Green Adeptness in the Synthesis and Stabilization of Copper Nanoparticles: Catalytic, Antibacterial, Cytotoxicity, and Antioxidant Activities

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Abstract
Copper nanoparticles (CuNPs) are of great interest due to their extraordinary properties such as high surface-to-volume ratio, high yield strength, ductility, hardness, flexibility, and rigidity. CuNPs show catalytic, antibacterial, antioxidant, and antifungal activities along with cytotoxicity and anticancer properties in many different applications. Many physical and chemical methods have been used to synthesize nanoparticles including laser ablation, microwave-assisted process, sol-gel, co-precipitation, pulsed wire discharge, vacuum vapor deposition, high-energy irradiation, lithography, mechanical milling, photochemical reduction, electrochemistry, electrospay synthesis, hydrothermal reaction, microemulsion, and chemical reduction. Phytosynthesis of nanoparticles has been suggested as a valuable alternative to physical and chemical methods due to low cytotoxicity, economic prospects, environment-friendly, enhanced biocompatibility, and high antioxidant and antimicrobial activities. The review explains characterization techniques, their main role, limitations, and sensitivity used in the preparation of CuNPs. An overview of techniques used in the synthesis of CuNPs, synthesis procedure, reaction parameters which affect the properties of synthesized CuNPs, and a screening analysis which is used to identify phytochemicals in different plants is presented from the recent published literature which has been reviewed and summarized. Hypothetical mechanisms of reduction of the copper ion by quercetin, stabilization of copper nanoparticles by santin, antimicrobial activity, and reduction of 4-nitrophenol with diagrammatic illustrations are given. The main purpose of this review was to summarize the data of plants used for the synthesis of CuNPs and open a new pathway for researchers to investigate those plants which have not been used in the past.

Keywords: Phytosynthesis, Copper nanoparticles, Phytochemicals, Cytotoxicity, Catalytic activity, Antibacterial activity

Background
Nanoparticles (NPs) have a number of interesting applications in the industrial field such as space technology, magnetism, optoelectronics and electronics, cosmetics, and catalytic, pharmaceutical, biomedical, environmental, and energy applications [1, 2]. The extraordinary properties of NPs such as ductility, high yield strength, hardness, flexibility, rigidity, high surface-to-volume ratio, macroquantum tunneling effect, and quantum size are attributable as compared to properties of bulk materials having the same chemical composition [3]. Indeed, the properties of NPs, which may considerably differ from those observed for fine particles, are higher specific surface area, specific optical properties, lower melting points, specific magnetizations, mechanical strength, and numerous industrial applications [4]. Copper nanoparticles (CuNPs) are of great interest due to easy availability, low cost, and their similar properties to those of noble metals [5–9]. CuNPs can also be used in sensors, heat transfer systems [10–12], and electronics (fuel cell and solar cell), as catalysts in many reactions and as bactericidal and antimicrobial agents used to coat hospital equipment [13–19].

Many physical and chemical methods including laser ablation [20], microwave-assisted process, sol-gel [21],...
co-precipitation [22], pulsed wire discharge [23], vacuum vapor deposition [24], high-energy irradiation [25], lithography [26], mechanical milling [27], photochemical reduction, electrochemistry [28–32], electrospray synthesis [33], hydrothermal reaction [34], microemulsion [35], and chemical reduction are used to synthesize nanoparticles. Although physical and chemical methods produce well-defined and pure nanoparticles, these methods are neither cost-effective nor eco-friendly due to the use of toxic chemicals. One of the most important criteria of nanotechnology is the development of eco-friendly, nontoxic, and clean green chemistry procedures [36]. Hence, biosynthesis of nanoparticles contains a green chemistry-based method which employs different biological bodies such as plants [37, 38], actinomycetes [39, 40], fungus [41–44], bacteria [45–49], yeast [50–52], and viruses [53, 54]. Biological entities offer a nontoxic, clean, and environment-friendly approach to synthesize the NPs with a wide range of size, physicochemical properties, shapes, and compositions [55].

Copper nanoparticles were synthesized and stabilized in the literature by using different plants such as Euphorbia esula [56], Panica granatum [57], Ocimum sanctum [58], Ginkgo biloba [59], Calotropis procera [60], Lawsonia inermis [61], Citrus medica [62], Camellia sinensis [63], Datura innoxia [64], Syzygium aromaticum [65], Sesamum indicum [66], Citrus limon, Turmeric curcumin [67], Gloriosa superba L. [68], Ficus carica [69], Aegle marmelos [70], Caesalpinia pulcherrima [71], Cassia fistula [72], Leucas aspera, Leucas chinensis [73], Delonix elata [74], Aloe barbadensis Miller [75], Thymus vulgaris [76], Phyllanthus emblica [77], Magnolia kobus [78], Eucalyptus [79], Artabotrys odoratisissimus [80], Capparis zeylanica [81], Vitis vinifera [82], Hibiscus rosa-sinensis [83], Zingiber officinale [84], Datura metel [85], Zea mays [86], Urtica, Matricaria chamomilla, Glycyrrhiza glabra, Schisandra chinensis, Inula helenium, Cinnamomum [87], Dodonaea viscosa [88], Cassia auriculata [89], Azadirachta indica, Lantana camera, Tridax procumbens [90], Allium sativum [91], Asparagus adscendens, Bacopa monnieri, Ocimum basilicum, Withania somnifera [92], Smithia sensitiva, Colocasia esculenta [93], Nerium oleander [94], and Psidium guajava [95]; by using different algae/fungi such as Phaeophyceae [96], Stereum hirsutum [97], and Hypocrea lixii [98]; and by using some microorganisms such as Pseudomonas fluorescens [99] and Enterococcus faecalis [100] cultures.

**Biosynthesis of Copper Nanoparticles**

**Parts of Plant Used for Extract**

Different parts of plants are used for the preparation of plant extracts such as leaves, seeds, barks, fruits, peel, coir, roots, and gum. Leaves and roots are used in two ways. Firstly, fresh leaves and roots are used for the preparation of plant extracts, and secondly, dry leaves and roots in powder form are used.

**Procedure for the Synthesis of CuNPs**

For the synthesis of CuNPs, plant extract was prepared by using different parts of different plants. For synthesis of the extract part of the plant of interest, leaves are collected and washed with tap water and then with distilled water to remove dust particles. The washed leaves are used further in two ways. First, these leaves are sun dried for 1–2 h to remove the residual moisture. Known weights of these sun-dried leaves are divided into small parts and soaked in deionized water or ethanol solution. This mixture is stirred for 24 h at room temperature by using a magnetic stirrer and then filtered for further use. Second, these leaves are sun dried for 4–7 days or dried in an oven at 50 °C for 1 day and powdered using a domestic blender. Known weight of plant powder is mixed in water or ethanol solution and then stirred and filtered.

For the synthesis of CuNPs, aqueous solution of precursor salts such as copper sulfate, copper chloride, copper acetate, and copper nitrate with different concentrations is mixed with plant extract. Aqueous solution of sodium hydroxide is also prepared and added to the reaction mixture to control the pH medium. The reaction mixture is strongly shaken for different time intervals in an electric shaker and heated in an oven at different time intervals and at different temperatures. The formation of CuNPs can also take place at room temperature and is confirmed by changing the color of the reaction mixture. At the end, nanoparticles were centrifuged and dried at different temperatures. Reaction optimizations take place by changing the pH of the mixture, concentration of precursor salt, heat, and temperature of reaction mixture. In the literature, different plants have been used for the formation of copper nanoparticles by using different precursor salts with different reaction conditions as shown in Table 1. From the table, it can be seen that the different reaction conditions affect the shape and size of copper nanoparticles.

**Effect of Reaction Parameters on Properties of NPs**

The concentration of plant extract plays a main role in reducing and stabilizing the CuNPs. It has been reported that by increasing the concentration of plant extract, the number of particles increased [88]. By increasing the concentration of plant extract, the concentration of phytochemicals increased and the reduction of copper salt also increased. Due to the fast reduction of the metal salt, the size of the nanoparticles also decreased [101].

The size and structure of CuNPs are highly affected by the copper salt. The morphology of nanoparticles changes when the salt (e.g., copper chloride, copper acetate, copper nitrate, or copper sulfate) is used in the
| Plants                  | Part of plant | Active compounds in plant                                                                 | Precursor salt     | Concentration of salt | Reaction conditions                                                                 | Characterization                        | Size       | Shape                  | References |
|------------------------|---------------|------------------------------------------------------------------------------------------|--------------------|-----------------------|-------------------------------------------------------------------------------------|------------------------------------------|------------|------------------------|------------|
| Euphorbia esula        | Leaves        | Flavonoids and phenolic acids                                                             | Copper chloride    | 5 mM                  | Temp 120 °C, pH 9, time 20 min                                                        | UV, FTIR, XRD, TEM                     | 20–110 nm  | Spherical              | [56]       |
| Punica granatum        | Peels         | –                                                                                        | Copper sulfate     | 50 mM                 | Temp 80 °C for 10 min and 40 °C for 4 h                                              | UV, FTIR, PSA, TEM                     | 15–20 nm   | Spherical              | [57]       |
| Ocimum sanctum         | Leaves        | Terpenoids, alcohols, ketones, esters, aldehydes, and carboxylic acids                    | Copper sulfate     | 1 mM                  | Room temp                                                                       | UV, FTIR, PSA, TEM, MZS                | 25 nm      | Rod, cylindrical, elliptical | [58]       |
| Euphorbia esula        | Leaves        | Flavonoids and phenolic acids                                                             | Copper chloride    | 1 mM                  | Room temp                                                                       | UV, FTIR, EDX, SEM                     | 150–200 nm | Spherical              | [115]      |
| Ginkgo biloba          | Leaves        | Polyphenols, quercetin                                                                    | Copper chloride    | 5 mM                  | Temp 80 °C, pH 9, time 30 min                                                        | UV, FTIR, EDS, TEM                     | 15–20 nm   | Spherical              | [59]       |
| Calatrops procera      | Latex         | Cysteine proteases                                                                        | Copper acetate     | 3 mM                  | Room temp                                                                       | UV, FTIR, XRD, TEM, EDAX               | 15 ± 1.7 nm | Spherical              | [60]       |
| Lawsonia inermis       | Leaves        | –                                                                                        | Copper sulfate     | 10 mM                 | Temp 100 °C, pH 11, time 30 min                                                      | UV, FTIR, HRTEM, SEM, DMOM             | –          | –                      | [61]       |
| Citrus medicinal        | Fruit juice   | Ascorbic acid, saponins, and flavonoids                                                  | Copper sulfate     | 100 mM                | Temp 60–100 °C                                                                     | UV, FTIR, NTA, XRD                     | 33 nm      | –                      | [62]       |
| Camella sinensis       | Leaves        | Flavonoids, phenolic acids, terpenoids, and polysaccharides                              | Copper chloride    | 1 mM                  | Temp 100 °C, time 3 h                                                               | UV, FTIR, EDX, TEM, SEM                | 15–25 nm   | Spherical              | [63]       |
| Ocimum sanctum         | Leaves        | –                                                                                        | Copper sulfate     | 10 mM                 | Temp 90 °C                                                                        | FTIR, EDX, TEM, SEM, XRD, NTA          | 10–40 nm   | Spherical              | [104]      |
| Datura innoxia         | Leaves        | –                                                                                        | Copper sulfate     | 1 mM                  | –                                                                                | UV, FTIR, EDX, FESEM                   | 90–200 nm  | Spherical              | [64]       |
| Syzygium aromaticum    | Flowers       | Eugenol                                                                                  | Copper sulfate     | 1 mM                  | Room temp, pH 3.43                                                                  | UV, FTIR, XRD, TEM, SEM                | 5–40 nm    | –                      | [65]       |
| Sesamum indicum        | Seeds         | –                                                                                        | Copper sulfate     | 10 mM                 | –                                                                                | UV                                        | –          | –                      | [66]       |
| Citrus limon and Turmeric curcinin | Fruit | Curcumonilineazomethine                                                                | Copper chloride    | 1 mM                  | –                                                                                | UV, FTIR, XRD, HRTEM, SEM              | 60–100 nm  | Spherical              | [67]       |
| Gloriosa superba L.    | Leaves        | –                                                                                        | Copper sulfate     | 1 mM                  | Room temp                                                                       | UV, FTIR                                | –          | –                      | [68]       |
| Gossypium              | Gum           | Hydroxyl, acetyl, carbonyl, and carboxylic groups                                        | Copper sulfate     | 10 mM                 | Room temp, pH 12                                                                  | TEM, SAXS, UV, XRD                      | 19 nm      | Spherical              | [116]      |
| Ficus carica           | Leaves        | –                                                                                        | Copper chloride    | 10 mM                 | Temp 25 °C, pH 8, time 30 min                                                       | UV, SEM, XRD                            | 50–120 nm  | –                      | [69]       |
| Aegle marmelos         | Leaves        | Polyphenols, alkenoids, phenylpropanoid, and terpenoids                                  | Copper chloride    | 1 mM                  | –                                                                                | UV, FTIR, XRD                           | 48 nm      | Spherical              | [70]       |
| Caesalpina pulcherima  | Flowers       | –                                                                                        | Copper nitrate     | 1 mM                  | –                                                                                | UV, FTIR, XRD, SEM, EDAX               | 18–20 nm   | Spherical              | [71]       |
| Cassia fistula         | Flowers       | –                                                                                        | Copper nitrate     | 1 mM                  | Room temp                                                                       | UV, FTIR, XRD, SEM                      | 20 nm      | –                      | [72]       |
Table 1: Data for synthesis of copper nanoparticles under different reaction conditions (Continued)

| Plants | Part of plant | Active compounds in plant | Precursor salt | Concentration of salt | Characterization | Size | Shape | References |
|--------|---------------|---------------------------|----------------|-----------------------|------------------|------|-------|------------|
| Leucas aspera | Leaves | – | Copper sulfate | 1 mM | UV | – | – | [73] |
| Leucas chinensis | Leaves | – | Copper sulfate | 1 mM | XRD, FESEM, EDX | 60.23 nm | – | [117] |
| Delonix elata | Flowers | – | Copper sulfate | 1 mM | UV, FTIR, XRD, SEM | 20 | – | [74] |
| Aloe barbadensis Miller | Flowers | – | Copper acetate | 5 mM | Temp 50 °C, time 30 min | UV, FTIR, FESEM | 40 nm | Spherical | [75] |
| Thymus vulgaris | Leaves | – | Copper sulfate | 0.2 M | Temp 80 °C, time 4 h | BET, TEM, SAED, FTIR, XRD, XRF, FESEM, EDS | – | – | [76] |
| Phyllanthus emblica | Fruit | Tannin, saponin, flavonoid, alkaloid, quinone, anthraquinone, anthocyanosides, phenols | Copper sulfate | 20 mM | Temp 60–80 °C, pH 10 | UV, FTIR, XRD, SEM, EDAX | 15–30 nm | Flakes | [77] |
| Magnolia kobus | Leaves | – | Copper sulfate | 1 mM | Temp 25–95 °C | ICP, EDS, XPS, SEM, HRTEM | 40–100 nm | Spherical | [78] |
| Eucalyptus | Leaves | Flavonoids and phenolic acids | Copper sulfate | 1 mM | UV, FTIR, XRD | 38.62 nm | – | [79] |
| Artabotrys odoratissimus | Leaves | – | Copper sulfate | 1 mM | Temp 95 °C | PSA | 35 nm | – | [80] |
| Capparis zeylanica | Leaves | – | Copper sulfate | – | UV, FTIR, SEM, EDX, XRD, TEM | 50–100 nm | Cubical | [81] |
| Vitis vinifera | Leaves | – | Copper sulfate | 1% | UV, FTIR, XRD | 3–6 nm | – | [82] |
| Hibiscus rosa-sinensis | Leaves | Polyphenols, flavonoids, proteins, lignins, xanthones | Copper nitrate | 50 mM | UV, FTIR, TEM | – | – | [83] |
| Zingiber officinalis | – | – | – | – | FTIR, XRD, EDX, TEM, SAED | 10.13 nm | Cubical | [84] |
| Datura metel | Leaves | Alkaloids, terpenoids, and phenolic groups | – | – | Time 10 min | UV, PSA, TEM, EDX, FTIR | – | – | [85] |
| Zea mays | Leaves | – | Copper sulfate | 10 mM | Room temp, time 1 h | UV, XRD, EDAX, FTIR | 40 nm | Mixed | [86] |
| Urtica | Leaves | Flavonoids, quercetin, rutin, morin | Copper sulfate | – | Temp 70 °C | UV, SEM, XRD | 6.5 nm | – | [87] |
| Matricaria chamomilla | Leaves | Flavonoids | Copper sulfate | – | Temp 70 °C | UV, SEM, XRD | 58.77 nm | – | [87] |
| Glycyrrhiza glabra | Leaves | Flavonoids | Copper sulfate | – | Temp 70 °C | UV, SEM, XRD | 28.21 nm | – | [87] |
| Schisandra chinensis | Leaves | Quercetin, rutin, morin | Copper sulfate | – | Temp 70 °C | UV, SEM, XRD | 32 nm | – | [87] |
| Plants          | Part of plant | Active compounds in plant | Precursor salt | Concentration of salt | Reaction conditions | Characterization | Size       | Shape     | References |
|-----------------|---------------|----------------------------|----------------|-----------------------|--------------------|-----------------|------------|-----------|------------|
| Inula helenium  | Leaves        | Flavonoids                 | Copper sulfate | –                     | Temp 70 °C         | UV, SEM, XRD     | 32.41 nm  | –         | [87]       |
| Cinnamomum      | Leaves        | Flavonoids                 | Copper sulfate | –                     | Temp 70 °C         | UV, SEM, XRD     | 48.8 nm   | –         | [87]       |
| Dodonaea viscosa| Leaves        | Santin, penduletin, alizarin, pinocembrin, tannins, saponins | Copper chloride | 1 mM                  | Temp 50 °C, pH 10  | UV, XRD, AFM, HRTEM, SAED | 30–40 nm  | Spherical | [88]       |
| Cassia auriculata| Leaves       | –                          | Copper sulfate | 1 mM                  | –                  | FESEM, XRD, FTIR | 38–43 nm  | Spherical | [89]       |
| Azadirachta indica| Leaves      | –                          | Fehling solution | –                   | –                  | UV              | –         | –         | [90]       |
| Lantana camera  | Leaves        | –                          | Fehling solution | –                   | –                  | UV              | –         | –         | [90]       |
| Tridax procumbens| Leaves      | –                          | Fehling solution | –                   | –                  | UV              | –         | –         | [90]       |
| Allium sativum  | –             | Copper sulfate             | 10 mM          | –                     | UV, FTIR, SEM, XRD, TEM | 100 nm   | Spherical | [91]       |
| Asparagus adscendens| Leaves    | copper sulfate             | 1 mM           | –                     | UV, FTIR, TEM, SAED | 10–15 nm | Spherical | [92]       |
| Bacopa monnieri | Leaves        | copper sulfate             | 1 mM           | –                     | UV, FTIR, TEM, SAED | 50–60 nm | Spherical | [92]       |
| Ocimum basilicum| Leaves        | copper sulfate             | 1 mM           | –                     | UV, FTIR, TEM, SAED | 40–60 nm | Spherical | [92]       |
| Withania somnifera| Leaves      | copper sulfate             | 1 mM           | –                     | UV, FTIR, TEM, SAED | 50–60 nm | Mixed     | [92]       |
| Smithia sensitiva| Leaves       | Tannin, saponin, flavonoid, antraquinone glycoside, steroids | Copper sulfate | 1 mM                  | –                  | UV, FTIR, SEM, NTA | 136 nm    | –         | [93]       |
|                | Leaves        | Tannin, saponin, flavonoid, antraquinone glycoside, steroids | Copper acetate | 1%                   | –                  | UV, FTIR, SEM, NTA | 50 nm     | –         | [93]       |
| Colocasia esculenta| Leaves      | Tannin, flavonoid, alkaloid, cardiac glycoside, terpenoids, phenols | Copper sulfate | 1 mM                  | –                  | UV, FTIR, SEM, NTA | 57 nm     | –         | [93]       |
|                | Leaves        | Tannin, flavonoid, alkaloid, cardiac glycoside, terpenoids, phenols | Copper acetate | 1%                   | –                  | UV, FTIR, SEM, NTA | 44 nm     | –         | [93]       |
| Nerium oleander | Leaves        | Copper sulfate             | 1 mM           | –                     | UV, FTIR           | –               | –         | –         | [94]       |
| Psidium guajava | Fruit         | Flavonoid, alkaloid, steroids, glycoside, terpenoids, phenols | Copper sulfate | 20 mM                 | Room temp, pH 10   | UV, FTIR, XRD, EDAX, TEM, SEM | 15–30 nm  | Flakes    | [95]       |
presence of sodium hydroxide. It was reported that the shape was triangular and tetrahedron in the case of copper chloride, rod-shaped in the case of copper acetate, and spherical in the case of copper sulfate [102]. By increasing the concentration of the precursor salt, the size of the CuNPs also increased.

The synthesis of CuNPs gives best results by varying the pH of the reaction medium within the preferred range. The size of nanoparticles was controlled by changing the pH value of the reaction mixture. At higher pH, smaller-sized nanoparticles were obtained compared to those obtained at low pH value. This difference can be attributed to the difference in reduction rate of the metal salts by plant extract. The inverse relation between the value of pH and the size of nanoparticle showed that an increase in pH value enables us to obtain small-sized spherical nanoparticles while a decrease in pH value gives large-sized (rod-shaped and triangular) nanoparticles. The effect on absorption spectra of different values of pH (4, 6, 8, 10, and 12) is represented in Fig. 1 [36]. It was reported that the addition of plant extract to CuCl₂ did not lead to the formation of CuNPs but, instead, the CuNPs were obtained by changing the pH of the reaction mixture to basic medium. The same behavior was observed by Wu and Chen, and it was concluded that pH plays an important role in the synthesis of CuNPs [103].

Mechanism for Phytosynthesis of Copper Nanoparticles

Phytochemical Screening: a Qualitative Analysis

Phytochemical screening analysis is a chemical analysis carried out for the detection of phytochemicals in different plants. Fresh plant extract with chemicals or chemical reagents is used for this analysis [77] as shown in Table 2.

Phytochemicals for Reduction of Metal and Stabilizing the NPs

Green synthesis of CuNPs by the use of phytochemicals offers more flexible control over the shape and size of the NPs (i.e., by changing reaction temperature, concentration of plant extract, metal salt concentration, reaction time, and pH of reaction mixture). Color change of the reaction medium indicates reduction of the metal ion and formation of NPs. The green reduction of the copper salts starts instantly, and the formation of copper nanoparticles is indicated by the color change of the reaction mixture. Phytochemicals have a main role in first reducing the metal ions and then stabilizing the metal's nuclei in the form of nanoparticles as shown in Fig. 2. The interaction of phytochemicals with metal ions and the concentration of these phytochemicals control the shape and size of CuNPs.

Flavonoids contain polyphenolic compounds, e.g., quercetin, catechins, flavanones, isoflavones, santin, pendeutin, alizarin, pinocembrin, anthocyanins, flavones, tannins, and saponins, which are present in different plants such as *Ginkgo biloba* [59], *Citrus medicalinn* [62], *Phyllanthus emblica* [77], *Hibiscus rosa-sinensis* [83], and *Dodonaea viscosa* [93]. These compounds play a main role in reducing and chelating the metal. Various functional groups present in the flavonoids are responsible for the reduction of the copper ion. It has been assumed that a reactive hydrogen atom in the flavonoids may be released during the tautomeric alterations of the enol form to the keto form which can reduce copper ions to form copper nuclei or CuNPs. For example, it is assumed that in the case of *Ginkgo biloba* plant extracts, it is the transformation of quercetin (flavonoid) which plays a main role in the reduction of copper metal ions into copper nuclei or CuNPs due to the change of enol form to keto form as shown in Fig. 3.

During the synthesis process of CuNPs, metal ions with monovalent or divalent oxidation states are converted into zero-oxidation copper nuclei and these nuclei are merged to obtain different shapes. During the nucleation, nuclei aggregate to form different shapes such as wires, spheres, cubes, rods, triangles, pentagons, and hexagons. Some flavonoids have an ability to chelate the CuNPs with their π electrons and carbonyl groups. Quercetin and santin are flavonoids with strong chelating activity due to the presence of two functional groups involving the hydroxyls and carbonyls. These groups chelate with copper nanoparticles by following the previous mechanism and also explain the ability of adsorption of santin (flavonoid) on the surface of CuNPs as shown in Fig. 4.

It was assumed that the protein molecules (superoxide dismutase, catalase, glutathione) in different plants such as *Hibiscus rosa-sinensis* [83] and *Camellia sinensis* [104] display a high reducing activity for the formation...
of nanoparticles from metal ions but their chelating activity is not excessive. Sugars such as monosaccharides (glucose), disaccharides (maltose and lactose), and polysaccharides in *Camellia sinensis* plant [63] can act as reducing agents or antioxidants and have a series of tautomeric transformations from ketone to aldehyde.

Other phytochemicals such as polyphenols (e.g., ellagic acid and gallic acid) which are present in *Hibiscus rosa-sinensis* [40], phenylpropanoids (phenylalanine, tyrosine) in *Aegle marmelos* [70], terpenoids in *Ocimum sanctum* and *Asparagus adscendens* [58, 92], cysteine proteases in *Calotropis procera* [60], curcininanilineazomethine in *Turmeric curcumin* [67], ascorbic acid in *Citrus medica-linn* [62], eugenol in *Syzygium aromaticum* [65], and alkaloids in *Aegle marmelos* [70] play the same role of reducing the copper ions and stabilizing the copper nanoparticles. Carbohydrates, anthraquinone, quinone, and anthocyanoside in *Phyllanthus emblica* [77]; lignins and xanthones in *Hibiscus rosa-sinensis* [83]; and cardiac glycoside, triterponoid, carotenoid glycoside, and anthraquinone glycoside in *Colocasia esculenta* plant [93] are also phytochemicals which are present in extracts of different plants and act as reducing and stabilizing agents. Examples of certain phytochemicals with structures are shown in Fig. 5.

### Characterization Techniques

For characterization of synthesized nanoparticles, different techniques were used such as ultraviolet-visible spectroscopy (UV-vis), transmission electron microscopy (TEM), small-angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy (FTIR), X-ray fluorescence spectroscopy (XRF), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), scanning

#### Table 2 Phytochemical screening analysis

| Test for phytochemicals | Amount of plant extract | Chemicals used | End point for confirmation of phytochemical |
|-------------------------|-------------------------|----------------|--------------------------------------------|
| Carbohydrate            | 2 mL                    | Few drops of concentrated sulfuric acid and 1 mL of Molisch’s reagent | Reddish or purple color |
| Tannins                 | 2 mL                    | 4 mL of 5% ferric chloride | Greenish black or dark blue color |
| Saponins                | 2 mL                    | 2 mL of distilled water and shake for 15 min | Layer of foam on surface |
| Flavonoids              | 2 mL                    | 1 mL of 2 N sodium hydroxide | Yellow color |
| Alkaloids               | 2 mL                    | Few drops of Mayer’s reagent and 2 mL of concentrated HCl | White precipitate or green color |
| Anthraquinone           | 1 mL                    | Few drops of 10% ammonia solution | Pink color precipitates |
| Anthocyanosides         | 1 mL of filtrate        | 5 mL HCl | Pale pink color |

![Fig. 2](image-url) A protocol for reducing the metal ions and then stabilizing the metal’s nuclei.
electron microscopy (SEM), field emission scanning electron microscopy (FESEM), particle size analysis (PSA), Malvern Zetasizer (MZS), energy-dispersive X-ray spectroscopy (EDX/EDS), nanoparticle tracking analysis (NTA), X-ray reflectometry (XRR), Brunauer-Emmett-Teller analysis (BET), selected area electron diffraction (SAED), and atomic force microscopy (AFM) (Table 3).

Applications of Copper Nanoparticles

Due to their outstanding chemical and physical properties, large surface-to-volume ratio, constantly renewable surface, low cost, and nontoxic preparation, CuNPs have been of great interest for applications in different fields. Copper nanoparticles show catalytic activity, antibacterial activity, cytotoxicity or anticancer activity, antioxidant activity, and antifungal activity in different applications. In catalytic activity, copper nanoparticles are used for the Huisgen [3 + 2] cycloaddition of alkynes and azides in many solvents under ligand-free conditions [59], 1-methyl-3-phenoxy benzene, 3,3-oxybis(methyl-benzene) [94], synthesis of 1-substituted 1H-1,2,3,4-tetrazole [76], adsorption of nitrogen dioxide, and adsorption of sulfur dioxide [66]. In most of the transition metals catalyzed, Ullmann coupling-reaction ligands, such as phosphines, are reported in the literature and most ligands are expensive, difficult to prepare, and moisture sensitive. For this work, synthesized copper nanoparticles are used for ligand-free Ullmann coupling
of diphenyl ether. Different dyes and toxic organic compounds and pesticides present in industrial waste are very harmful for the environment and living organisms. Copper nanoparticles are used for degradation of different dyes such as methylene blue [73], degradation of atrazine [86], and reduction of 4-nitrophenol [76].

Among the antimicrobial agents, copper compounds have been commonly used in agriculture as herbicides [105], algaecides [106], fungicides [107], and pesticides as well as in animal husbandry as a disinfectant [108] (shown in Table 4). The biogenic copper nanoparticles showed powerful antibacterial activity against gram-positive and gram-negative pathogens such as Pseudomonas aeruginosa (MTCC 424), Micrococcus luteus (MTCC 1809), Enterobacter aerogenes (MTCC 2832) [57], Salmonella enterica (MTCC 1253), Xanthomonas solani, Xanthomonas axonopodis pv. citri, Xanthomonas axonopodis pv. punicea [58], Escherichia coli (ATCC 14948) [62], Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 6633), Pediococcus acidilactici [69], and Klebsiella pneumoniae (MTCC 4030). In antifungal activity, copper nanoparticles are used against Alternaria carthami, Colletotrichum gloeosporioides, Colletotrichum lindemuthianum, Drechslera sorghicola, Fusarium oxysporum f.sp. carthami, Rhizopus stolonifer, Fusarium oxysporum f.sp. ciceris, Macrophomina phaseolina, Fusarium oxysporum f.sp. udum, Rhizoctonia bataticola [58], Candida albicans, Curvularia, Aspergillus niger, and Trichophyton simii [67]. In cytotoxicity, copper nanoparticles are used for a study on HeLa, A549, MCF7, MOLT4, and BHK21 cell lines (cancer tumors) [60, 104].

Hypothetical Mechanism of Antimicrobial Activity
It was observed that CuNPs have an excellent antimicrobial activity and only limited reports presented the mechanism of the antibacterial activity of copper nanoparticles in the literature, but these mechanisms were hypothetical. It was observed that bacteria and enzymes/proteins were destroyed due to the interaction of CuNPs with –SH (sulfhydryl) group [109, 110]. It was also reported that the helical structure of DNA molecules become disturbed by the interaction of CuNPs [111]. The interaction of CuNPs with the cell membrane of bacteria decreased the transmembrane electrochemical potential, and due to the decrease in transmembrane electrochemical potential, it affected the membrane integrity [112]. It was assumed that metal NPs release their respective metal ions. Copper nanoparticles and copper ions accumulate on the cell surface of the bacteria and form pits.
in the membrane, causing leakage of the cellular component from the cell and inside the cell, causing oxidative stress which leads to cell death [112–114]. A hypothetical mechanism of antibacterial activity representing the above possibilities is shown in Fig. 6.

**Catalytic Activity for Reduction of 4-Nitrophenol**

4-Nitrophenol (4-NP) which is usually found in agricultural wastewaters and industrial products is hazardous and not environment-friendly. Hydrogenation or reduction of 4-NP, which is converted into 4-aminophenol (4-AP), takes place in the presence of CuNPs. CuNPs can catalyze the reaction to overcome the kinetic barrier by assisting electron transfer from the donor borohydrate ions to the acceptor 4-NP.

Catalytic activity of the synthesized CuNPs has been studied in the reduction of 4-nitrophenol in aqueous medium at room temperature in the presence of aqueous solution of sodium borohydride [56]. The reduction of 4-NP by using CuNPs is a simple and environment-friendly process. Catalytic efficiency of CuNPs for the reduction of 4-NP was examined by using a UV-vis spectrometer. It was observed that the maximum absorption peak for 4-NP in aqueous medium was at 317 nm and the adsortion peak shifted to 403 nm by adding sodium borohydride due to the formation of 4-nitrophenolate ions. A peak at 403 nm remained unaffected even after 2 days, which indicated that the reduction of 4-NP cannot take place in the absence of a catalyst. After adding the CuNPs, the absorption peak of the solution shifted

| Table 3 Characterization techniques and limitations |
|---------------------------------|-----------------|-----------------|-----------------|------------------|
| Technique                        | Main role                        | Limitations                                    | Sensitivity       | Ref.          |
| Ultraviolet-visible spectroscopy (UV-vis) | Concentration and shape of NPs can be measured | Only for liquid samples                          | UV-visible regions 200–800 nm | [22]          |
| Fourier transform infrared spectroscopy (FTIR) | Nature of bonds and functional groups can be determined | Structure and size of NPs cannot be measured | 20 Å–1 μm | [22]          |
| X-ray diffraction (XRD)         | Size and crystallinity of nanoparticles can be measured | Composition of NPs and plasmon cannot be found | 1 nm            | [36]          |
| Scanning electron microscopy (SEM) | Shape and size of nanostructures can be determined | Samples must be solid and cannot detect elements with atomic number < 1 | < 1 nm | [115]          |
| Field emission scanning electron microscopy (FESEM) | All structural and morphological investigations are carried out by this technique | Does not give a concentration of NPs | < 1 nm | [117]          |
| Transmission electron microscopy (TEM) | Shape and size of nanostructures can be determined | Particles with size < 1.5 nm cannot be determined | < 1.5 nm | [92]          |
| Particle size analysis (PSA)    | Measured the distribution of size in the sample of solid or liquid particulate materials | – | 1 nm–1 μm | [57, 58]       |
| Malvern Zetasizer (MZS)         | Measured the size of NPs, zeta potential, and protein mobility | In nanorange | – | [58]          |
| Energy-dispersive X-ray spectroscopy (EDX/EDS) | Composition of NPs can be analyzed | Particles with size < 2 nm cannot be analyzed | < 2 nm | [59, 60]       |
| Nanoparticle tracking analysis (NTA) | Visualize and measure particle size, concentration, and fluorescent properties of a nanoparticle | – | 30–10 nm | [62]          |
| Small-angle X-ray scattering (SAXS) | Size and shape conformation | Lower resolution range | 50–10 Å | [116]          |
| X-ray reflectometry (XRR)       | Determination of thickness, density, and roughness | Layer thickness 0.1–1000 nm | – | [116]          |
| X-ray fluorescence spectroscopy (XRF) | Chemical composition and concentration can be measured | Limited in their ability to measure precisely and accurately | – | [76]          |
| X-ray photoelectron spectroscopy (XPS) | Elemental composition of nanoparticles can be analyzed | Decomposition of samples occurred | 3–92 nm | [78]          |
| Brunauer-Emmett-Teller analysis (BET) | Specific surface area is measured | 0.35–2 nm | – | [76]          |
| Selected area electron diffraction (SAED) | Technique that can be performed inside a TEM | Cannot be recommended for quantitative identification techniques | – | [76]          |
| Atomic force microscopy (AFM)   | Particle size and characterization | For gas and liquid samples | 1 nm–8 μm | [88]          |
| Biological entity    | Activity                  | In/against | Concentration of NPs | References |
|----------------------|---------------------------|------------|----------------------|------------|
| Euphorbia esula      | Catalytic                 |            | 25 μL                | [56]       |
| Punica granatum      | Antibacterial             |            | 100 μg/L             | [57]       |
| Ocimum sanctum       | Antibacterial             |            | –                    | [58]       |
| Ginkgo biloba        | Catalytic                 |            | 10 μM               | [59]       |
| Calotropis procera   | Cytotoxicity              |            | 120 μM              | [60]       |
| Citrus medica din    | Antibacterial             |            | 20 μL               | [62]       |
| Ficus carica         | Antibacterial             |            | 80 μg/mL             | [64]       |
| Sesamum indicum      | Catalytic                 |            | 0.01-0.06 g         | [66]       |
| Citrus limon and     | Antibacterial             |            | –                   | [67]       |
| Ficus carica         | Catalytic                 |            | 10 μg/mL            | [69]       |
| Thymus vulgaris      | Catalytic                 |            | 50 g and 15 mg, respectively | [76] |
| Phyllanthus emblica  | Antibacterial             |            | –                   | [77]       |
| Moringa oleifera     | Antibacterial             |            | –                   | [78]       |
| Capparis zeysnica    | Antibacterial             |            | –                   | [81]       |
| Vitis vinifera       | Antibacterial             |            | –                   | [82]       |
| Hibiscus ros-sinensis| Antibacterial             |            | –                   | [83]       |
| Zingiber officinalis | Antibacterial             |            | –                   | [84]       |
| Zea mays             | Catalytic                 |            | 30 mg               | [86]       |
| Dodonaea viscosa     | Antibacterial             |            | –                   | [88]       |
| Azadirachta indica   | Antibacterial             |            | –                   | [90]       |
| Lantana camera       | Antibacterial             |            | –                   | [90]       |
| Tridax procumbens    | Antibacterial             |            | –                   | [90]       |
| Allium sativum       | Antibacterial             |            | 75 and 50 μL, respectively | [91] |
| Asparagus adscendens  | Antibacterial             |            | –                   | [92]       |
| Bacopa monnieri      | Antibacterial             |            | –                   | [92]       |
| Nerium oleander      | Antibacterial             |            | 35 μL               | [94]       |
| Psidium guajava      | Antibacterial             |            | –                   | [95]       |
to 300 nm and the peak at 403 nm completely disappeared which indicated the reduction of 4-NP to 4-AP without any side product. A hypothetical mechanism for the reduction of 4-NP is shown in Fig. 7. In the mechanism, 4-NP and sodium borohydride are present in the solution in the form of ions. The protons of the borohydride ion are adsorbing on the surface of the copper nanoparticles and BO₂ produced. 4-Nitrophenolate ions also adsorb on the surface of the CuNPs. Due to the adsorption of both protons and 4-nitrophenolate ion, CuNPs overcome the kinetic barrier of reactants and 4-nitrophenolate ion is converted into 4-aminophenolate ion. After conversion, desorption of the 4-aminophenolate ion takes place and it is converted into 4-aminophenol.

**Conclusions**

This paper has reviewed and summarized recent information of biological methods used for the synthesis of
copper nanoparticles (CuNPs) using different plants. Green synthesis of CuNPs has been proposed as a valuable alternative to physical and chemical methods with low cytotoxicity, economic prospects, environment-friendly, enhanced biocompatibility, feasibility, and high antioxidant activity and high antimicrobial activity of CuNPs. The mechanism of biosynthesis of NPs is still unknown, and more research needs to be focused on the mechanism of formation of nanoparticles and understanding of the role of phytochemicals in the formation of NPs. This review gives data of plants used in the synthesis of copper nanoparticles, synthesis procedure, and the reaction parameters which affect the properties of synthesized CuNPs. A phytochemical screening analysis is a chemical analysis used to identify the phytochemicals such as detection of carbohydrates, tannins, saponins, flavonoids, alkaloids, anthraquinones, and anthocyanosides in different plants. The mechanism of reduction of copper ion by quercetin and stabilization of copper nanoparticles by santin is described in this paper. Characterization techniques used in the literature for copper nanoparticles are UV–vis, FTIR, XRD, SEM, FESEM, TEM, PSA, MZS, EDX, NTA, SAXS, XRR, XRF, XPS, BET, SAED, and AFM. Copper nanoparticles show catalytic activity, antibacterial activity, cytotoxicity or anticancer activity, antioxidant activity, and antifungal activity in different applications. Hypothetical mechanisms of antimicrobial activity and reduction of 4-nitrophenol with diagrams are shown in this paper.

CuNPs with different structural properties and effective biological effects can be fabricated using new green protocols in the coming days. The control over particle size and, in turn, the size-dependent properties of CuNPs will open the new doors of their applications. This study provides an overview of synthesis of CuNP by using plant extract, microbial extract, and naturally occurring biomolecules. Although all these green protocols for CuNP synthesis have their own advantages and limitations, the use of plant extract as a reductant is more beneficial as compared to the use of microbial extract because of the rapid rate of production of nanoparticles with former green reductant.

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The authors agreed to share data to any recommended repositories.

Authors’ Contributions

MID collected all the data and write the whole manuscript. FA also contributes to this article by studying more than 100 relevant articles and also helped in collecting some data. ZH helped in the writing of this manuscript. She is a native speaker of English from UK. She revised the whole manuscript and improved its English language. MM helped in the writing of the interpretation of antimicrobial effect. She also helped in explaining the green mechanism. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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