Green Synthesis of Copper Nano-particles using leaf extract of Indian medicinal plants and their phytochemical analysis

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Abstract— Due to its cost-effectiveness and eco-friendliness, plant-mediated nano-material production has become more and more popular. An emerging area in nano-technology and nanoscience in former years is the green and environmentally acceptable technique of production for metal nano-particles. The majority of the uses for copper nanoparticles (Cu NPs) are in the fields of medicine, electronics, Biosensors, lubricants, reagents in various methods, and antimicrobial, antifungal, and antimicrobial compounds, among other things. In the current study, copper nitride nanoparticles (Cu NPs) were synthesized in an environmentally friendly and green manner utilizing Murraya koenigii (Curry leaves), Laurus nobilis (Bay leaves), Trigonella foenum graecum (Fenugreek leaves), Curcuma longa (Turmeric) leaf extract and a 1mM copper sulphate (CuSO4.5H2O) solution. Using the use of FTIR and UV-visible spectroscopy, the produced Cu NPs were evaluated. The current study's objective is to look into the phyto screening chemicals found in aqueous curry leaf, with the goal of determining their antibacterial activity. Tannins, saponins, alkaloids, quinones, terpenoids, cardio glycosides, and phenols were found in the aqueous leaf extract, according to the research.

Keywords: Aqueous leaves extract, Characterization, Green Synthesis, Nano Particles, Phyto chemical screening

I. INTRODUCTION

One of the fastest growing branches of science and technology is nanotechnology. This branch covers with the production of different metallic or non-metallic nanostructured materials that differ in structure, material or size [1].

Nanoparticles are particles with a nanometer size between 1 and 100 nm. In comparison to their bulk structure, substances at the nano-scale have new, got better, and unique physical and chemical properties because of their increased surface area per unit volume. Because they are simpler to synthesis, metal nanoparticles are the most researched kind of nanoparticle material [2, 3]. This material can also be used as catalysts and detectors, among other things. Because of their versatility, metal nanoparticles are used extensively in both industry and medicine for a variety of purposes, such as drug delivery, cancer treatment, wastewater treatment, and biosensors that use antibiotics, DNA analysis, solar energy, and catalysis [4, 5].

Green synthesis of metal nanoparticles is proposed as an environmentally friendly and cost-effective an alternative to physical and chemical techniques. The properties of nanomaterials have been shown to be different and better than the macroscopic properties of the same material. This increases the importance of scientific interest in this information. However, the use of expensive reagents, long and tedious procedures, and environmental toxicity are the main problems with this method. On the other hand, green synthesis of metal nanoparticles follows a biological process involving bacteria, yeast, mold, enzymes, waste products, essential oils and plant extracts. Plant-mediated green nanoparticle synthesis is a fast, reliable, economical and environmentally friendly one-step method [5].

Researchers have been interested in Cu nanoparticles lately because of their potential uses in both business and medicine. Copper nanoparticles (Cu NPs) are the most commonly used nanoparticles as they can be easily prepared by reducing copper ions in aqueous copper sulfate solution [3]. The primary process for creating these nanoparticles has been shown to utilize the phytochemicals in the extract acting as reducing agents to lower metal ions in water. Many plants, or entire plants, have been employed for the environmentally friendly synthesis of Cu NPs since plants are rich in bioactive chemicals. Plant extracts have been used effectively for this purpose. Copper nanoparticles are more effective against bacteria than silver nanoparticles, according to research on their antibacterial efficacy using Bacillus subtilis and Escherichia coli. Therefore, this study aims to evaluate the synthesis of green copper nanoparticles (g-Cu NPs) using Indian medicinal plant extracts [6].

I.I. COPPER NANOPARTICLES (CU NPS)

Topper is an alkaline metal used to make copper nanoparticles. Like many other types of nanoparticles, copper nanoparticles can be produced chemically or naturally. They have special needs because they are used not only as biomedical materials, colorants, and also have special antimicrobial properties [7]. Copper nanoparticles have unique properties not found in copper, including catalytic and antifungal/antibacterial activity. Copper nanoparticles are also added to some basic materials used by dentists, such as cement, brackets, adhesives, resins, and some dental implants. The application of copper nanoparticles is shown in figure no. 1

I.II. LEAF PHYTOCHEMICALS

Plants contain chemicals called phytochemicals. (Phyto means "plant" in Greek.) Foods that contain this chemical include fruits, vegetables, whole grains, nuts, seeds, leaves, and legumes. They are responsible for the color, taste and aroma of the plant. Different leaves contain different phytochemicals that may help boost the immune system and prevent cancer [8]. This study used leaf extracts of four different Indian medicinal plants to understand their properties and role in healthcare.

I.II.I. CURRY LEAVES EXTRACT

There is now a need to use herbs as medicine in most of the developing countries as they are not expensive and provide good treatment. Murraya koenigii, also known as curry leaf, is a plant belonging to the Rutaceae family [9]. Gurifolia leaves were used in early times and were also used to season and season foods. These leaves are highly valued for their medicinal properties and aroma. Phytochemicals found in curry leaf extract act as natural antioxidant substances for humans [10].

I.II.II. BAY LEAVES EXTRACT

Laurus nobilis L., also known as laurel or laurel leaf, is an evergreen tree belonging to the Lauraceae family. Scientists have studied the aqueous solution of the leaves for their other beneficial properties, such as cytotoxicity, wound healing, and killing trypanosomes. Previously studied phytochemical analysis of leaves and fruits of L. nobilis has shown that it is good for isolating some plant components such as sesquiterpene lactones, alkaloids, glycosylated flavonoids, as well as monoterpenes and germanium Enol [11].

I.II.III. FENUGREEK LEAVES EXTRACT

In 2017, Zameer et al. conducted research on fenugreek leaves and proved that fenugreek has pleiotropic effects and some biological properties such as antioxidant activity, anti-cancer properties, antibacterial. product and also stomach protector. Trigonella foenum graecum (Family: Leguminosae) (Family: Fabaceae) is used in the treatment of many diseases in traditional medicine due to its anti-inflammatory, antibacterial and antifungal properties [11]. Fenugreek is widely used in humans. Preliminary research in people with type 1 diabetes suggests that consuming fenugreek may play an important role in lowering LDL cholesterol and lowering insulin levels [12].

I.II.IV. TURMERIC EXTRACT

Turmeric, also known as Curcuma longa (Zingiberaceae), is grown in tropical regions around the world. Turmeric is an important herb with many activities such as anti-inflammatory. It is also used to treat wounds and common diseases. It has many properties such as free radical scavenger, anti-protozoal, anti-snake venom, anti-proliferative, anti-angiogenic, anti-tumor, anti-aging, anti-arthritic, anti-Alzheimer's disease, hypoglycemic, and antiulcer properties [13].

I.III. CHARACTERIZATION TECHNIQUES

For the characterization of nanoparticles, the commonly used techniques include UV- Vis spectroscopy, FT-IR.

I.III.I. UV-VIS SPECTROSCOPY

The technique known as UV/Visible Spectroscopy is used to quantify the amount of light that a sample absorbs and scatters (called extinction, defined as the consequent effect of its absorption and scattering) technology (light). In its simplest form, a sample is placed between the light source and the photodetector and the intensity of the UV/visible light is measured before and after passing through the sample [14].

I.III.II. FT-IR

By scanning the sample with infrared light, FTIR analysis is used to identify organic, inorganic, and polymeric components. Changes in the properties of dust particles clearly indicate changes in composition. This method is used only to identify functional groups in specific models. FT-IR spectra were captured between 4000 and 400 cm [15-18]. A corresponding functional groups and spectral range helps to analyse FT-IR band.

II. MATERIALS AND METHODS

II.I. SAMPLE COLLECTION

Fresh leaves (bay leaves), Trigonella foenum graecum (fenugreek leaves), Curcuma longa (turmeric) and Murraya koenigii (Curry leaves) It was purchased from local vegetable vendors in Vadodara, Gujarat [18,19].

II.II. PREPARATION OF LEAF EXTRACT

Examine newly cut leaf samples that have been twice-distilled to get rid of bacteria, after first being cleaned with tap water. Additionally, these leaves are dried for 4-5 days to remove moisture and used for extraction and further use. For extraction preparation, approximately 10 g of each dry and powdered sample is boiled in 150 ml of distilled water for approximately 15-20 minutes and applied to Whattman filter paper No. It is filtered using 1. 1. 1 After cooling. The results were stored at 4°C for later use [3, 19]. Provide appropriate sterile conditions for accuracy of results and extraction of pure content.

II.III. PHYTOCHEMICAL ANALYSIS

Plants produce phytochemicals that insects and other animals eat and use to protect themselves from fungi, bacteria and plant diseases. Four different leaf extracts were prepared for this study and used in various preliminary phytochemical tests [21].

The results obtained after phytochemical analysis were determined to examine the functional groups found in the chemical compounds analyzed by FT-IR.

II.IV. GREEN SYNTHESIS OF COPPER NANOPARTICLES

To prepare Cu NPs, an aqueous solution of 5 mM copper sulfate (CuSO4.5H2O) is first 250 ml you need it ready-made in bottles. To reduce copper ions, add 2 ml of leaf extract to 5 mM copper sulfate solution in a ratio of 2:2 and 2:4, stir constantly and store the solution at room temperature in the dark for 24 hours. A gradual yet noticeable shift in color from light blue to green was noticed, signifying the creation of copper nanoparticles.. The created nanoparticles were also used for characterization by UV–visible spectroscopy and Fourier transform infrared spectroscopy [22, 23].

II.V. CHARACTERIZATION OF COPPER NANOPARTICLES

Characterize composite copper nanoparticles by UV-visible spectroscopy and Fourier transform infrared spectroscopy.

II.V.I. SAMPLE PREPARATION

For UV-visible light analysis and Fourier transform infrared spectroscopy, the synthesized green nanoparticles were collected in different tubes and all of the test tubes were covered with aluminum foil to reduce ions. The tubes were employed for additional characterisation after being shielded from light for one hour at room temperature. Characterization by UV-Vis spectrophotometer by using the Thermo-fisher UV spectrophotometer for UV-visible spectrophotometric analysis with 1 nm resolution between 200 and 600 nm. Metal nanoparticles contain free electrons that give rise to characteristic surface plasmon resonance absorption bands in the UV-visible spectrum [17, 22, 23].

II.VI. CHARACTERIZATION OF COPPER NANOPARTICLES BY FT-IR

FT-IR spectra of all samples were obtained using the Vertex 70 model (Bruker, Germany) for nanoparticles synthesized in the range of 500 -3500 cm-1. Utilizing FT-IR characterization, connections between functional groups connected to nanoparticles were examined [22, 23].

III. RESULTS AND DISCUSSIONS

III.I. SAMPLE COLLECTION

Curry leaves, bay leaves, fenugreek leaves and turmeric leaves were collected as of their high medicinal value, antibacterial, antifungal, antioxidant property.

III.II. PREPARATION OF LEAF EXTRACT

Fig 1. Leaf extract of aqueous solution

As we stated earlier that we have collected four different leaf samples of Indian medicinal plants for the extraction purpose. The visual representation of the extracts obtained after the extract preparation is shown in the figure no. 1

III.III. PHYTOCHEMICAL ANALYSIS

In the present study, phytochemical constituents of aqueous extracts of Curry leaves, Bay leaves, Fenugreek leaves and Turmeric leaves were determined.

The secondary metabolites present in the plants are responsible for the therapeutic potential of the plants. These includes Tannins, Alkaloids, Saponins, Quinones, Terpenoids, Phenols and Cardio glycosides.

The phytochemical analysis results of the aqueous leaf extract showed the following metabolites listed in Table 1, and an overview of the phytochemical analysis results is shown in Figure 2 to 6.

Table no 1. Results of phytochemical analysis

Constituents	extract Presence / absence			
	Curry leaves	Bay leaves	Fenugreek leaf	Turmeric leaf
Tannins	++	++	+	-
Saponins	++	++	++	--
Alkaloids	++	++	++	+
Quinones	--	--	--	++
Terpenoids	++	++	--	++
Phenols	++	++	--	--
Cardio glycosides	++	+	+	--

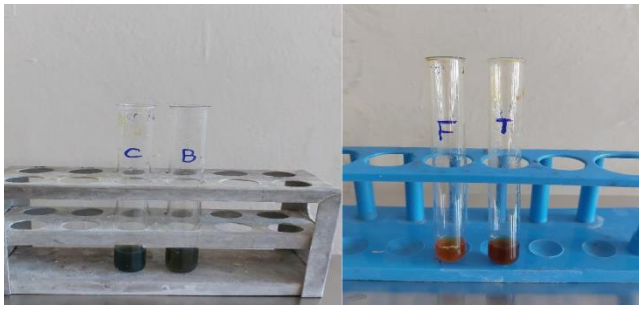

Fig 2. Test for Alkaloids

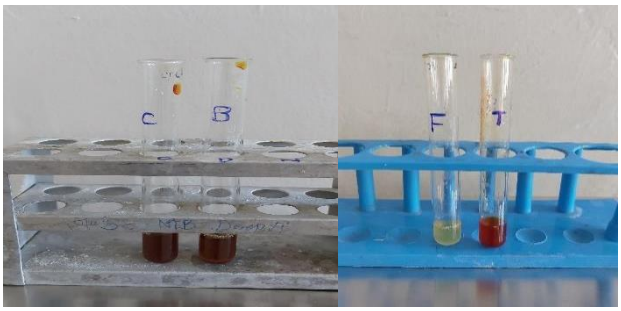

Fig 3. Test for Quinones

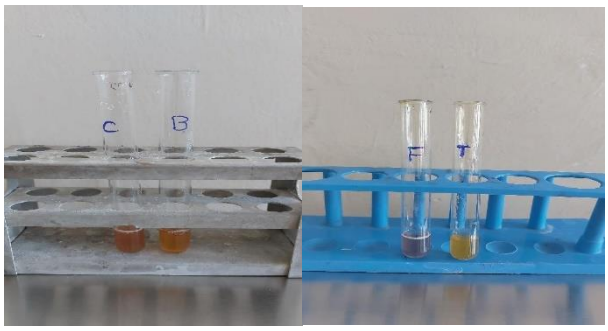

Fig 4. Test for Terpenoids

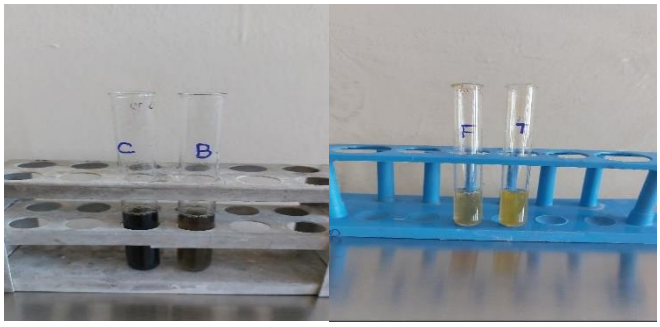

Fig 5. Test for Phenols

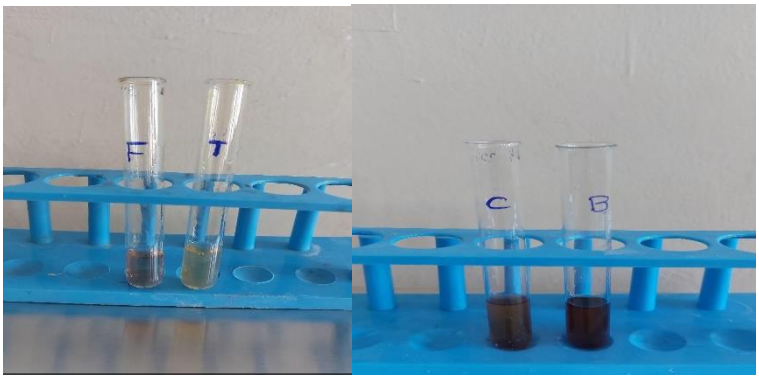

Fig 6. Test for Cardio Glycosides

Out of the seven tested phytochemical constituents, alkaloid is the only constituents present in all the extracted samples. Tannins, saponins and cardio glycosides were present in three extracts except turmeric. Quinones was only present in the turmeric extract. Phenols was present in curry and bay leaf extract but absent in fenugreekand turmeric extract. Lastly terpenoids was present in all three except the fenugreek extract.

III.IV. GREEN SYNTHESIS OF COPPER NANOPARTICLES

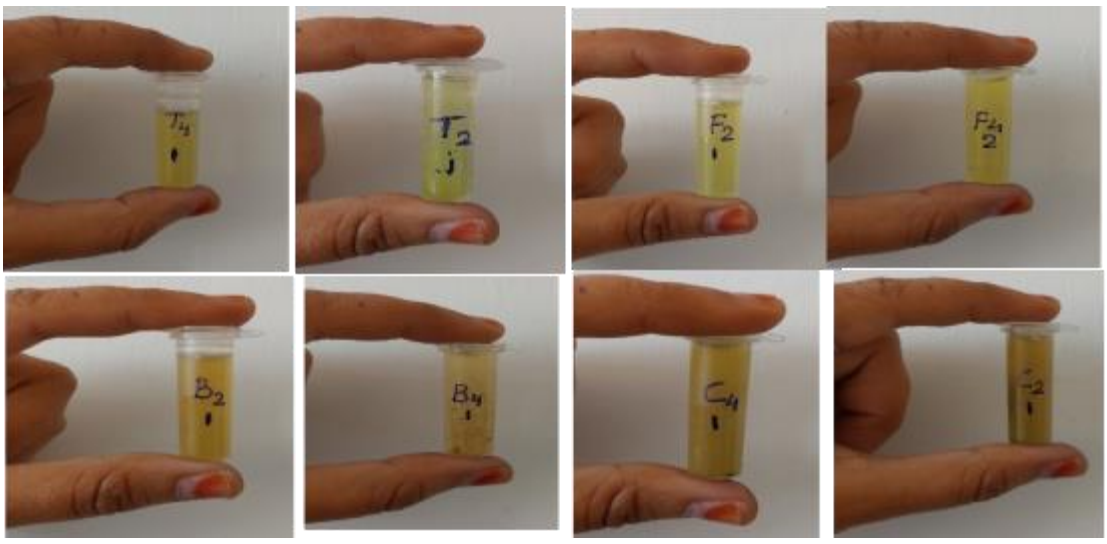

Fig 7. Green Synthesized Copper Nanoparticles

All four leaf extracts were tested and found to have the following phytochemicals: tannins, saponins, alkaloids, quinones, terpenoids, phenols and cardiac glycosides, etc. It is accountable for the synthetic Cu NPs' reduction, capping, and stabilization. The solution was mixed and left in the dark for 24 hours. The blue to green color of copper sulfate confirms the formation of Cu NPs. An overview of the nanoparticles synthesized from four different sheets is shown in Figure 7.

III.V. CHARACTERIZATION OF THE SYNTHESIZED NANOPARTICLES

III.VI. FT-IR ANALYSIS

IR Studies found that potential biomolecules found in leaf extracts are responsible. To reduce and stabilize CuNPs. An image showing the results of the FT-IR analysis is shown in Figure no 8.

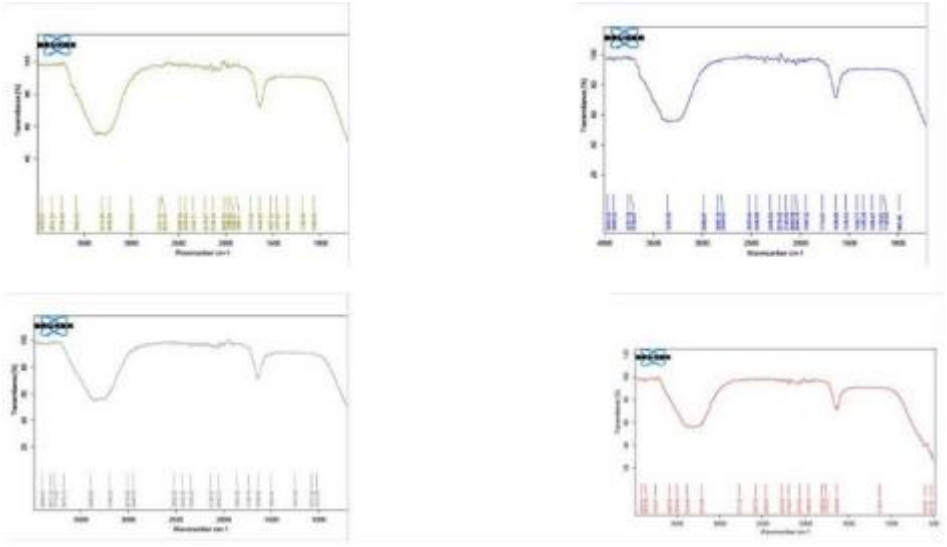

Fig 8. FT-IR images of synthesized Cu NP's

III.IV.I. UV-VIS ANALYSIS

The formation of CuNPs was confirmed by adding sulfate to the aqueous leaf extract to change the color from yellow to green. In addition, the synthesis of Cu NPs was confirmed by the characteristic peaks obtained on the peak, and it was observed that the peak gradually decreased as the material increased. The image shown in Figure no 9 represents the UV-Vis spectrum of the synthesized copper nanoparticles.

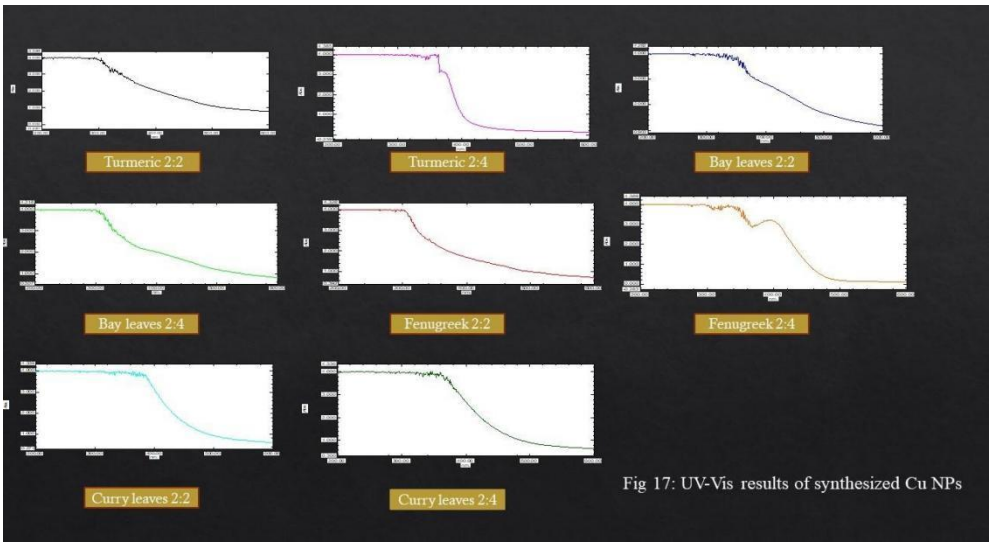

Fig 9. UV-Vis results of synthesized CU NP's

IV. CONCLUSION

In the domains of nanoscience as well as nanotechnology, there is a great need for the creation of a dependable, straightforward, inexpensive, safe, non-toxic, and environmentally friendly method for the production of metal nanoparticles.. Copper nanoparticles (Cu NPs) were successfully obtained from the bio-reduction of the copper sulphate solution using leaves of Murrayakoenigii (Curry leaves), Laurus nobilis (Bay leaves), Curcuma longa (Turmeric) and Trigonella foenum-graecum (Fenugreek leaves). The phytochemical studies showed the presence of common phyto- constituents in the four leaves extracts collected for the synthesis of copper nanoparticles. Due to the different characteristics of these four types of medicinal herbs, the size of the Cu NPs extracted from them also differed. The synthesized Cu NPs were successfully characterized by UV-Vis spectroscopy and FT-IR. FTIR studies and the phytochemical analysis confirmed the green synthesis of Cu NPs in the extract solution via the action of various phytochemicals with different functional groups. The antibacterial activity by Disc diffusion method (Bauer and colleagues) showed the zone of inhibition against the four selected test organisms. This study provided an opportunity to synthesise Cu NPs using natural products, which could be useful in the future for drug formulation, drug-delivery systems, biomedical applications, and so on.

REFERENCES

1. Rehana, D., Mahendiran, D., Kumar, R. S., & Rahiman, A. K. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomedicine & Pharmacotherapy, 89, 1067-1077, 2017.

2. Ashtaputrey, S. D., Ashtaputrey, P. D., & Yelane, N. Green synthesis and characterization of copper nanoparticles derived from Murraya koenigii leaves extract. J. Chem. Pharm. Sci, 10(3), 1288-1291, 2017.

3. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1, 1-10, 2014.

4. Billacura, M. P., & Mimbesa, H. S. 026: leaf extract mediated green synthesis of silver nanoparticles from widely available Wedelia trilobata: synthesis, partial characterization and antimicrobial property analysis. BMJ Open, 5(Suppl 1), bmjopen2015.

5. Aritonang, H. F., Koleangan, H., & Wuntu, A. D. Synthesis of silver nanoparticles using aqueous extract of medicinal plants'(Impatiens balsamina and Lantana camara) fresh leaves and analysis of antimicrobial activity. International journal of microbiology, 2019.

6. Chandraker, S. K., Lal, M., Ghosh, M. K., Tiwari, V., Ghorai, T. K., & Shukla, R. Green synthesis of copper nanoparticles using leaf extract of Ageratum houstonianum Mill. and study of their photocatalytic and antibacterial activities. Nano Express, 1(1), 010033, 2020.

7. Rashmi, J. B., & Naveen, G. Phytochemical analysis and antibacterial activity of different leaf extracts of Murraya koenigii. IJBB, 1, 5, 2016.

8. Deepika, T., & Noorjahan, C. M. Phytochemical Screening and Antioxidant Property of Aqueous Murraya Koenigii (Curry Leaf) Extract. Int J Res Advent Technol, 7(2), 738-43, 2019.

9. Padma, P. N., Banu, S. T., & Kumari, S. C. Studies on green synthesis of copper nanoparticles using Punica granatum. Annual Research & Review in Biology, 1- 10, 2018.

10. Soshnikova, V., Kim, Y. J., Singh, P., Huo, Y., Markus, J., Ahn, S., & Yang, D. C. Cardamom fruits as a green resource for facile synthesis of gold and silver nanoparticles and their biological applications. Artificial cells, nanomedicine, and biotechnology, 46(1), 108-117, 2018.

11. Kumar, N., Ahmad, A. H., Singh, S. P., Pant, D., Prasad, A., & Rastogi, S. K. Phytochemical analysis and antioxidant activity of Trigonella foenum-graecum seeds. Journal of Pharmacognosy and Phytochemistry, 10(1), 23-26, 2021.

12. Amaliyah, S., Pangesti, D. P., Masruri, M., Sabarudin, A., & Sumitro, S. B. Green synthesis and characterization of copper nanoparticles using Piper retrofractum Vahl extract as bioreductor and capping agent. Heliyon, 6(8), e04636, 2020.

13. Kumar, H., Bhardwaj, K., Dhanjal, D. S., Nepovimova, E., Şen, F., Regassa, H., & Kuča, K. Fruit extract mediated green synthesis of metallic nanoparticles: A new avenue in pomology applications. International journal of molecular sciences, 21(22), 8458, 2020

14. Nordin, N. R., & Shamsuddin, M. Biosynthesis of copper (II) oxide nanoparticles using Murayya koeniggi aqueous leaf extract and its catalytic activity in 4-nitrophenol reduction. Malaysian Journal of Fundamental and Applied Sciences, 15, 218-224, 2019.

15. Gavade, S. M., Nikam, G. H., Dhabbe, R. S., Sabale, S. R., Tamhankar, B. V., & Mulik, G. N. Green synthesis of silver nanoparticles by using carambola fruit extract and their antibacterial activity. Advances in Natural Sciences: Nanoscience and Nanotechnology, 6(4), 045015, 2015.

16. Elia, P., Zach, R., Hazan, S., Kolusheva, S., Porat, Z. E., & Zeiri, Y. Green synthesis of gold nanoparticles using plant extracts as reducing agents. International journal of nanomedicine, 9, 4007, 2014.

17. Teimuri-Mofrad, R., Hadi, R., Tahmasebi, B., Farhoudian, S., Mehravar, M., & Nasiri, R. Green synthesis of gold nanoparticles using plant extract: Minireview. Nanochemistry Research, 2(1), 8-19, 2017.

18. Soshnikova, V., Kim, Y. J., Singh, P., Huo, Y., Markus, J., Ahn, S., ... & Yang, D. C. Cardamom fruits as a green resource for facile synthesis of gold and silver nanoparticles and their biological applications. Artificial cells, nanomedicine, and biotechnology, 46(1), 108-117, 2018.

19. Khanal, L. N., Sharma, K. R., Paudyal, H., Parajuli, K., Dahal, B., Ganga, G. C., ... & Kalauni, S. K. Green synthesis of silver nanoparticles from root extracts of Rubus ellipticus Sm. and comparison of antioxidant and antibacterial activity. Journal of Nanomaterials, 2022, 1-11, 2022.

20. AL Qahtani, F. S., AlShebly, M. M., Govindarajan, M., Senthilmurugan, S., Vijayan, P., & Benelli, G. Green and facile biosynthesis of silver nanocomposites using the aqueous extract of Rubus ellipticus leaves: toxicity and oviposition deterrent activity against Zika virus, malaria and filariasis mosquito vectors. Journal of Asia-Pacific Entomology, 20(1), 157-164, 2017.

21. Mali, S. C., Dhaka, A., Githala, C. K., & Trivedi, R. Green synthesis of copper nanoparticles using Celastrus paniculatus Wild. leaf extract and their photocatalytic and antifungal properties. Biotechnology Reports, 27, e00518, 2020.

22. Murthy, H. C., Desalegn, T., Kassa, M., Abebe, B., & Assefa, T. Synthesis of green copper nanoparticles using medicinal plant Hagenia abyssinica (Brace) JF. Gmel. leaf extract: Antimicrobial properties. Journal of nanomaterials, 2020.

23. Jayarambabu, N., Akshaykranth, A., Rao, T. V., Rao, K. V., & Kumar, R. R. Green synthesis of Cu nanoparticles using Curcuma longa extract and their application in antimicrobial activity. Materials Letters, 259, 126813, 2020.