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Review Article

BIOGENIC SYNTHESIS OF COPPER NANOPARTICLES AND THEIR BIOLOGICAL APPLICATIONS: AN OVERVIEW

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ABSTRACT

Copper nanoparticles are one of the most promising agents in the field of nanotechnology which has the widest range of applications in various fields. Copper is an inorganic and safest material to humans, extensively used as an anti-bacterial, anti-fungal, anti-cancer agent and also as catalysts and sensors in high potential, peculiarly in nanosize. This emerged the preparation of CuNPs using various techniques. Many conventional methods have been employed for the synthesizing CuNPs which include electron beam lithography, inert gas condensation, ion implantation, laser ablation, mechanical milling, mechanical grinding, pulsed wire discharge, spray pyrolysis, vacuum vapour deposition, chemical reduction method, electrochemical method, microemulsion method, microwave method and solvothermal decomposition method. Relatively the biological method is highly recommended for the synthesis of CuNPs due to the absence of harmless chemicals, enhanced biocompatibility, eco-friendly, greater biological activity and low toxicity. This review is focussing on the biogenic synthesis of CuNPs using plants and micro-organisms, reaction conditions, characterization techniques and their applications.

Keywords: Nanotechnology, Copper nanoparticles, Green synthesis, Plant extracts, Microorganisms, Biological applications

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INTRODUCTION

In modern material science and technology, one of the most active areas of research is nanotechnology. Nanotechnology is a transformation tool used to enhance the development of highly valuable products from renewable and biocompatible raw materials. Nanotechnology mainly aims in the study of particles ranging from 1-100 nm approximately and these particles are said to be Nanoparticles (NPs). Nanoparticles are useful for delivering medications to the target specific locations. Interaction of the nanoparticles with humans and the diversity of organisms in an environment is an essential thing to be considered [1-5]. Nanoparticles are found to exhibit enhanced optical and catalytic activity due to the quantum size effect. NPs have an enhanced effect on various properties due to their Surface Plasmon Resonance (SPR) enhanced Rayleigh scattering and surface-enhanced Raman scattering (SERS) which makes them more constant as compared to bulk metals. NPs have distinct chemical, physical, electrical, electronic, magnetic, mechanical, optical and biological properties. Metal nanoparticles are widely employed in various fields because of their unique characters including the large surface area to volume ratio, large surface energies, plasmon excitation, short-range ordering and quantum confinement. Among the transition metals, copper has a better view of both science and technology because it is a reusable metal [3].

Copper is one of the most extensively utilized materials on the planet and is found in both plant and animal tissues. It is a prominent metal of therapeutics which can be utilized for various pathological conditions like wound, bacterial and fungal infections. It would be an anti-fouling, anti-bacterial, anti-fungal agent etc. that can be used to purify the water. It also helped in crosslinking of collagen, formation of bone matrix and preventing the wound from infection. According to the U. S. EPA (United States Environmental Protection Agency), copper is the only solid surface material that destroys microorganisms. Due to the fascinating physical, optical and electronic properties, it is subjected to the intense research of nanoscience [7-9].

Copper nanoparticles (CuNPs) are more advantageous because they can be easily synthesised at a low cost, show an intense colour and exhibit a strong tunable absorption band in the visible region, which is not present in the bulk metals. CuNPs are highly toxic to microorganisms, exhibits a strong bactericidal effect on many species of bacteria, also act as antifungal, anti-inflammatory agents and works in preventing infecting and wound healing. The drawback of CuNPs is the severe susceptibility to oxidation that makes their catalytic and optical reactions non-reproducible [6-9]. But CuNPs can resist oxidation or aggregation, by stabilizing through the adsorption or by the covalent attachment of the organic compounds on the surface of the NPs which provides the electrostatic or electrostatic repulsive forces between particles. NPs can be synthesised in two ways: top-down and bottom-up approach (fig. 1).

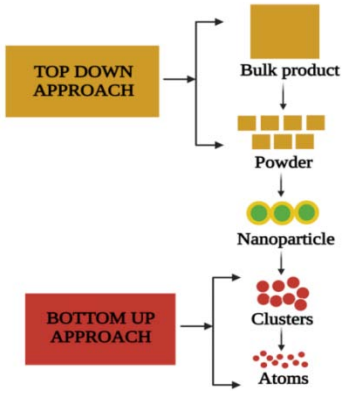

Fig. 1: Approaches for the synthesis of nanoparticles

The top-down approach is a process of breaking down the bulk material into nano-sized particles. The bottom-up approach is a process of building nanoparticles using atoms. There are three different processes to generate nanoparticles based on these two approaches viz., physical, chemical and biological methods. Among these three methods, the physical method is classified as a top-down approach, whereas the other two ways are classified as bottom-up approaches (fig. 2).

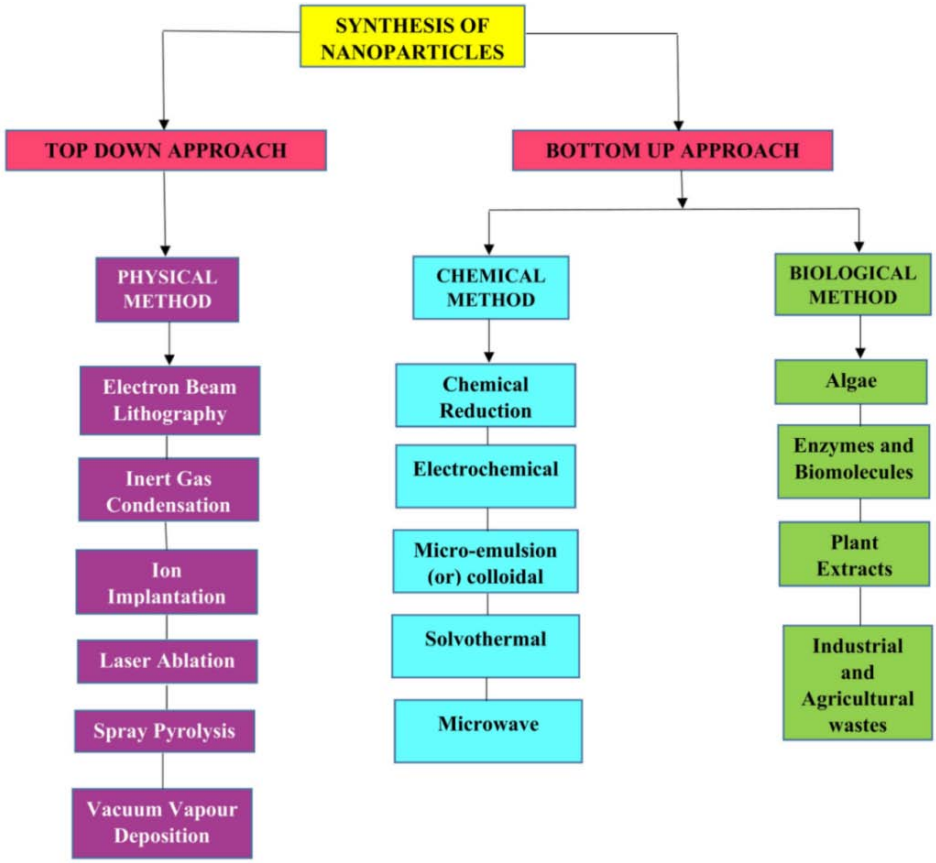

Fig. 2: Methods for the synthesis of nanoparticles

In comparison with the conventional physical and chemical methods, the biological method plays a greater role in the synthesis of NPs because it is a simple, eco-friendly, non-toxic and economical method [11] (fig. 3).

Fig. 3: Advantages of green synthesised CuNPs

In the biological method, either plants or microorganisms can be mediated for synthesizing CuNPs. The plant-mediated synthesis is an eco-friendly method which requires less drastic reaction conditions and inexpensive reagents. Three main steps involved in the green synthesis of CuNPs are choice of solvent used, choice of the eco-friendly reducing agent and the choice of a non-toxic material for the stabilization of the nanoparticles. Most of the synthetic methods have reported on organic solvents due to the hydrophobicity of the capping agents used. The synthesis of CuNPs using biological methods is more compatible with the green synthesis because the methods are eco-friendly; same components act as reducing and capping agents [12].

In this review, only the literature indexed in ScienceDirect, PubMed, Springer, Google Scholar, ResearchGate, Research square and Royal Society of Chemistry databases between the time period of 2015 and 2021 were surveyed. The keywords for this survey include copper nanoparticles, green synthesis, microwave irradiation, biological synthesis, characterization, applications of copper nanoparticles, both individually and in combination were applied and shortlisted according to the purpose of this study. This review focuses on various plant extracts and micro-organisms employed for the biological synthesis of the CuNPs along with their reaction conditions, characterization techniques and their various biological applications (fig. 4).

Mechanism of synthesis of nanoparticles

In the biosynthesis of CuNPs, extracts from biological sources may act as both reducing and capping agents. Combinations of biomolecules included in these extracts, such as proteins, amino acids, vitamins, and polysaccharides, reduce Cu^{+} ions in an environmentally favourable but chemically complex. Copper ions were bound on the surface of proteins in extract via electrostatic interactions, which served as a reduction process [11, 19]. (fig. 5).

Plant mediated synthesis of copper nanoparticles

The main advantage of the green synthesis of CuNPs is that they are easily available, safe to handle and possess a broad variability of metabolites. In the light of IR spectroscopic research, the primary phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. The main

water-soluble phytochemicals like quinones, flavones and organic acids were responsible for immediate reduction. Redial tautomerization occurs in anthraquinone compounds, resulting in the formation of nanoparticles. The stability of the green synthesized CuNPs is enhanced and thereby it increases the rate of reaction of CuNPs by preventing the formation of agglomerates [13, 17]. The part of the plants such as leaf, fruit, flower, bark, root and stem along with the precursor copper salts such as copper acetate, copper nitrate, copper sulphate and copper chloride were processed as per the time and temperature is given in table 1 and fig. 6.

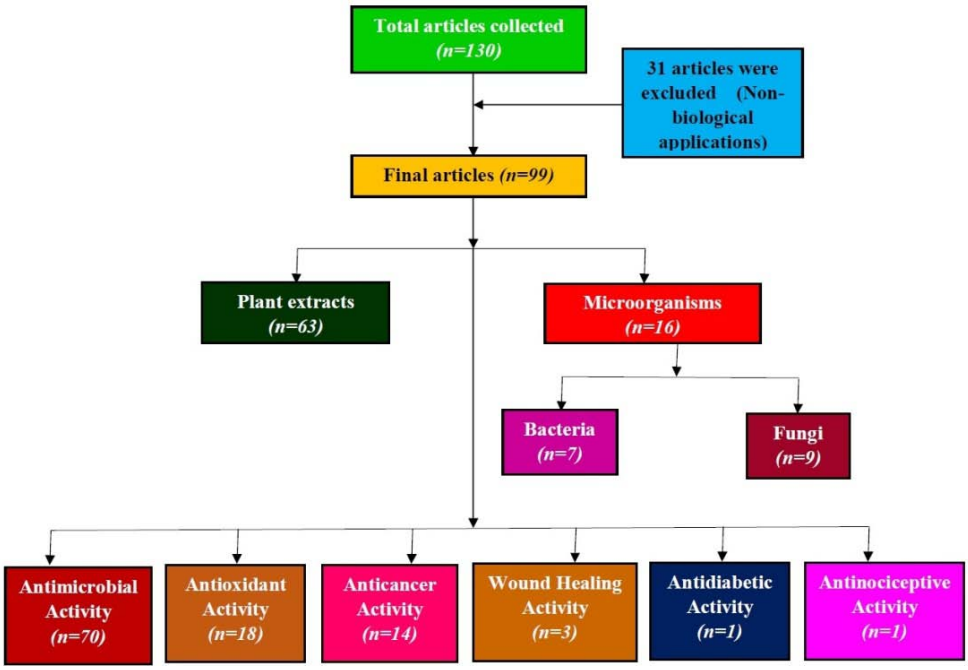

Fig. 4: Selection strategy of this review

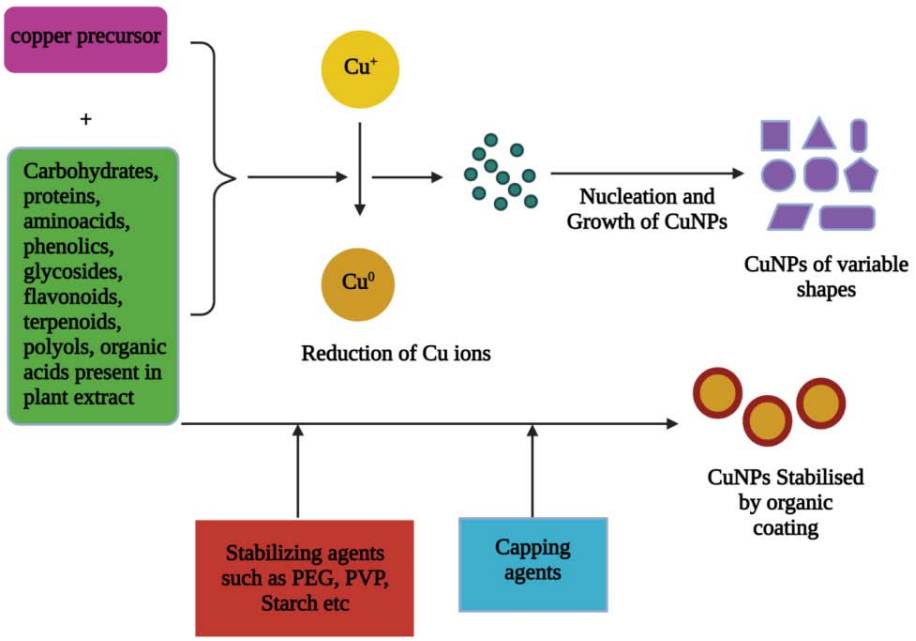

Fig. 5: Probable mechanism for the synthesis of CuNPs

Microorganisms mediated synthesis of copper nanoparticles

For the biological synthesis of CuNPs, various green algae, bacteria, viruses and fungi were used. Microorganisms are a good source for the production of CuNPs because of their metabolism and ease of growth in laboratory conditions. Initially, bacteria were used to synthesize NPs and this was later succeeded with the use of fungi because they are easier to handle on comparing with the other group of microorganisms [5]. Microbiological methods synthesize nanoparticles at a slower rate of reaction than that observed when plant extracts are used. CuNPs were prepared from various bacteria and fungi along with the precursor copper salts are given in table 2 and table 3, respectively. The probable mechanism of the formation of copper nanoparticles is shown in fig. 7.

Table 1: Plant mediated synthesis of copper nanoparticles

Plant name	Parts used	Phyto constituents	Precursor	Temp (°C)/time	Activity	References
Allium sativum	Leaves	Steroid saponins	1 mmol Copper sulphate	RT, 48 h	Antibacterial	[1]
Allium sativum	Herb	Sulphides, thiosulfinates, vinyldithiins	2 mmol Copper chloride	RT, Nil	Antibacterial, anticancer	[4]
Zingiber officinale	Rhizome	Phenols and terpenes	2 mmol Copper chloride	RT, Nil	Antibacterial, anticancer	[4]
Cissus quadrangularis	Leaves	Quercitin, quercitrin, beta-sitosterol	1 mmol Copper acetate	300-400, Nil	Antifungal	[10]
Moringa oleifera	Leaves	Flavonoids, alkaloids, phenols, vitamins, minerals	0.04M Cu^{+2} solution	60, 3 h	Antibacterial, antifungal, antioxidant	[13]
Azadirachta indica	Leaves	Azadirachtin, nimbin, nimbidin, quercetin	0.2 M Copper acetate	120, 2 h	Anticancer, antioxidant	[14]
Hibiscus rosa-sinensis	Leaves	Tannins, anthraquinones, quinines, phenols, flavonoids, alkaloids	0.2 M Copper acetate	120, 2 h	Anticancer, antioxidant	[14]
Murraya koenigii	Leaves	Polyphenols	0.2 M Copper acetate	120, 2 h	Anticancer, antioxidant	[14]
Tamarindus indica	Leaves	Tannins, alkaloid, flavonoids, sesquiterpenes, glycosides	0.2 M Copper acetate	120, 2 h	Anticancer, antioxidant	[14]
Eclipta prostrata	Leaves	P-caryophyllene, α-humulene	3 mmol Copper acetate	RT, 24 h	Anticancer, antioxidant	[15]
Abutilon Indicum	Leaves	Carbohydrates, steroids, glycosides, flavonoids, tannins, phenolic compound	Copper nitrate	200, 2 h	Antibacterial, antifungal, anticancer, antioxidant	[16]
Clerodendrum inerme	Leaves	Beta sitosterol	Copper nitrate	200, 2 h	Antibacterial, antifungal, anticancer, antioxidant	[16]
Clerodendrum infortunatum	Leaves	Saponin	Copper nitrate	200, 2 h	Antibacterial, antifungal, anticancer, antioxidant	[16]
Eryngium caucasicum	Leaves	Octane, carvone, beta ionene, beta bisaboline	10 mmol cupric nitrate	RT, 72 h	Antibacterial, antioxidant	[17]
Curcuma longa	Rhizome	Curcumin, deoxy curcumin	Copper sulphate	RT, 30 min	Antifungal	[18]
Vaccinium myrtillus	Fruit	Phenols	0.1 M Copper chloride, Copper acetate, Copper nitrate	RT, 14 h	Antibacterial, antifungal	[19]
Vaccinium uliginosum	Fruit	Anthocyanin	0.1 M Copper chloride, Copper acetate, Copper nitrate	RT, 14 h	Antibacterial, antifungal	[19]
Cucumis sativus	Root	Anthocyanin	CuNPs purchased	25, 12 h	Anticancer, antioxidant	[20]
Anethum graveolens	Seeds	Volatile oil, flavonoids, coumarins, xanthones, triterpenes	Copper chloride	35, 24 h	Antifungal	[21]
Thymus daenensis	Leaves	Thymol, carvacrol, linalool, a-terpineol	Copper chloride	35, 24 h	Antifungal	[21]
Persea Americana	Seeds	Flavonol glycoside	Copper sulphate	45-50, 6-7 h	Antibacterial, antifungal, antioxidant	[22]
Trigonella foenum-graecum	Seeds	Carbohydrates, proteins, lipids, alkaloids, flavonoids, steroidal saponins	2.0 mmol Copper sulphate	121, 20 min	Antibacterial, antifungal, antioxidant	[23]
Punica granatum	Peel	Flavonoids, ellagitannin, punicalagin, ellagic acid	Copper sulphate	40, 48 h	Antibacterial	[24]
Fagus sylvatica	Sapwood	Epicatechin, catechin, protocatechuic acid, isoquercitrin	Copper sulphate	Vacuum 80 KPa, 2 h	Antifungal	[25]
Pinus sylvestris	Sapwood	α-terpineol, linalool, limonene	Copper sulphate	Vacuum 80 KPa, 2 h	Antifungal	[25]
Cissus vitiginea	Leaves	Tannin, phenol, flavonoid, terpenoids, saponin	10 mmol Copper sulphate	RT, Nil	Antibacterial, antioxidant	[26]
Citrus medica	Juice of matured fruits	Vitamin C, pectin, citral, limonene, phenolics	100 mmol Copper sulphate	60-100, Nil	Antibacterial, antifungal	[27]

Plant name	Parts used	Phyto constituents	Precursor	Temp (°C)/time	Activity	References
Azadirachta indica	Leaves	Azadirachtin, nimbin, nimbidin, gedunin, salannin, quercetin	1 mmol Copper sulphate	70-90, 24 h	Antifungal	[29]
Ocimum sanctum	Leaves	Linalool, carvacrol, beta caryophyllene, germacrene	1 mmol Copper sulphate	RT, Nil	Antibacterial, antifungal	[30]
Allium saralicum	Leaves	Neophytadiene, phytol, vitamin E, tocopherol	Copper sulphate	RT, 1 h	Antibacterial, anticancer, antifungal, antioxidant, wound healing	[31]
Allium eriophyllum	Leaves	Carvacrol, geranyl acetone, beta ionone	0.04 M Copper sulphate	60, 15 min	Antibacterial, anticancer, antifungal, antioxidant, wound healing	[32]
Zingiber officinale	rhizome	Phenols, terpenes	Copper sulphate	50, 20 min	Antibacterial, antifungal, antioxidant	[33]
Celastrus paniculatus	Leaves	Alkaloids, sterols	5 mmol Copper sulphate	60, 20 min	Antifungal	[34]
Triticum aestivum	Seeds	Protein, starch	Copper sulphate	25, 12 h	Antioxidant	[35]
Tilia cordata	Leaves	Flavonoids	Copper sulphate	100, 12 h	Antibacterial, anticancer	[36]
Syzygium aromaticum	Bud	Terpenes, phenols, hydrocarbons	Cupric acetate	80, 5 min	Antibacterial, antifungal	[37]
Falcaria vulgaris	Leaves	Carvacrol, Spatulenul	0.04 M Copper sulphate	40, 30 min	Antibacterial, anticancer, antifungal, antioxidant, wound healing	[38]
Camellia sinensis	Leaves	Flavonoids, polyphenols	1 mmol Copper sulphate	80, 10 min	Antibacterial, antifungal	[39]
Manilkara zapota	Leaves	Vitamin C, niacin, stearic acid, pantothenic acid	5 mmol Copper sulphate	100, 10 min	Antibacterial, antifungal, anticancer	[40]
Citrus limon	Fruits	Limonene, citronellol, geranial	Copper sulphate	27, 4 h	Antibacterial	[41]
Zizipus spina-christi	Fruit	Alpha and beta pinene, trans-caryophyllene	0.02M Copper sulphate	80, 1 h	Antibacterial	[42]
Piper retrofractum	Fruit	Alkaloids, phenylpropanoids, alkyl glycoside, lignans	Copper sulphate	60, 1h	Antibacterial	[43]
Piper longum	Powder	Sesquiterpene hydrocarbons, ethers	Copper sulphate	60, 30 min	Antibacterial	[44]
Piper nigrum	Powder	Piperine	Copper sulphate	60, 30 min	Antibacterial	[44]
Syzygium cumin	Leaves	Anthocyanin, glucoside, isoquercetin	0.1 M Copper sulphate	100, 30 min	Antibacterial	[45]
Mitragyna parvifolia	Bark	Alkaloid	Copper sulphate	80, 4-5 h	Antibacterial	[46]
Cissus arnotiana	Leaves	Saponins, flavonoids, alkaloids, steroids, anthraquinones	10 mmol Copper sulphate	RT, 4 h	Antibacterial, antioxidant	[47]
Capparis spinosa	Fruit	Flavonoids, proteins	0.01 M Copper sulphate	60, 24 h	Antinociceptive	[48]
Garcinia mangostana	Leaves	Tannins	0.001 M Copper nitrate	70, 1 h	Antibacterial	[49]
Quisqualis indica	Flower	Alkaloid, flavonoid	Copper acetate	RT, Nil	Anticancer	[50]
Gnidia glauca	Flower, stem and leaf	Alkaloids, steroids, saponins, coumarin, flavonoids	1 mmol Copper sulphate	100, 5 h	Antidiabetic	[51]
Plumbago zeylanica	Leaves	Flavonoids, alkaloids, steroids, tannins, phenols	1 mmol Copper sulphate	100, 5 h	Antidiabetic	[51]
Syzygium alternifolium	Stem bark	Anthocyanins, glucose, ellagic acid, isoquercetin	5 mmol Copper sulphate	50, 2 h	Antibacterial, antifungal, anticancer	[52]
Ctenolepis garcinii	Powder	Anthocyanin, alkaloids, steroids, tannins, saponins, flavonoids	1 mmol Copper nitrate	RT, 24 h	Antibacterial	[53]
Blumea balsamifera	Leaves	Terpenes, flavonoids, esters, alcohol, sterol	1 mmol Copper sulphate	100, 8 h	Antibacterial	[54]
Prosopis cineraria	Leaves	Alkaloids, flavonoids, tannins, saponins	5 mmol Copper acetate	-20, 10 min	Antibacterial, anticancer	[55]
Cinnamomum zeylanicum	Bark	Tannins, mucilage, calcium oxalate, starch	100 mmol Copper sulphate	60-100, Nil	Antibacterial	[56]

Plant name	Parts used	Phyto constituents	Precursor	Temp (°C)/time	Activity	References
Bougainvillea	Flower	Phenol, flavonoid, saponin	Copper acetate	80, 10 min	Antifungal	[57]
Citrus reticulate	Peel	Limonene, myrcene	0.001 M Copper sulphate	45, Nil	Antibacterial	[58]
Olea europea	Leaves	Flavonoids, steroids, tannins, terpenes	2 mmol copper sulphate	100, 24 h	Anticancer, antioxidant	[59]
Artemesia haussknechtii	Leaves	Fibre, protein, tannin, tocopherol	0.01 M Copper sulphate	RT, 24 h	Antibacterial, antioxidant	[60]
Leucas aspera	Leaves	Phenols, proteins, tannins	Copper sulphate	100, 2 h	Antibacterial	[61]
Morinda tinctoria	Leaves	Proteins and amino acids, diterpenes	Copper sulphate	100, 2 h	Antibacterial	[61]
Morus alba	Leaves	Protein, saccharose, xylose, galactose	Copper acetate	60, 5 min	Antioxidant	[62]
Avicennia mariana	Leaves	Triterpenoids, hydrocarbons	Copper sulphate	65, 3 h	Antibacterial, antifungal	[63]
Datura stramonium	Leaves	Tropane alkaloids	Copper sulphate	65, 3 h	Antibacterial, antifungal	[63]
Eucalyptus camaldulensis	Leaves	Aromatic phenol, alcohol, oxides, esters	Copper sulphate	65, 3 h	Antibacterial, antifungal	[63]
Rosa rubiginosa	Leaves	Proteins, flavonoids, tocopherol	Copper sulphate	65, 3 h	Antibacterial, antifungal	[63]
Stachys lavandulifolia	Flower	Alpha pinene, linalool, acetoside	Copper chloride	RT, Nil	Antibacterial	[64]
Echinops sphaerocephalus	Roots	Apigenin, hesperidin, kaempferol, rutin	0.5 M Copper nitrate	RT, Nil	Antibacterial	[65]
Cardiospermum helicacabum	Leaves	Saponin, phytosterol, polyphenol	10 mmol Copper chloride	90, 1 h	Antibacterial	[66]
Asparagus adscendens	Root and leaf	Steroidal saponins	1 mmol Copper sulphate	RT, 1 h	Antibacterial	[67]
Passiflora foetida	Leaves	Amino acid alpha alanine, organic acids	20 mmol copper sulphate	80, 4 h	Antibacterial	[68]
Majorana hortensis	Leaves	Monoterpenes	Copper chloride	70, 24 h	Antibacterial	[69]
Magnolia champaca	Flower	Phenol, phenyl acetonitrile	3 mmol copper acetate	37, 24 h	Antioxidant	[70]
Citrus aurantifolia	Leaves	Terpenes	Copper sulphate	80, 10 h	Antibacterial	[71]
Capparis spinosa	Fruit	Alkaloids, flavonoids, phenolics, triterpenoids, steroids	Copper sulphate	60, 24 h	Antibacterial	[72]
Terminalia chebula, Terminalia belerica, Emblica officinalis	Fruits	Phenols	1 mmol Copper sulphate	37, 5 h	Antibacterial, antifungal	[73]

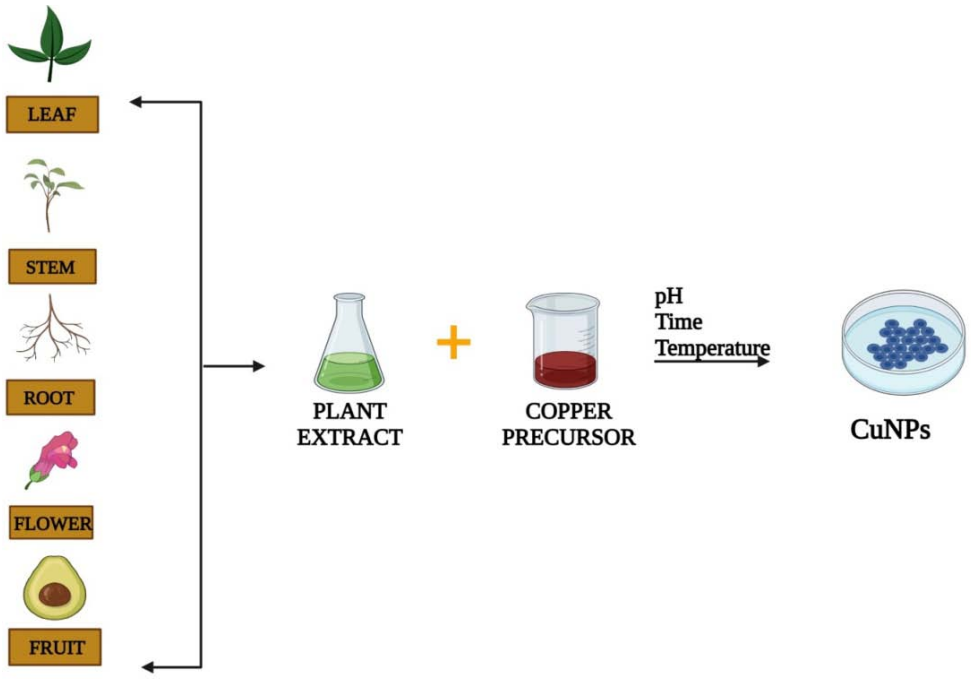

Fig. 6: Plant mediated synthesis of copper nanoparticles

Table 2: Bacteria mediated synthesis of copper nanoparticles

Bacteria	Precursor	Temp (°C)/time	Reference
Staphylococcus aureus	0.5 M Copper sulphate	RT, Nil	[8]
Staphylococcus epidermis	0.5 M Copper sulphate	RT, Nil	[8]
Streptomyces griseus	1 mmol Copper sulphate	37, 72 h	[76]
Escherichia coli	Copper sulphate	30, 1 h	[83]
Morganella morgana	Copper sulphate	30, 24-48 h	[84]
Bacillus Euplotes focardii	4 mmol Copper sulphate	RT, 48 h	[87]
Brevundimonas Euplotes focardii	5 mmol Copper sulphate	RT, 48 h	[87]
Marinomonas Euplotes focardii	5 mmol Copper sulphate	RT, 48 h	[87]
Pseudomonas Euplotes focardii	3.5 mmol Copper sulphate	RT, 48 h	[87]
Rhodococcus Euplotes focardii	4.5 mmol Copper sulphate	RT, 48 h	[87]
Pseudomonas fluorescens	1 mmol Copper sulphate	35, 48 h	[92]

Table 3: Fungi mediated synthesis of copper nanoparticles

Fungi	Precursor	Temp (°C)/Time	Reference
Candida albicans	0.5 M Copper sulphate	RT, Nil	[8]
Candida parapsilosis	0.5 M Copper sulphate	RT, Nil	[8]
Aspergillus versicolor	Copper sulphate	25, 72 h	[12]
Fusarium solani	Copper sulphate	95, 90 min	[74]
Neofusicoccum sp.	Copper sulphate	95, 90 min	[74]
Fusarium oxysporum	Copper sulphate	95, 90 min	[74]
Botrytis cinerea	Copper oxide (purchased)	22, 7-14 d	[77]
Candida albicans	0.01 M Copper acetate	140, 10 h	[78]
Penicillium olsonii	0.02 M Copper sulphate	25, 3 d	[78]
Fusarium sp.	Copper chloride	80, Nil	[79]
Fusarium culmorum	0.001-0.0100 M Copper nitrate	28±2, 72 h	[80]
Fusarium oxysporum	0.001-0.0100 M Copper nitrate	28±2, 72 h	[80]
Fusarium equiseti	0.001-0.0100 M Copper nitrate	28±2, 72 h	[80]
Aspergillus flavus	100 mmol Copper sulphate	20, 20 min	[81]
Coniophora puteana	Cupric carbonate. Copper hydroxide	20, 14 d	[82]
Gleophyllum trabeum	Cupric carbonate. Copper hydroxide	20, 14 d	[82]
Trametes versicolor	Cupric carbonate. Copper hydroxide	20, 14 d	[82]
Agaricus bisporus	1 mmol Copper nitrate	60, 20 min	[85]

Table 4: Characterization of the biosynthesized CuNPs

Plants/Organisms	Characterization	Size (nm)	Shape	λ max (nm)	References
Allium sativum	UV-VIS, FTIR, XRD, SEM, TEM	100	Spherical	580	[1]
Allium sativum	UV-VIS, FTIR, XRD, TEM	10-40	Spherical	575	[4]
Zingiber officinale	UV-VIS, FTIR, XRD, TEM	25-50	Spherical	610	[4]
Cissus quadrangularis	UV-VIS, FTIR, XRD, SEM, TEM, EDX	33±2	Spherical	260	[10]
Moringa oleifera	UV-VIS, FTIR, XRD, TEM, HRTEM	35.8-49.2	Spherical	260	[13]
Azadirachta indica	UV-VIS, XRD, SEM, TEM, SAED. EDX	12	Spherical	220-235	[14]
Hibiscus rosasinensis	UV-VIS, XRD, SEM, TEM, SAED, EDX	12	Spherical	220-235	[14]
Murraya koenigii	UV-VIS, XRD, SEM, TEM, SAED, EDX	12	Spherical	220-235	[14]
Tamarindus indica	UV-VIS, XRD, SEM, TEM, SAED, EDX	12	Spherical	220-235	[14]
Eclipta prostata	UV-VIS, FTIR, XRD, SEM, HRTEM, SAED	28-50	Spherical, Hexagonal, Cubical	565	[15]
Abutilon indicum	FTIR, XRD, SEM, EDX	<100	Spherical	-	[16]
Clerodendrum inerme	FTIR, XRD, SEM, EDX	<100	Spherical	-	[16]
Clerodendrum infortunatum	FTIR, XRD, SEM, EDX	<100	Spherical	-	[16]
Eryngium caucasicum	UV-VIS, FTIR, XRD, SEM	40	Spherical	580	[17]
Curcuma longa	UV-VIS, FTIR, XRD, SEM, TEM, ^{1}HNMR, ^{13}CNMR	20-30	Spherical	436	[18]
Vaccinium myrtillus	TEM, UV-VIS, XPS	2-10	Tiny globular	540-550	[19]
Vaccinium uliginosum	TEM, UV-VIS, XPS	2-10	Tiny globular	550-565	[19]
Cucumis sativus	SEM, XRF	10-30	Spherical	-	[20]
Anethum graveolens	SEM, FTIR	100-250	Spherical	-	[21]
Thymus daenensis	SEM, FTIR	100-250	Spherical	-	[21]
Persea Americana	UV-VIS, FTIR, XRD, SEM,TEM	45-100	Spherical	357	[22]
Trigonella foenum-graecum	UV-VIS, FTIR, XRD, TEM, DLS	31.7-35	Spherical	400	[23]
Punica granatum	UV-VIS, FTIR, TEM, PSA	15-20	Spherical	585	[24]
Fagus sylvatica	TEM	15.6	Spherical	-	[25]
Pinus sylvestris	TEM	15.6	Spherical	-	[25]
Cissus vitiginea	UV-VIS, FTIR, XRD, SEM, TEM, AFM, XPS	20	Spherical	340	[26]
Citrus medica	UV-VIS, XRD	33	Spherical	631	[27]
Azadirachta indica	UV-VIS, FTIR, XRD, SEM, TEM	100	Spherical	260	[29]
Ocimum sanctum	UV-VIS, FTIR, TEM, HRTEM, PSA, SAED	25	Cylindrical, rod	345	[30]

Plants/Organisms	Characterization	Size (nm)	Shape	λ max (nm)	References
			and elliptical		
Allium saralicum	UV-VIS, FTIR, FESEM, TEM, AFM	45-50	Spherical	576	[31]
Allium eriophyllum	UV-VIS, FTIR, XRD, FESEM,TEM	30-35	Spherical	572	[32]
Zingiber officinale	UV-VIS, FTIR, XRD, TEM, NTA	50	Spherical	618	[33]
Celastrus paniculatus	UV-VIS, FTIR, SEM-EDX, TEM, DLS	2-10	Spherical	269	[34]
Triticum aestivum	SEM, DLS	15.6 µm	Spherical	-	[35]
Tilia cordata	UV-VIS, FTIR, XRD, SEM, TEM	4.7-17.4	Spherical	563	[36]
Syzygium aromaticum	UV-VIS, FTIR, XRD, FESEM, TEM	15	Spherical	580	[37]
Falcaria vulgaris	UV-VIS, FTIR, XRD, FESEM, TEM	20-25	Spherical	572	[38]
Camellia sinensis	UV-VIS, FTIR, SEM, EDX	10-20	Spherical	563-582	[39]
Manilkara zapota	UV-VIS, FTIR, XRD, SEM, EDX	18.9-42.5	Spherical	580	[40]
Citrus limon	UV-VIS, FTIR, XRD, SEM, TEM	28	Spherical	579	[41]
Zizipus spinachristi	UV-VIS, FTIR, XRD, FESEM, TEM	5-20	Spherical	551	[42]
Piper retrofractum	UV-VIS, FTIR, XRD, SEM-EDX, TEM	2-10	Spherical	207	[43]
Piper longum	UV-VIS, FTIR, XRD, FESEM, TEM, EDX	15-30	Spherical	225	[44]
Piper nigrum	UV-VIS, FTIR, XRD, FESEM, TEM, EDX	15-30	Spherical	245	[44]
Syzygium cumin	UV-VIS, FTIR, XRD, SEM, EDX	10 µm	Spherical	190	[45]
Mitragyna parvifolia	UV-VIS, FTIR, XRD, SEM, TEM	12-23	Spherical	565-570	[46]
Cissus arnotiana	UV-VIS, XRD, SEM, TEM	60-90	Spherical	350-380	[47]
Capparis spinosa	UV-VIS, FTIR, SEM, EDX	17-41	Spherical	414	[48]
Garcinia mangostana	XRD, SEM, TEM, TGA, DTA	20-25	Spherical	-	[49]
Quisqualis indica	UV-VIS, XRD, FESEM, TEM, AFM	39.3±5.45	Spherical	309	[50]
Gnidia glauca	UV-VIS, FTIR, XRD, FESEM, DLS	1-5	Spherical	550	[51]
Plumbago zeylanica	UV-VIS, FTIR, XRD, FESEM, DLS	1-5	Spherical	600	[51]
Syzygium alternifolium	UV-VIS, FTIR, XRD, TEM, DLS	5-13	Spherical	285	[52]
Ctenolepis garcinii	UV-VIS, FTIR, XRD, SEM, EDX	67-82	Spherical	330	[53]
Blumea balsamifera	FTIR, SEM, EDX	1 µm	Spherical	-	[54]
Prosopis cineraria	UV-VIS, FTIR, XRD, FESEM, EDX	18.9-32.09	Spherical	420	[55]
Cinnamomum zeylanicum	UV-VIS, FTIR, TEM	66.14	Spherical	252.55	[56]
Bougainvillea	UV-VIS, FTIR, XRD, TEM	12±4	Spherical	274	[57]
Citrus reticulate	UV-VIS, FTIR, XRD, TEM, DLS	54-72	Spherical	442	[58]
Olea europea	FTIR, XRD, SEM, TEM	20-50	Spherical	-	[59]
Artemesia haussknechtii	UV-VIS, FTIR, XRD, FESEM, AFM, EDX	35.36±444	Spherical	200-300	[60]
Leucas aspera	UV-VIS, FTIR, XRD, SEM	30-32	Spherical	319	[61]
Morinda tinctoria	UV-VIS, FTIR, XRD, SEM	18-72	Spherical	412	[61]
Morus alba	UV-VIS, FTIR, XRD, TEM, SEM	40-50	Spherical	285	[62]
Avicennia mariana	UV-VIS, FTIR, SEM, TEM,EDX	64	Spherical	563-582	[63]
Datura stramonium	UV-VIS, FTIR, SEM, TEM,EDX	43	Spherical	563-582	[63]
Eucalyptus camaldulensis	UV-VIS, FTIR, SEM, TEM,EDX	65	Near spherical	563-582	[63]
Rosa rubiginosa	UV-VIS, FTIR, SEM, TEM,EDX	55	Spherical	563-582	[63]
Stachys lavandulifolia	UV-VIS, FTIR, XRD, TEM	80±8	Spherical	590	[64]
Echinops sphaerocephalus	UV-VIS, FTIR, XRD, FESEM	20-100	Spherical	441	[65]
Cardiospermum helicacabum	UV-VIS, FTIR, XRD, FESEM, TEM, DLS	30-40	Spherical	553	[66]
Asparagus adscendens	UV-VIS, FTIR, HRTEM, SAED	50-60	Spherical	500-700	[67]
Passiflora foetida	UV-VIS, FTIR, XRD, SEM, EDX	40	Spherical	407	[68]
Majorana hortensis	UV-VIS, FTIR, XRD, SEM, EDX	3	Irregular, agglomerated particles	280	[69]
Magnolia champaca	UV-VIS, FTIR, XRD, SEM, TEM, EDX	20-40	Spherical	285	[70]
Citrus aurantifolia	UV-VIS, FTIR, XRD, SEM	20-90	Spherical	240-300	[71]
Capparis spinosa	UV-VIS, FTIR, SEM, EDX	17-41	Spherical	414	[72]
Terminalia chebula, Terminalia belerica, Emblica officinalis	XRD, SEM	20-25	Spherical	-	[73]
Staphylococcus aureus, Staphylococcus epidermis, Candida albicans, Candida parapsilosis	UV-VIS, XPS, DLS, NTA	50-70	Spherical	550	[8]
Aspergillus versicolor	UV-VIS, FTIR, SEM, TEM, DLS	22.09±0.6	Round, polygonal	460	[12]
Fusarium solani, Neofusicoccum sp, Fusarium oxysporum	XRD, TEM, PDF, XPS	200-500	Spherical	-	[74]
Streptomyces griseus	UV-VIS, FTIR, XRD, TEM	5-50	Spherical	590	[76]
Botrytis cinerea	TEM	40-100	Spherical	-	[77]
Candida albicans	XRD, FESEM, HRTEM	10.7	Spherical	-	[78]
Penicillium olsonii	UV-VIS, FTIR, SEM	6-26	Spherical	631	[78]
Fusrium sp.	UV-VIS, FTIR, XRD, TEM	20-50	Spherical	500-600	[79]
Fusarium culmorum, Fusarium oxysporum, Fusarium equiseti	UV-VIS, FTIR, XRD, TEM	3-30	Spherical	560	[80]
Aspergillus flavus	UV-VIS, FTIR, XRD, TEM, NTA	5-12	Spherical	602	[81]
Coniophora puteana, Gleophyllum trabeum,	UV-VIS, XRD, TEM, SAD, EDX	15-20	Spherical	360	[82]

Plants/Organisms	Characterization	Size (nm)	Shape	λ max (nm)	References
Trametes versicolor					
Escherichia coli	SEM, EDX	10-50	Spherical	-	[83]
Morganella morgana	UV-VIS, FTIR, XRD, SEM, EDX	13.5±0.6	Spherical	540	[84]
Agaricus bisporus	UV-VIS, FTIR, XRD, SEM, TEM, EDX	10	Spherical	551	[85]
Spirulina platansis	UV-VIS, FTIR, XRD, SEM	5	Crystal	641	[87]
Bacillus Euplotes focardii	UV-VIS, FTIR, XRD, TEM, DLS	10-70	Monodispersed, spherical, oval	381-383	[87]
Brevundimonas Euplotes focardii	UV-VIS, FTIR, XRD, TEM, DLS	10-70	Monodispersed, spherical, oval	381-383	[87]
Marinomonas Euplotes focardii	UV-VIS, FTIR, XRD, TEM, DLS	10-70	Monodispersed, spherical, oval	381-383	[87]
Pseudomonas Euplotes focardii	UV-VIS, FTIR, XRD, TEM, DLS	10-70	Monodispersed, spherical, oval	381-383	[87]
Rhodococcus Euplotes focardii	UV-VIS, FTIR, XRD, TEM, DLS	10-70	Monodispersed, spherical, oval	381-383	[87]
Pseudomonas fluorescens	UV-VIS, FTIR, XRD, TEM	15.6-34.2	Spherical	420-560	[92]
Scenedesmus obliquus	SEM, AFM	100	Spherical	-	[95]

Note: UV-VIS, Ultra Violet Visible Spectroscopy; FTIR, Fourier Transform Infrared Spectroscopy; SEM, Scanning Electron Microscopy; FESEM, Field Emission Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; HRTEM, High-Resolution Transmission Electron Microscopy; DLS, Dynamic Light Scattering; ZP, Zeta Potential; PSA, Particle Size Analyzers; XRD, X-Ray Diffraction; XPS, X-Ray Photon Spectroscopy; XRF, X-Ray Fluorescence; EDX, Energy Dispersive X-Ray; AFM, Atomic Force Microscopy; TGA, Thermogravimetric Analysis; SAED, Selected Area Electron Diffraction; VSM, Vibrating Sample Magnetometer; NTA, Nanoparticle Tracking Analysis.

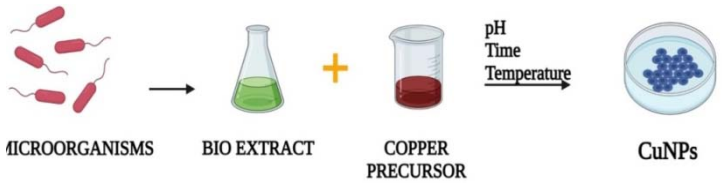

Fig. 7: Microorganisms mediated synthesis of copper nanoparticles

Characterization

The primary step of characterization after the synthesis of CuNPs was to determine the size, shape and morphology of the synthesized nanoparticles. The crystal structure and the chemical composition of the synthesized nanoparticles were analyzed by using various analytical techniques. The techniques like Scanning electron microscopy (SEM), Field emission scanning electron microscopy (FESEM), Transmission electron microscopy (TEM), High-resolution transmission electron microscopy (HRTEM), Dynamic light scattering (DLS), Zeta potential (ZP), Particle size analyzers (PSA) were used to determine their morphology. The various spectral, thermal and other techniques like UV-Vis spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), X-Ray Photon spectroscopy (XPS), X-Ray fluorescence (XRF), Atomic force microscopy (AFM), Thermogravimetric analysis (TGA), Energy-dispersive X-ray (EDX), Selected area electron diffraction (SAED), Vibrating sample magnetometer (VSM) and Nanoparticle tracking analysis (NTA) were used to determine the elemental composition and other features of the synthesized CuNPs. Based on the previous studies, CuNPs exhibited the size between the range of 1–250 nm with spherical, oval, tiny globular, cylindrical, irregular, polygonal, hexagonal, rod, elliptical, agglomerated and mono-dispersive shapes. All the synthesized CuNPs showed their excitation at the range between 190-631 nm. The characterization results of the synthesized CuNPs are given in table 4.

Biological applications

Copper nanoparticles are most commonly used in the emerging interdisciplinary field of nanobiotechnology and in biomedical technology. CuNPs have extensive applications in various fields due to their constant renewable surface, nontoxic and low cost of preparation [88, 89]. This review suggests that CuNPs can act as antioxidant, anticancer, antibacterial, antifungal, anti-diabetic, anti-nociceptive and wound healing agents (fig. 8).

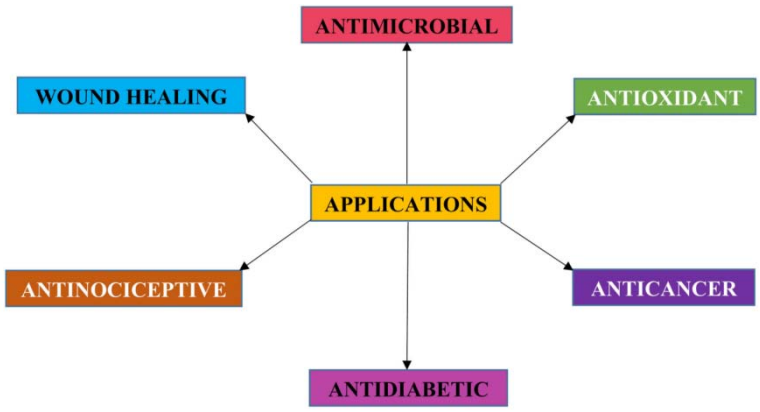

Fig. 8: Biological applications of copper nanoparticles

Antibacterial activity

Gram-positive and Gram-negative bacteria are distinguished by the structure of their cell walls. Gram-positive bacteria have a thick peptidoglycan layer in their cell wall, whereas Gram-negative bacteria have a thin peptidoglycan layer with a periplasm membrane layer. Due to the difference in cell walls, Gram-positive bacteria develop resistance to the nanoparticle's mechanism. CuNPs had a superior antibacterial effect against the causative agents. For analysing the anti-bacterial activity of the synthesised copper nanoparticles, the zone of inhibition is to be considered [90, 91]. CuNPs generated from the various plant extracts showed greater activity against pathogens such as *Bacillus subtilis, Escherichia coli, Klebsiella sp., Pseudomonas aeruginosa and Staphylococcus aureus.* The susceptible organisms and the zone of inhibition are shown in table 5.

Table 5: Antibacterial activity of biosynthesized CuNPs

Plant extracts	Tested organisms	Media/Assay used	Concentration (µg/ml)	Diameter of zone of inhibition (µg/ml) /inhibition (%)	References
Trigonella foenum-graecum	*Acinetobacter calcoaceticus*	Agar well diffusion method	62.0	15.0±0.5	[23]
Eryngium caucasicum	*Bacillus cereus*	Agar diffusion method	60	21.1	[17]
Syzygium aromaticum	*Bacillus sp.*	Kirby–Bauer disc diffusion assay	16	8	[37]
Allium sativum	*Bacillus subtilis*	Agar well diffusion method	75	18	[1]
Clerodendrum inerme		Agar well diffusion method	0.08	0.04	[16]
Trigonella foenum-graecum		Agar well diffusion method	62.0	13.0±0.1	[23]
Allium saralicum		Agar well diffusion method	64	43.4±0.89	[31]
Allium eriophyllum		Disc diffusion method	64	34.2±0.83	[32]
Falcaria vulgaris		Disc diffusion method	64	26.6±0.89	[38]
Piper longum		Agar well diffusion method	60	11	[44]
Mitragyna parvifolia		Agar disc diffusion method	75	17.50	[46]
Cinnamomum zeylanicum		Agar well diffusion method	10	18	[56]
Trigonella foenum-graecum	*Citrobacter freundii*	Agar well diffusion method	62.0	11.0±1.0	[23]
Trigonella foenum-graecum	*Enterobacter agglomerans*	Agar well diffusion method	62.0	12.0±0.6	[23]
Trigonella foenum-graecum	*Enterobacter cloacae*	Agar well diffusion method	62.0	9.0±1.0	[23]
Cinnamomum zeylanicum	*Enterobacteria*	Agar well diffusion method	10	19	[56]
Moringa oleifera	*Enterococcus faecalis*	Resazurin microtiter assay	10	250	[13]
Cissus vitiginea	*Enterococcus sp.*	Agar disc diffusion method	75	20.3	[26]
Allium sativum	*Escherichia coli*	Agar well diffusion method	75	13	[1]
Allium sativum		Disc diffusion method	10	19	[4]
Zingiber officinale		Disc diffusion method	10	18	[4]
Moringa oleifera		Resazurin microtiter assay	10	500	[13]
Clerodendrum inerme		Agar well diffusion method	0.30	0.80	[16]
Eryngium caucasicum		Agar diffusion method	60	23.3	[17]
Vaccinium sp.					
Persea americana		Broth dilution method	0.4	117±27	[19]
Trigonella foenum-graecum		Disc diffusion method	75	15.06±0.13	[22]
Cissus vitiginea		Agar well diffusion method	62.0	14.0±0.6	[23]
Citrus medica		Kirby–Bauer disc diffusion assay	75	22.2	[26]
Allium saralicum		Disc diffusion method	20	28	[27]
Allium eriophyllum		Agar well diffusion method	64	34.2±0.44	[31]
Syzygium aromaticum		Agar well diffusion method	64	29.2±0.83	[32]
Falcaria vulgaris		Agar disc diffusion method	16	6.0	[37]
Citrus limon		Disc diffusion method	64	22.2±0.44	[38]
Piper retrofractum vahl		Agar well and disc diffusion method	25	4.5	[41]
Piper longum		Kirby–Bauer disc diffusion assay	0.2	2.0	[43]
Mitragyna parvifolia		Agar well diffusion method	60	12	[44]
Cissus arnotiana		Agar disc diffusion method	75	13.80	[46]
Prosopis cineraria		Nutrient agar medium	50	19.20±0.11	[47]
Cinnamomum zeylanicum		Disc diffusion method	50	22.6±2.0	[55]
Artemesia haussknechti		Agar well diffusion method	10	25	[56]
		Agar disc diffusion method	0.1	34±2.64	[60]
Moringa oleifera	*Klebsiella pneumoniae*	Resazurin microtiter assay	10	500	[13]
Trigonella foenum-graecum		Agar well diffusion method	62.0	16.0±1.0	[23]
Citrus medica		Disc diffusion method	20	20	[27]
Prosopis cineraria		Disc diffusion method	50	22.7±1.0	[55]
Clerodendrum inerme	*Klebsiella sp.*	Agar well diffusion method	0.14	0.09	[16]
Persea Americana		Agar well diffusion method	75	20.16±0.13	[22]
Cissus vitiginea		Agar disc diffusion method	75	18.5	[26]
Cissus arnotiana		Nutrient agar medium	50	15.20±0.12	[47]
Zingiber officinale	*Listeria monocytogenes*	Kirby–Bauer disc diffusion assay	20	55±1.25	[33]
Punica granatum	*Micrococcus luteus*	Agar well diffusion method	100	20.33±1.53	[24]
Citrus medica	*Propionibacterium acne*	Disc diffusion method	20	20	[27]
Cissus vitiginea	*Proteus sp.*	Agar disc diffusion method	75	16.33	[26]

Plant extracts	Tested organisms	Media/Assay used	Concentration (µg/ml)	Diameter of zone of inhibition (µg/ml) /inhibition (%)	References
Prosopis cineraria	*Proteus vulgaris*	Disc diffusion method	50	17.7±0.7	[55]
Allium sativum	*Pseudomonas*	Disc diffusion method	10	14	[4]
Zingiber officinale	*aeruginosa*	Disc diffusion method	10	14	[4]
Trigonella foenum-graecum		Agar well diffusion method	62.0	14.0±0.6	[23]
Punica granatum		Agar well diffusion method	100	18.67±1.53	[24]
Allium saralicum		Agar well diffusion method	64	39.4±0.54	[31]
Allium eriophyllum		Agar well diffusion method	64	30.6±0.89	[32]
Piper longum		Agar well diffusion method	60	13	[44]
Prosopis cineraria		Disc diffusion method	50	18.1	[55]
Zingiber officinale	*Pseudomonas fluorescens*	Kirby–Bauer disc diffusion assay	20	35±1.21	[33]
Syzygium aromaticum	*Pseudomonas sp.*	Kirby–Bauer disc diffusion assay	16	7	[37]
Cissus arnotiana	*Rhizobium sp.*	Nutrient agar medium	50	16.07±0.25	[47]
Persea americana	*Rizhobacterium*	Agar well diffusion method	75	12.09±0.16	[22]
Vaccinium sp.	*Saccharomyces cerevisiae*	Broth dilution method	0.4	60	[19]
Punica granatum	*Salmonella enterica*	Agar well diffusion method	100	18.67±1.53	[24]
Citrus medica	*Salmonella Typhi*	Disc diffusion method	20	22	[27]
Eryngium caucasicum	*Salmonella typhimurium*	Agar diffusion method	100	23.1	[17]
Artemesia haussknechtii	*Serratia marcescens*	Agar disc diffusion method	0.1	4±1.52	[60]
Moringa oleifera	*Staphylococcus*	Resazurin microtiter assay	10	500	[13]
Clerodendrum inerme	*aureus*	Agar well diffusion method	0.10	0.95	[16]
Eryngium caucasicum		Agar diffusion method	60	21.33	[17]
Trigonella foenum-graecum		Kirby–Bauer disc diffusion assay	62.0	15±0.6	[23]
Allium saralicum		Agar well diffusion method	64	35±1.22	[31]
Allium eriophyllum		Agar well diffusion method	64	32±0.7	[32]
Zingiber officinale		Disc diffusion method	20	40±0.87	[33]
Falcaria vulgaris		Disc diffusion method	64	24.2±0.44	[38]
Citrus limon		Agar well and disc diffusion	25	2.2	[41]
Piper retrofractum		method			
Piper longum		Kirby–Bauer disc diffusion assay	0.2	1.4	[43]
		Agar well diffusion method	60	12	[44]
Prosopis cineraria	*Staphylococcus epidermidis*	Disc diffusion method	50	23.0±1.0	[55]
Allium saralicum	*Streptococcus*	Agar well diffusion method	64	40.4±0.54	[31]
Falcaria vulgaris	*pneumonia*	Disc diffusion method	64	27.2±0.83	[38]
Persea americana	*Streptococcus sp.*	Agar well diffusion method	75	22.23±0.15	[22]
Cissus arnotiana		Nutrient agar medium	50	20.59±0.12	[47]
Ocimum sanctum	*Xanthomonas axonopodis pv. citri*	Potato dextrose agar media	0.03	13.5±1.29	[30]
Ocimum sanctum	*Xanthomonas axonopodis pv. Punicae*	Potato dextrose agar media	0.03	17.25	[30]

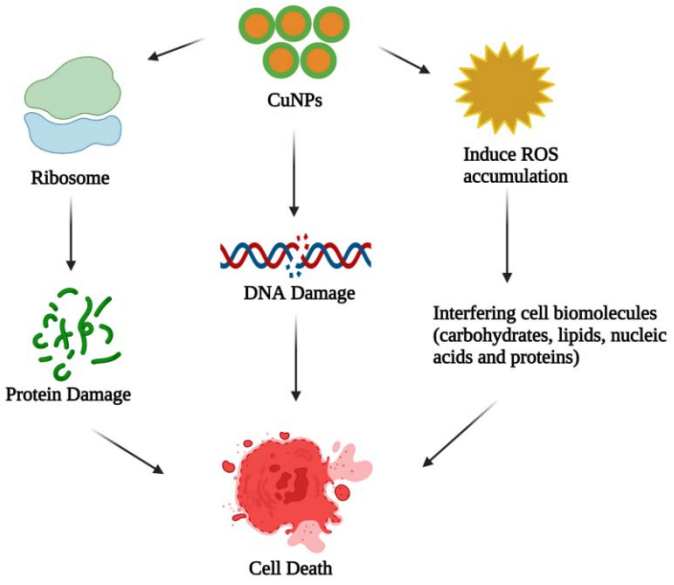

Fig. 9: Graphical representation for the hypothetical mechanism of antibacterial activity of copper nanoparticles

Table 6: Antifungal activity of biosynthesized CuNPs

Tested organisms	Plant extracts	Media/Assay used	Concentration (µg/ml)	Diameter of zone of inhibition (mm) /Inhibition (%)	References
Alternaria carthami	*Ocimum sanctum*	Potato dextrose agar media	0.06	18.5±1.7	[30]
Alternaria mali	*Azadirachta indica*	Modified Food Poisoning Technique	0.05	80	[29]
Aspergillus flavus	*Cissus quadrangularis*	potato dextrose broth	500 ppm	86	[10]
	Moringa oleifera	Resazurin Microtiter Assay	15.6	125	[13]
	Clerodendrum inerme	Potato dextrose agar	0.10	24±0.08	[16]
	Persea americana	Agar well diffusion method	75	9.5±0.2	[22]
	Camellia sinensis	Broth dilution method	10	11.3±1.2	[39]
	Syzygium alternifolium	Disc diffusion assay	40	8.2	[52]
Aspergillus fumigatus	*Persea americana*	Agar well diffusion method	75	10	[22]
Aspergillus niger	*Cissus quadrangularis*	potato dextrose broth	500	85	[10]
	Moringa oleifera	Resazurin Microtiter Assay	7.8	125	[13]
	Clerodendrum inerme	Potato dextrose agar	0.29	17±0.07	[16]
	Ocimum sanctum	Potato dextrose agar media	0.06	12.75±1.7	[30]
	Zingiber officinale	Kirby–Bauer disc diffusion assay	20	25±0.29	[33]
	Syzygium alternifolium	Disc diffusion assay	40	9.0	[52]
	Blumea balsamifera	Disc diffusion assay	80	12±4	[57]
Aspergillus parasiticus	*Camellia sinensis*	Broth dilution method	10	18.4±1.6	[39]
Botryosphaeria dothidea	*Azadirachta indica*	Modified Food Poisoning Technique	0.25	85	[29]
Candida albicans	*Moringa oleifera*	Resazurin Microtiter Assay	31.2	62.5	[13]
	Trigonella foenum-graecum	Agar well diffusion method	0.5	15.0	[23]
	Allium saralicum	Disc diffusion method	60	39.6±0.89	[31]
	Allium eriophyllum	Agar well diffusion method	60	37.8±0.44	[32]
	Falcaria vulgaris	Agar well diffusion method	64	22.6±1.34	[38]
Candida glabrata	*Moringa oleifera*	Resazurin Microtiter Assay	62.5	31.2	[13]
	Allium saralicum	Disc diffusion method	60	38.4±0.54	[31]
	Allium eriophyllum	Agar well diffusion method	60	39.6±1.14	[32]
	Falcaria vulgaris	Agar well diffusion method	64	24.6±1.34	[38]
Candida guilliermondii	*Allium saralicum*	Disc diffusion method	60	42.8±1.09	[31]
	Allium eriophyllum	Agar well diffusion method	60	39.6±1.14	[32]
	Falcaria vulgaris	Agar well diffusion method	64	26±1	[38]
Candida krusei	*Allium saralicum*	Disc diffusion method	60	39±1.22	[31]
	Allium eriophyllum	Agar well diffusion method	60	41.0±1.0	[32]
	Falcaria vulgaris	Agar well diffusion method	64	27.8±1.09	[38]
Colletotrichum gloeosporioides	*Ocimum sanctum*	Potato dextrose agar media	0.03	11.5±1.0	[30]
Colletotrichum lindemuthianum	*Ocimum sanctum*	Potato dextrose agar media	0.03	15.25±0.5	[30]
Diplodia seriata	*Azadirachta indica*	Modified Food Poisoning Technique	0.10	90	[29]
Fusarium culmorum	*Citrus medica*	Disc diffusion assay	20	34	[27]
Fusarium graminearum	*Citrus medica*	Disc diffusion assay	20	22	[27]
Fusarium moniliforme	*Zingiber officinale*	Kirby–Bauer disc diffusion assay	20	20±0.93	[33]
Fusarium oxysporum	*Curcuma longa*	Agar diffusion method	10	65	[18]
	Persea americana	Agar well diffusion method	25	12.2±0.03	[22]
	Citrus medica	Disc diffusion assay	20	29	[27]
	Celastrus paniculatus	Food poison method	0.24	76.29±1.52	[34]
Fusarium oxysporum f. sp. carthami	*Ocimum sanctum*	Potato dextrose agar media	0.06	14.75±1.25	[30]
Fusarium oxysporum f. sp. cicero	*Ocimum sanctum*	Potato dextrose agar media	0.03	13.5±1.25	[30]
Macrophomina phaseolina	*Ocimum sanctum*	Potato dextrose agar media	0.03	12.5±0.5	[30]
Penicillium sp.	*Syzygium aromaticum*	Kirby–Bauer disc diffusion assay	16	6	[37]
Rhizoctonia bataticola	*Ocimum sanctum*	Potato dextrose agar media	0.03	10.5±0.5	[30]
Rhizoctonia solani	*Manilkara zapota*	Potato dextrose agar media	50	24.4	[40]
			100	56.6	
			200	65.5	
Rhizopus stolonifer	*Ocimum sanctum*	Potato dextrose agar media	0.03	11.75±1.5	[30]
Sclerotium oryzae	*Manilkara zapota*	Potato dextrose agar media	50	61.1	[40]
			100	88.9	
			200	100	

For the antibacterial mechanism, CuNPs intracellularly permeate the Cu^{2+}ions by interacting with the bacterial cell membrane. Many plant-derived CuNPs with antibacterial effects also have antioxidant characteristics. Likewise, CuNPs produced by *C. vitiginea* has antioxidant activity, which helps to limit the growth of bacteria that cause urinary tract infections. CuNPs from the extract *Allium sativum* and *Allium eriophyllum* leaf extract, on the other hand, have antibacterial properties, which could be owing to their antioxidant properties [11, 26, 31, 32]. The hypothetical antibacterial mechanism of CuNPs is given in fig. 9.

Antifungal activity

Among the various species of fungi, *Aspergillus* and *Fusarium* species play a major role in influencing the yield of small grains. The plant extracts contain proteins found to protect the plants from fungal infection [92-95]. CuNPs generated from the various plant extracts showed better activity against fungal pathogens. CuNPs synthesized from *Aspergillus flavus, Aspergillus niger, Candida sp., Fusarium sp.* and *Rhizoctonia solani* exhibited better activity. Table 6 shows the susceptible fungal species, minimum inhibitory concentration and diameter zone of inhibition of fungal medicated biogenic CuNPs.

For the antifungal mechanism, CuNPs intracellularly permeates the Cu^{2+}ions by interacting with the fungal cell membrane. According to a recent study, the caused cell wall damage and accumulated reactive oxygen species (ROS) in *Aspergillus flavus*, demonstrating an antifungal activity. Furthermore, the CuNPs made from *Allium sativum* extract has antioxidant activity, which could contribute to the antifungal properties [11, 32]. The hypothetical antifungal mechanism of CuNPs is given in fig. 10.

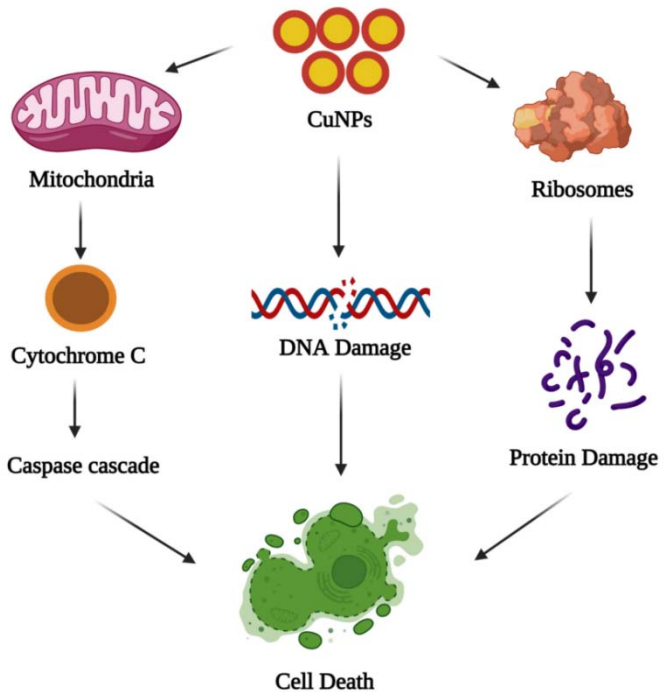

Fig. 10: Graphical representation of the hypothetical mechanism for the antifungal activity of copper nanoparticles

Table 7: Antioxidant activity of biosynthesized CuNPs

Plant	Methods involved	Concentration (µg/ml)	% Scavenging activity	References
Moringa oleifera	DPPH radical scavenging assay and phosphomolybdate assay	500	29.3	[13]
Azadirachta indica	ABTS, DPPH and H_2O_2 radical scavenging assay	80	38	[14]
Hibiscus rosa-sinensis	ABTS, DPPH and H_2O_2 radical scavenging assay	80	21.06	[14]
Murraya koenigii	ABTS, DPPH and H_2O_2 radical scavenging assay	80	25.89	[14]
Tamarindus indica	ABTS, DPPH and H_2O_2 radical scavenging assay	80	34.82	[14]
Eclipta prostrata	DPPH radical scavenging assay	500	53	[15]
Abutilon indicum	DPPH radical scavenging assay	60	90±0.23	[16]
Clerodendrum inerme	DPPH radical scavenging assay	60	83±0.23	[16]
Clerodendrum infortunatum	DPPH radical scavenging assay	60	78±0.25	[16]
Eryngium caucasicum trautv	DPPH radical scavenging assay	100	58.98	[17]
Persea americana	DPPH radical scavenging assay	80	22	[22]
Trigonella foenum-graecum	DPPH radical scavenging assay	20 kGy (radiation source)	43	[23]
Cissus vitiginea	DPPH radical scavenging assay	40	21	[26]
Allium saralicum	DPPH radical scavenging assay	250	228	[31]
Allium eriophyllum	DPPH radical scavenging assay	250	206	[32]
Zingiber officinale	DPPH and H_2O_2 radical scavenging assay	20	75±0.87	[33]
Falcaria vulgaris	DPPH radical scavenging assay	125	109	[38]
Cissus arnotiana	DPPH radical scavenging assay	40	21±2	[47]
Olea europea	DPPH radical scavenging assay	400	45	[59]
Artemesia haussknechtii	DPPH radical scavenging assay	500	74.45	[60]
Magnolia champaca	ABTS and DPPH radical scavenging assay	500	76.30	[70]

Antioxidant activity

Anti-oxidant activity is a capability of a biological compound to inhibit lipid oxidation reaction and to maintain the function and structure of cells by destroying the free radicals. Flavanoids, particularly naringin, naringenin, hesperidin, quercetin and rutin, have antioxidant activity by inhibiting oxidant enzymes in the body, enhancing antioxidant enzyme activity, scavenging ROS directly, anti-lipid oxidation and decreasing the quality of peroxide formation [96-97]. CuNPs generated from the various plant extracts showed a greater scavenging activity. CuNPs generated from the leaves extract *Abutilon indicum, Clerodendrum infortunatum* and *Clerodendrum inerme* showed better scavenging activity [16]. The percentage of scavenging activity prior to its plant extract concentration is given in table 7.

Anticancer activity

Apoptosis induction and inhibition of tumor cell proliferation are the approaches engaged in the treatment of cancer. Anti-cancer agents exhibit high toxicity to the tumor cell and also to the normal cells of the body where cancer developed [98, 99]. CuNPs obtained from the various plant extracts exhibited anticancer activity, particularly in breast, cervical, colon, epithelial, liver, lung and skin cancers. The CuNPs obtained from the species like *Tilia cordata* [36], *Manilkara zapota* [40] and *Prosopis cineraria* [55] exhibited better cytotoxicity against MCF-7 (breast) cell line. CuNPs from the leaves extract of *Olea europea* [59] showed inhibition against AMJ-13 (breast) cancer cell line Against MDA-MB-231, *Abutilon indicum, Clerodendrum inerme, Clerodendrum infortunatum* [16] and *Syzygium alternifolium* [52] showed the activity. *Zingiber officinale* [4], *Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii* and *Tamarindus indica* [14] showed anticancer activity against HeLa (cervical) cell line. *Tilia cordata* [36] exhibited cytotoxicity against Caco-2 (colon) cell line. The growth inhibition of the Hep-2 (epithelioma) cell line was observed by the CuNPs prepared using the leaves extract of *Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii* and *Tamarindus indica* [14]. The CuNPs from the green extract of *Allium saralicum* [31], *Allium eriophyllum* [32] and *Falcaria vulgaris* [38] inhibited the growth of the HUVEC (umbilical vein) cell line. Against the HepG2 (liver) cell line, the CuNPs prepared from the extracts of *Allium sativum, Zingiber officinale* [4], *Eclipta prostrate* [15] and *Tilia cordata* [36] exhibited better inhibition. *Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii, Tamarindus indica* [14] and *Quisqualis indica* [50] exhibited better anticancer activity against A549 (lung) and B16F10 (melanoma) cell lines, respectively. The activity against various cell lines is given in table 8.

Table 8: Anticancer activity of biosynthesised CuNPs

Types of cell	Cell lines	Plant extracts	Assay/Method involved	Ic_{50} value (µg/ml)	Reference
Breast cancer	AMJ-13	*Olea europea*	MTT assay	1.47	[59]
	MCF-7	*Tilia cordata*	MTT assay	12.21	[36]
	MDA-MB-231	*Manilkara zapota*	MTT assay	53.89	[40]
		Prosopis cineraria	MTT assay	65.27	[55]
		Clerodendrum inerme	MTT assay	85±0.05	[16]
		Syzygium alternifolium	MTT assay	50	[52]
Cervical cancer	HeLa	*Zingiber officinale*	MTT assay	<80	[4]
		Azadirachta indica	MTT assay	20.32±1.16	[14]
Colon cancer	Caco-2	*Tilia cordata*	MTT assay	11.21	[36]
Epithelioma	Hep-2	*Azadirachta indica*	MTT assay	21.66±1.22	[14]
Endothelial cell	HUVEC	*Allium saralicum*	MTT assay	85	[31]
		Allium eriophyllum	MTT assay	95	[32]
		Falcaria vulgaris	MTT assay	85	[38]
Liver cancer	HepG2	*Zingiber officinale*	MTT assay	<80	[4]
		Eclipta prostrate	MTT assay	500	[15]
		Tilia cordata	MTT assay	19.88	[36]
Lung cancer	A549	*Azadirachta indica*	MTT assay	18.11±0.93	[14]
Melanoma	B16F10	*Quisqualis indica*	MTT assay	102	[50]

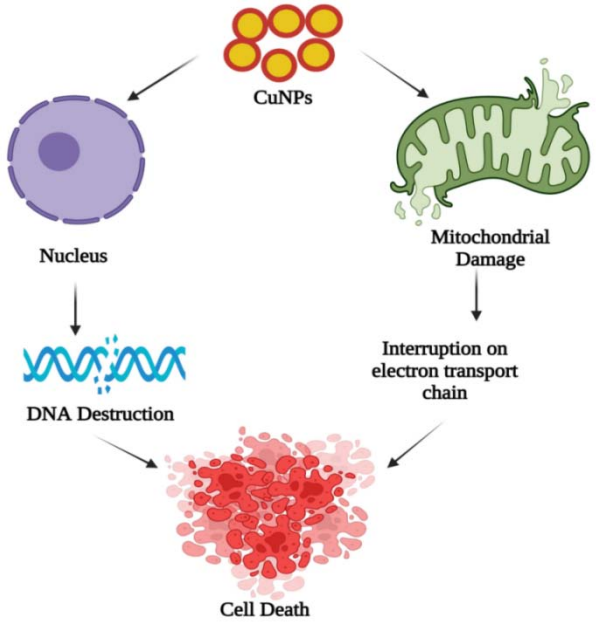

Fig. 11: Graphical representation of the hypothetical mechanism for the anticancer activity of copper nanoparticles

Most of the plant extracts induced apoptosis by the generation of ROS and nitrogen oxide. The uptake of the synthesized CuNPs regulated the nitrogen oxide level in various cancer cell lines. Some of the extracts caused G2/M cell cycle arrest and increased p53 expression, as well as inhibiting histone deacetylase, which removes the acetyl group on histones to form a non-transcriptional compact chromatin structure. The Bak/Bax expression, BCl-2, caspase-9 and caspase-7 were up regulated on treating with CuNPs. Some of the extracts decreased the level of various enzymes and increased tumor suppressing genes [11]. The hypothetical mechanism of CuNPs in the anticancer activity is given in fig. 11.

Antidiabetic activity

α-amylase and *α-glucosidase* are the most favourable candidates for the prevention and treatment of T2DM. The two most significant methods for diabetes control are inhibition of *α-amylase* and *α-glucosidase* and scavenging of free radicals. CuNPs obtained from the leaves extract of *Gnidia glauca* and *Plumbago zeylanica* against porcine pancreatic *amylase* inhibition assay exhibited the most promising inhibition as that of standard acarbose. From the *α-glucosidase* inhibition assay, the synthesised CuNPs showed the highest *α-glucosidase* inhibition as that of standard acarbose. The circular dichroism analysis was also performed and it revealed the nature of the interaction of CuNPs with Porcine pancreatic *α-amylase* and *α-glucosidase* [51].

Antinociceptive activity

Pain is a sensory and defensive system that alerts the living organism to the dangers in its environment and allows it to respond appropriately. The antinociceptive effect of the synthesized CuNPs from the fruit extract of *Capparis spinosa* was evaluated by tail-flick method, hot plate method and rotarod method using mice model. The antinociceptive effect was achieved in combination with morphine. As a dose-dependent response, CuNPs at the concentration of 25, 50 and 75 mg/kg had potent antinociceptive activity [48].

Wound healing activity

Physical damage, water loss and harmful chemical invasion are all protected by the skin. A wound is when the integrity of the skin's normal anatomical structure is compromised. The term "healing" refers to the return of normal anatomical structure and function. The phases of haemostasis, inflammation, proliferation, and remodelling, which involve cutaneous cell-cell and cell-matrix interactions, make up wound healing [32]. The CuNPs obtained from the extracts of *Allium saralicum* [31], *Allium eriophyllum* [32] and *Falcaria vulgaris* [38] exhibited notable cutaneous wound healing activity. The CuNPs/CuNPs ointment obtained from the above extracts increased the concentration of hydroxyproline, hexosamine, hexuronic acid and fibrocyte/fibroblast ratio significantly.

LIMITATIONS

There is insufficient data to compare the various precursors and their effects on the green synthesis of CuNPs. Several investigations indicate that more research is needed to establish the effect of precursors on the size and form of NPs generated from plants. Increased quantities of plant extract have been used to accelerate the reduction of copper ions in solution, which increases the rate of synthesis of CuNPs. Due to the addition of an excess amount of plant extracts, the morphology of the particles will be changed. During long-term storage, NPs can aggregate, shrink, or expand and they also have a shelf life that impacts their total potential. The size of NPs depends upon the reaction time and temperature. i.e., a higher temperature is required to synthesize the smaller NPs [100-103].

FUTURE DIRECTIONS

NPs have recently been used as nanomedicines that can be used as delivery agents by encapsulating or attaching therapeutic pharmaceuticals and more effectively delivering them to the targeted tissues or cells. Due to their advantages like cost-effectiveness, quick, and non-hazardous nature, it can be used in commercial productions and used to treat various diseases and disorders using targeted drug delivery concept. They are made at tiny sizes to allow unrestricted mobility in the human body while destroying cancer cells and also by means of transdermal delivery, the treatment of diabetes, wounds, burns, etc. will be achieved.

CONCLUSION

Using traditional physical and chemical processes necessitated the use of hazardous substances at a significant expense. The synthesis of nanoparticles using a biological approach is an eco-friendly, non-toxic, cost-effective and rapid approach. This review has focussed on the greater benefit of the biological method of synthesizing CuNPs. It gives the summarized data of the plants and micro-organisms used in the preparation of CuNPs, along with its characterization techniques. Copper nanoparticles synthesized by biological method express anti-oxidant, anti-cancer, anti-bacterial, anti-fungal, antidiabetic, antinociceptive and cutaneous wound healing activities. The limitations like the effect of the precursors, extracts along with time and temperature, are also discussed.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

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