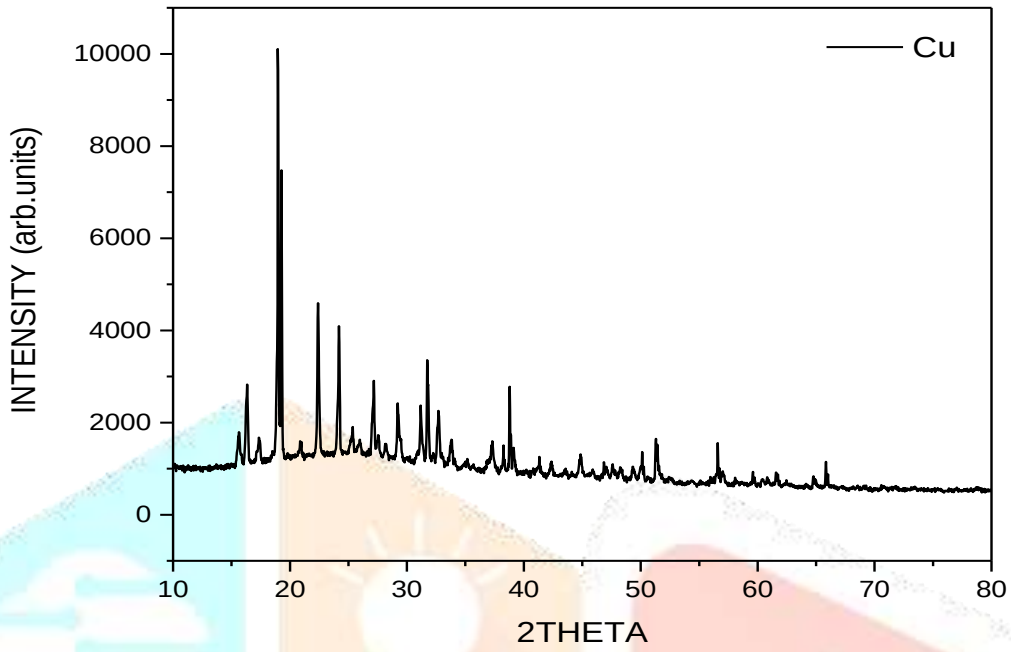

Figure 5. XRD Patterns of $CuSo_4$ nanoparticle

Scanning Electron Microscopy (SEM) analysis

The morphology of $CuSo_4$ nanoparticles were studied using SEM (JSM-6480 LV) microscopy. The results showed that the nanoparticles synthesized are spherical.

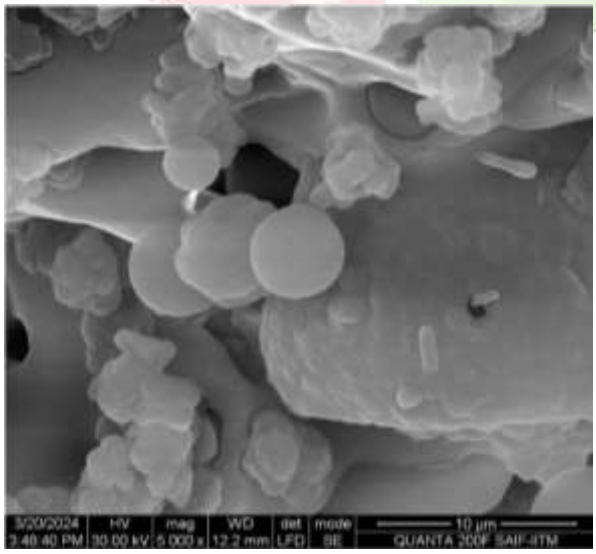

Figure 6. SEM image of $CuSo_4$ nanoparticle

ANTIMICROBIAL ACTIVITIES:

Antibacterial activity result:

The study on antibacterial activity of copper nanoparticles on bacteria showed a zone of inhibition in copper nanoparticles. Different quantities were taken at 50µl,100µl,150µl and blank. The zone of inhibition showed different concentrations and no zone appeared on the blank. The zones were measured in *Bacillus spp* - 1.6mm,1.9mm,2.1mm and *Streptococcus aureus* -0.7mm, 1.3mm, 1.5mm and *Pseudomonas spp* - 1mm, 1.3mm, 1.9mm.

Figure 7. *Bacillus spp.*

Figure 8. Pseudomonas spp.

Antifungal activity result:

The study on antifungal activity of copper nanoparticles on fungi showed a zone of inhibition in copper nanoparticles. Different quantities were taken at 50µl,100µl,150µl and blank. The zone of inhibition showed different concentrations and no zone appeared on the blank. the zone were measured in *Aspergillus niger* - 0.5mm, 1.5mm,2mm (Vardhana et al., 2022)

Figure 9: Aspergillus niger

Cytotoxicity test

The cytotoxic effect of the VERO cell line was tested. That showed the toxic level in green synthesis copper nanoparticles. The Grape seed extract contains 76.25, Metal salt contains 62.09, copper nanoparticles contains 41.98 . The green synthesis of nanoparticles contains 92.9% of viability. This result showed the less toxic compound in the copper nanoparticles. It proved that the copper nanoparticles used in many food based applications (Naz et al., 2020).

Figure 10: Cytotoxicity test

Anticancer activity result:

%cell viability = 1.144/1.815X100

= 63.0%

The study of anticancer activity of copper nanoparticles was done using the MFC-7 cell line. This test showed the anticancer activity of copper nanoparticles. Grape seed extract showed o.168, Metal Salt showed 0.615, copper nanoparticles showed 1.144 in the test. The cell viability were calculated by Inhibition (%) = [(Abs1-Abs2)/Abs1] X 100 where

Abs1 = sample and Abs2 = control. The % of cell viability was observed as grape seed extract showed 9.256, Metal Salt showed 33.88, copper nanoparticles showed 63.030 in the test. The cell viability percentage was reduced (Naz et al., 2020).

Figure 11: Anticancer activity

Anti-diabetic study-α amylase inhibitory assay result:

Antidiabetic assay was carried out at different concentrations of copper nanoparticles and %inhibition was calculated using the standard curve. The antidiabetic assay was done using α

And this study was performed to understand the glucose uptake by the copper nanoparticle. From the % inhibition, the mean value was 77.94 and SD was **± 2.10** (Faisal et al., 2022).

Antioxidant assay -DPPH free radical scavenging activity of biosynthesized copper nanoparticles:

This work showed the antioxidant assay was done using DPPH method and the antioxidant potential in copper nanoparticles. The OD value of the sample was taken twice, and their antioxidant activity observed. Antioxidant activity indicates that the cells can be protected from damage caused by free radicals. These results were compared to ascorbic acid, a conventional antioxidant agent possessed potency with a value. The antioxidant assay was calculated based on % inhibition using a standard curve. Antioxidant assays were calculated by

DPPH Inhibitory%=A0-A1/A0X100 **(Kaningini et al., 2023).**

APPLICATION RESULT:

Copper nanoparticles were coated on the bean seed. Leave it to dry for a few hours. Seeded in the soil for germination. After 1 week the growth was observed.

Figure 12. BEAN SEED COAT WITH COPPER NANOPARTICLES BEAN SEED

CONCLUSION:

Biosynthesis of copper nanoparticles using Grape seed extract was prepared successfully. Phytochemical Analysis of Grape Seed Extract as done for both the Qualitative determination and Quantitative determination were done. The characterization studies such as UV-VIS spectra, FTIR, XRD, SEM were done with the green synthesis copper nanoparticles. The nanoparticles' anti -diabetic efficacy was tested. Antimicrobial activity was studied in bacteria and fungi, zones of inhibition were observed. Antioxidant activity was also performed. Anticancer and Cytotoxicity tests were done to understand the toxicity of the nanoparticles. The application of nanoparticles was done on bean seed, seed coating method.

The other applications are Reduction of hazardous chemicals by green synthesis of nanoparticles. Copper nanoparticles used in soil remediation, Nano pesticides. Plant based nanoparticles play an important role in the environment. The green synthesis method has more benefits to the environment than chemical synthesis method. Reduce the cost, toxicity, eco-friendly, and has lot of food-based application

Acknowledgements:

We extend our heartfelt thanks to DST FIST and UGC for the instruments in Advanced Instrumentation Centre in Women's Christian College

Reference:

Aswathi, V. P., Meera, S., Maria, C. G. A., & Nidhin, M. (2023). Green synthesis of nanoparticles from biodegradable waste extracts and their applications: A critical review. *Nanotechnology for Environmental Engineering*, *8*(2), 377–397. https://doi.org/10.1007/s41204-022-00276-8

2.De, M., Ghosh, P. S., & Rotello, V. M. (2008). Applications of Nanoparticles in Biology. *Advanced Materials*, *20*(22), 4225–4241. https://doi.org/10.1002/adma.200703183

Demet Gultekin, D. D. G., Nadaroglu, H., Alayli Gungor, A., & Horasan Kishali, N. (2017). Biosynthesis and Characterization of Copper Oxide Nanoparticles using Cimin Grape (Vitis vinifera cv.) Extract. *International Journal of Secondary Metabolite*, 77–84. https://doi.org/10.21448/ijsm.362672

3. Din, M. I., & Rehan, R. (2017). Synthesis, Characterization, and Applications of Copper Nanoparticles. *Analytical Letters*, *50*(1), 50–62. https://doi.org/10.1080/00032719.2016.1172081

4.Faisal, S., Jan, H., Abdullah, Alam, I., Rizwan, M., Hussain, Z., Sultana, K., Ali, Z., & Uddin, M. N. (2022). In Vivo Analgesic, Anti-Inflammatory, and Anti-Diabetic Screening of *Bacopa monnieri* -Synthesized Copper Oxide Nanoparticles. *ACS Omega*, *7*(5), 4071–4082. https://doi.org/10.1021/acsomega.1c05410

5.Gupta, M., Dey, S., Marbaniang, D., Pal, P., Ray, S., & Mazumder, B. (2020). Grape seed extract: Having a potential health benefits. *Journal of Food Science and Technology*, *57*(4), 1205–1215. https://doi.org/10.1007/s13197-019-04113-w

6.Hussain, I., Singh, N. B., Singh, A., Singh, H., & Singh, S. C. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnology Letters*, *38*(4), 545–560. https://doi.org/10.1007/s10529-015-2026-7

7.Ibrahim, F. M., Najeeb, D. A., & ThamerSadeq, H. (2023). Green preparation of Cu nanoparticles of the avocado seed extract as an adsorbent surface. *Materials Science for Energy Technologies*, *6*, 130–136. https://doi.org/10.1016/j.mset.2022.12.006

8.Kaningini, A. G., Motlhalamme, T., More, G. K., Mohale, K. C., & Maaza, M. (2023). Antimicrobial, antioxidant, and cytotoxic properties of biosynthesized copper oxide nanoparticles (CuO-NPs) using Athrixia phylicoides DC. *Heliyon*, *9*(4), e15265. https://doi.org/10.1016/j.heliyon.2023.e15265

9.Kim, S., Jeong, S., Park, W., Nam, K., Ahn, D., & Lee, S. (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. *Food Chemistry*, *97*(3), 472–479. https://doi.org/10.1016/j.foodchem.2005.05.027

10.Kitsiou, M., Purk, L., Gutierrez-Merino, J., Karatzas, K. A., Klymenko, O. V., & Velliou, E. (2023). A Systematic Quantitative Determination of the Antimicrobial Efficacy of Grape Seed Extract against Foodborne Bacterial Pathogens. *Foods*, *12*(5), 929. https://doi.org/10.3390/foods12050929