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Green Synthesis and Characterization of Cu-Based Nanoparticles by Apple Peel and Assessment of their Antioxidant Activity

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Article Details

ABSTRACT

Keywords: Apple Peel, Copper, Nanoparticles, Green Chemistry, Antibacterial

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For the nanoparticle's synthesis, green chemistry is a recent development and has drawn a lot of interest due to its capacity to develop advanced, reliable, attentive, and unadventurous methods. By manipulating green synthesis, formation of Cu-NPs is carried out with apple peels extracts which also acts as reducing substance. Due to its distinctive optical, electrical, imaging and catalytic properties, Cu-NPs rather than other metal nanoparticles have received an abundance of interest in a wide range of applications such as fabrication of gas detectors, photovoltaic cells as well as delivery of therapeutic material. The green process offers a lot of benefits comprehended are easily operated, harmless and environmentally secure. Cu-NPs exhibits activities like anti-oxidant, antibacterial, anti-fungal in fields of bio-medicine. Apple also contain anti-oxidative, anti-proliferative, anti-carcinogenic and anti-inflammatory properties. Epi-catechin, oligomer, trimer, glycosides quercetin, chlorogenic acid, phloridzin-3-hydroxy, pro-cyanidin, are the significant phenolic compounds that are found in apple peels. To analyzed the copper based nanoparticles different characterization techniques are employed includes UV-Visible spectrometer, X-Ray diffraction, FTIR-spectroscopy, EDX, TEM and SEM techniques. UV-Visible verified the absorption peak of NPs, XRD analysis of metallic NPs showed peaks that directly linked to its shaped. It shows the crystalline nature of Cu-NPs. According to FTIR results, bio-active functional groups are served as reducing and capping agents. Cu-NPs formation verified by EDAX. The homogenous spherical particles produced by green approach are visible by using TEM and SEM techniques.

INTRODUCTION

Nanoparticles are illustrated as very small tiny particles whose size is less than 100nm and possess a remarkable approach in the fields of therapeutic drug dispensing, science of chemicals and analyzing matter (Hamelian, Hemmati, Varmira, & Veisi, 2018). Some considerable innovative qualities are present in inorganic nanoparticles because of their Nano-size which carried out physical, chemical and biological characteristics and due to its broad qualities, these particles of Nano-sizes are mostly employed (Sharma *et al.*, 2019). That's why complexes of metal components and their derivatives are concentrated towards the synthesis of Nano size bio-particles (Aazadfar, Solati, & Dorranean, 2018).

Nano-particles are categorized in many ways on the base of their size, origin and material qualities (Jeevanandam, Barhoum, Chan, Dufresne, & Danquah, 2018). It is sorted on the base of material which includes Nano-materials that relate to carbon, nanotubes, nanofibers and etc. Those Nano-materials that are inorganic in nature comprehends silver, gold and metal and metal oxide nanoparticles, and those Nano-sizes particles that own organic nature undergoes weak interactivity. Composite nano-materials have complex structural framework of metal and organic and they are multi-component. It could include metal, carbon and organic compound in addition to other metal.

Zero dimensional Nano-particles are among those that are codified on their dimension which comprise quantum dots and boxes. Nano-particles that are one dimensional structure includes poly-ethylene oxide fiber's and etc. In two-dimensional Nano-material, nanotubes like materials are sorted. Nanowires of Zinc oxide are three dimensional nanoparticles. Origin based nanoparticles are natural and synthetic. Nano-particles that are naturally created by living things or found on planetary surfaces are regarded as natural nanoparticles.

Nano-particles are produced by the several processes such as physical, chemical, biological and hybrid reduction techniques (Harishchandra *et al.*, 2020). Elevated temperature, pressure and high cost are demanded in physical technique while in chemical technique, noxious and dangerous chemicals are used which are unsafe for the environment that causes the formation of hazardous products (Tahvilian *et al.*, 2019). Not long ago, many empirical researches that comprehends with the nanoparticles using the synthesis of plant extract accompanied by the evolution of nanotechnology (Khane *et al.*, 2022). It is highly demanded to develop high effective approaches, reasonable, and harmless and safe techniques for forming nanoparticles and for this, biological method is considered significant and highly demanded.

Contrary to both chemical and physical techniques, biological process is steady, uncomplicated, easy, efficient and environmentally safe (Khane *et al.*, 2022). For the production of metal nanoparticle by using plant extracts, extensive records are available (Tahvilian *et al.*, 2019). Particles of Nano sizes that are synthesized by the extraction of plants have a significant impact as they are biodegradable, productive and superficial. It is much emphasized for the biosynthesis of metal particles of Nano sizes according to the phenomenon of green synthesis and on the account of environment safety and security, harmless and retarding agents are used in accordance to Nano-particles stability (Parthibavarman, Bhuvaneshwari, Jayashree, & BoopathiRaja, 2019).

Chemically, copper is a substance having a nuclear number 29 (Reddy, 2017) and having a red-orange color (Al-Hakkani, 2020). It contains some significant qualities which are gentle, flexible and ductile and possess heat and electrical conduction (Shanmugam Rajeshkumar *et al.*, 2019). Because of its remarkable role as a part of innumerable enzymes, it is considered a crucial element for maintaining the human health. It is also engaged in several body activities as being a component of the elastic tissue that are found in skin, bone and in further organs of body (Al-Hakkani, 2020). Being taken into analysis in the middle of all the metals comparison, copper considered as more efficient, economical and harmless material (Reddy, 2017). It is considered the essential element for the human beings (Parthasarathy *et al.*, 2020).

From the old days, copper and its derivatives are notable for owing a wide range of spectrum and having antioxidant and antimicrobial activities, that opposed the bacteria, fungi, yeast etc. (Hamad, Atiyea, Hameed, & Dalaf, 2023). It can be employed for the production of metal particles of Nano-sizes, which have the high antibiotic potency (Beyth, Hourri-Haddad, Domb, Khan, & Hazan, 2015). Copper based Nano-particles are extensively utilized in the various fields such as pharmaceutical for the purpose of drug delivery and in cosmetics for the Nano-powder (Schröfel *et al.*, 2014) and used for packaging food (Longano *et al.*, 2012).

Nanoparticles of copper have drawn a lot of concentration on account of their properties which are optical, mechanical, catalytic, and electrical. Furthermore, green synthesis of copper nanoparticles has a beneficial and efficient influence. A diverse amount of techniques and processes are present for the preparation of the copper based nanoparticles such as electrical, thermal and reduction by chemicals (Amer & Awwad, 2020). Nano-particles of inorganic metal copper have an adverse effect in many aspects such as in agriculture, technology and industry. Over the span of years, the branch of bio-sciences possess more approachable and predominantly over all methods for copper Nano-particles which exhibit the efficient activities like antifungal,

antimicrobial and antioxidant (Chung *et al.*, 2017). In contrast to further Nano-particles like as silver and gold, copper based nanoparticles have drawn interest and attention on the basis of its effective qualities (Chung *et al.*, 2017). The importance of copper is also increased in era of COVID-19, in comparison to other metals, due to the faster decomposition on the surface of copper of SARS-COVID (2) (Van Doremalen *et al.*, 2020).

In order to create the particles of Nano-sizes, a range of natural mechanism has devised, that inspired the researches for the production of Nano-particles by consuming the extracts the of plants, bacteria and fungi (Yu et al., 2019). In a short time, for the production of metal nanoparticles, the utilization of plant extracts has gained much novelty on the account of its specialty for being convenient and beneficial. Its use is economical and secure. Extracts that are excluded from the plants exhibit some agents, Reducing agent and stabilizing agent in the preparation of particles of Nano-sizes (Umoren, Obot, & Gasem, 2014).

Apples are planted in the area which are affluent with minerals, and the growth of the plant is medium. It's originated from *Rosacea* family. They are cultivated all over the world. Substantially, grown on the regions that are plenty in minerals, and the tree of Apple was initially cultivated in the area of *Kazakhstan*. The shape of the apple is in oval form and resembles with pears. The outer peel of the apple exhibits the variety of hues depending upon the region of cultivation. Those who are in off white cream color are luscious pulp and its flavor is a mild combination of sweet and sour. Anti-oxidants, phytonutrients, flavonoids and polyphenolics are abundantly present in apple peels (Lee, Kim, Lee, Lee, & chemistry, 2003). Nonetheless, the extraction of pectin and fiber is used in industrial applications (Riaz *et al.*, 2020).

Among other flavonoids, apples contain some significant quantity of *quercetin*, *cate-chin* and *pro-cyanides*, *chloro-genic acid*. Furthermore, they also comprise of tartaric content, which makes them to have a sour taste (Lee *et al.*, 2003). It has been analyzed that synthesis of nanoparticles can be facilitated by using apple peel extracts. As the peels of apple comprise the higher constituents of flavonoids, polyphenolic in comparison to its pulp (K. L. Wolfe, Liu, & Chemistry, 2003) with abundant source of reducing and antioxidant activity (Ting, Chin, & Pollution, 2020) and the apples that had not been peeled has greater effects of anti-oxidant and anti-proliferative activities than that of peeled apples. Moreover, these activities reduce the maturation of cancer cells in the human beings (K. Wolfe, Wu, Liu, & chemistry, 2003). The fruit peels especially apple peel is considered the attractive substitute for the production of the metal Nano-particles (Ting *et al.*, 2020).

Forthwith, to provide the better environment to people and to prevent the humanity, synthetic components by green manufacturing is very essential. Green chemistry is regarded as the better choice for the production of Nano-particles that are non-toxic as comparison to the production of toxic and harmful materials (Agarwal, Menon, Kumar, & Rajeshkumar, 2018); (Shanmugam Rajeshkumar *et al.*, 2019). In the most recent research, the ability of Nano-particles and its composites have gained a center stage to compete with medicinal agents, not only for the development of the innovative medications but also in the treatment of the disease.

Copper based Nano-particles have antimicrobial activity. For instance, active oxygen species produced by the copper based Nano-particles that interconnect with the cell membrane of bacteria to perforate the cells and impede the development of bacteria by inhibiting the cells and maybe even causing the death (Hasanin, Al Abboud, Alawlaqi, Abdelghany, & Hashem, 2021). To combat drug resistance to many drugs, such as harmful bacteria produce bio-films, metal oxide Nano-particles are considered effective, where nickel and iron based Nano-particles are regarded as potent than the copper based Nano-particles (Harishchandra *et al.*, 2020).

Applications for copper based Nano-particles comprehends biomedical, in the treatment of cancer, fungus, bacteria, viruses, photo-catalytic and agriculture which are effectual, inexpensive and comprehensive (Santhoshkumar, Agarwal, Menon, Rajeshkumar, & Kumar, 2019). Precisely, using copper based nanoparticles buildings are constructed because it smoothens the surface of the which increases its resistance to corrosion that improves its properties like thermal conductivity and heat transfer, copper based nanoparticles that are coated by polymer also exhibit the anti-bacterial and hydrophobicity properties (Shah, Lu, & Materials, 2018). Not long ago, inorganic Nano-particles that comprised of both metals and non-metals (such as silver, gold, iron, zinc) are being synthesized with increasing sophistication. They have demonstrated the widespread use across various industries.

Fundamentally, for the synthesis of Nano-particles, there are two processes are utilized which are Top-down, and bottom-up approaches. With the bottom-up approach green synthesis has sustained the contemplation, for the reason that, it is reasonable, hypoallergenic, safe and ecological for the green-synthesis of Nano-particles (Santhoshkumar *et al.*, 2019). High pressure, temperature and energy and dangerous chemicals is indispensable in green-synthesis (Devatha & Thalla, 2018). For the green-synthesis, bottom-up strategy is comparable to the chemical Nano-particles reduction, with the exception of that chemical reducing agent is being swapped out by the extractions of plants, fruits, algae and fungi (Hussain, Singh, Singh, Singh, & Singh, 2016);

(Pal, Rai, & Pandey, 2019). Collecting the atoms, molecule's or other microscopic particles to create the particles of Nano-sizes is known as bottom-up strategy (Thakkar, Mhatre, & Parikh, 2010).

Plants extract has received supremacy as compared to bacteria and fungi for the large-scale production of Nano-particles via green synthesis. It includes the elimination of requirement for the preparation of the culture and prevention, quick production periods, stability and environment friendly (Gowramma, Keerthi, Rafi, & Muralidhara Rao, 2015);(Makarov *et al.*, 2014). By using fruit and plant extract, copper based Nano-particles that are spherical and 5-10nm size, are synthesized by taking the homogenous mixture of cupric solution and fruit extract at elevated temperature and pressure with time (Naika et al., 2015). These nanoparticles have effective effect against harmful micro-organisms like *Staphylococcus aureus* and *Klebsiella aerobe-genes* (Jayalakshmi, Yogamoorthi, & Biostructures, 2014).

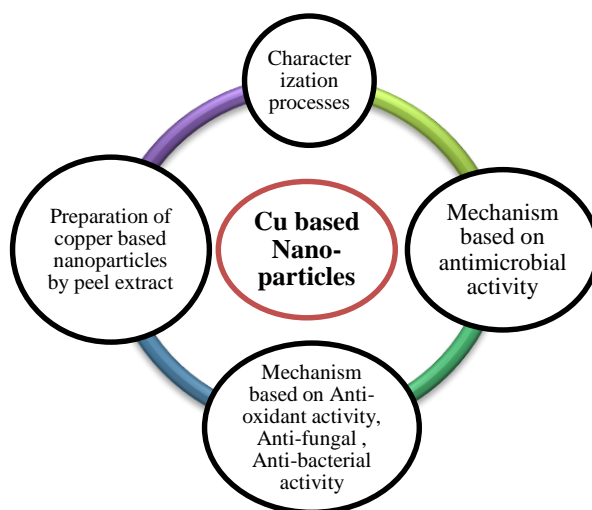


FIGURE 1.1: GENERAL FRAMEWORK OF COPPER-BASED NANOPARTICLES

Apple peels are enticing substitutes for the production of copper Nano-particles due to their abundant source of reducing and anti-oxidant chemicals (Ting *et al.*, 2020). The ability of synthesized Nano-particles is then exploded by the dyes such as malachite green dye. It is a cationic, non-volatile, *diamino-triphenylmethane* color which is made of *triphenylmethane* (Yang *et al.*, 2011) mostly used as coloring agent for food, paper, cotton and etc. and in aqua-culture as fungicide, bactericide and parasiticide (Fischer, Werner, & Goss, 2011).

Nano-technology focuses on the creating of nanoparticles with sizes between 1nm to 100nm. In

the discipline of biological findings, chemical research, it indicated as developing and alluring topic for study and exploration (LewisOscar *et al.*, 2016). All the world, a great interest has shown on the researches that are based on the Nano technology (Chandra, Kumari, Bontempi, Yadav, & Biotechnology, 2020). In the fields of biological and medical sciences, conversion of energy by solar sources, treatment of water and etc. Nano-technology provides the solution of all the issues related to environment and technology (Okafor *et al.*, 2013).

GRADING OF NANOPARTICLES

Nanoparticles are usually codified into organic, inorganic, origin and carbon-based.

I. INORGANIC

NANOPARTICLES:

Inorganic nanoparticles are the non-carbon based nano-particles which typically comprised on metal and metal oxide. Metal includes;

- Gold which is visible with light, reacts and interacts (Syed *et al.*, 2016).
- Silver that is stable, anti-septic in nature and reflects light (Ealia *et al.*, 2017).
- Iron that is brittle, reactive in nature, dependent on breathable air and liquid (Harshiny *et al.*, 2015).
- Aluminum that has a vast array of surfaces, high level of reactivity and susceptible to humidity (Geetha *et al.*, 2016) and copper-based nanoparticles that's highly ignited substance, soft and exhibits an exceptionally high thermo and electrical properties (Ryu *et al.*, 2016). And metal oxide includes;
- Titanium that's prevents from bacterial development (Laad *et al.*, 2016).
- Iron oxide that's volatile due to reactive behavior (Ruales-Lonfat *et al.*, 2015).
- Zinc oxide that has antibiotic, non-lethal, anti-mycotic properties (Bajpai *et al.*, 2016).
- Silicon dioxide that are durable and safe to use (Kaynar *et al.*, 2016), etc. and contains semiconductor like silicon etc. or those that aren't biocompatible. Their particle size is less than 100 nm (Sannino *et al.*, 2021).

II. ORGANIC NANOPARTICLES

Organic nanoparticles are derived from the source of material that are organic in nature with the exception of non-carbon-based particles. It can be made into desirable structures and polymers like liposomes, micelles, dendrimers through weak interactions (Jeevanandam *et al.*, 2018). Some

of these nanoparticles like liposomes that are also known as nano-capsules are non-poisonous, safe and reusable. Heat and electro-magnetic rays can affect it (Tiwari *et al.*, 2008). Due to their effectiveness and the fact that they can be inoculated into certain body sections, widely employed in the field of medicine as drug delivery system.

III. CARBON BASED NANO-PARTICLES

Nanoparticles that are based on carbon are formed entirely from carbon (Bhaviripudi *et al.*, 2007). They are spherical and in hollow-tubes structure. These carbons-based nanoparticles are derived significantly from the graphene, carbon nano-tubes, nano-fiber, fullerenes (carbon-60) and carbon black by using such techniques like laser ablation, electric arc, vapor deposition (Jeevanandam *et al.*, 2018).

▪ FULLERENES

Fullerenes are a spherical carbon-molecule having a discrete number of carbon atoms in their layout (Sannino *et al.*, 2021) with sp^2 -hybridization. Fullerenes transmits light according to its intensity and it acts cautious due to non-reactive behavior. It functions as conductor, semi-conductor and super-conductor (Tenne *et al.*, 2002). *Graphene* contains two-dimensional planar surface forms a hexagram network of shape honey-comb. Graphene sheets are 1nm thick in width. Extremely strong strength, thermal and electrical conductivities and absorption of light are all the attributes of graphene (HUANG *et al.*, 2010).

▪ CARBON-NANOTUBES

Carbon nano-tubes is basically a foil of nanoparticles make up the tube of carbon atoms to create a nano-tubes. The range of its length diversified from few microns to various millimeters. Its single layer diameter is about 0.7nm while multi-layers are about 100nm. Its characteristics include strong, ductile, malleable and show high conductivities (De Volder *et al.*, 2013).

▪ CARBON NANO-FIBER

Carbon based nanofibers sometimes known as carbon-nanotubes, made from the same graphene foil which used for the carbon nanotubes. In spite of that, twisted in a structure of cup shaped rather than cylindrical tubes.

▪ CARBON-BLACK

Carbon black is an unformed structure of spherical shape and have diameter between 20 to 70nm. They are typically in form of aggregate (Ealia *et al.*, 2017).

ORIGINALITY OF NANO-PARTICLES

The foundation for the development of the nanomaterials is both natural and artificial. Nanomaterials on the basis of nature and nanomaterials on the basis of its artificially synthesis.

▪ NATURAL NANOMATERIALS

Natural nano-particles come in nature either from organic class or by human actions. It is simple to accomplish the unnatural surface using natural resource with scale that's are extremely small in capability. Naturally present nano-materials can be found across the spheres of the earth (Hochella *et al.*, 2015) ;(Sharma *et al.*, 2015).

▪ ARTIFICIAL NANOMATERIALS

These types of nano-materials are created by vapor, fumes, gas and grating methods. Moreover, by the physical, biological and chemical methods (Wagner *et al.*, 2014).

PHYSICAL AND CHEMICAL PROPERTIES OF NPS

The physical features of nanoparticles include their color, their ability for absorption of light and reflection along with their potential for absorbing ultraviolet rays and reflecting in fluids when deposited on substrates. It also comprises the mechanical characteristics of the material, such as malleable, soft, brittle, and mobility, that are crucial in how they're employed. Many currently available elements, have more attributes like water-holding capacity, water resistance, and floating form. dispersion, and settling traits.

The employment of nanoparticles in the field of thermal conductivity in projects using renewable energy has been assisted by attracting the magnetic and electromagnetic properties of conducting capacity, semi-conductivity capacity, and resistance.

The chemical properties of nanoparticles employed their ability to endure and sensibility to constituents which include water, surroundings, temperatures & radiation. For medicinal and ecological uses, the nanoparticles antibacterial, antifungal, disinfectant, and toxicological features are suitable (Ealia *et al.*, 2017).

COPPER & CU-NPS ATTRIBUTES

Copper is a crucial component of society and served a variety of functions. The aspects of copper have elevated it to the top spot among substances used in the home like water pipe systems and most notable metal used in air conditioner and radiator in car. Since ancient times it is employed as a biocide (Dollwet *et al.*, 2001). Among the numerous metal Nano-particles, including gold, silver, zinc, palladium, quantum dots, iron, copper is the frequently utilized substance of all on the account of its application (Tian *et al.*, 2012) such as in optics, electronic, bi-omedicine and in the fabrication of conductive metals, emollient and fluids of nano-sizes (Din *et al.*, 2017). As

compared to other conventional catalyst, it can increase yields and reaction rate in favorable reaction condition (Saranyaadevi et al., 2014).

Metals whose selection is remarkably determined by the surface-area that constitute the most of the catalysts. For choosing the catalysts, the substantial framework is a ratio of the high surface to volume that's why this characteristic is extensively researched (Lisiecki *et al.*, 2000). Cu-based nanoparticles alloy usually used as catalysts, since they are less expensive and have higher surface and volume ratio in contrast to noble metals which also serve as catalyst for the water gas transition and gas-detoxification (Barrabes *et al.*, 2006). The ability to manage size, shape and surface is the properties of copper-based nanoparticles that's the important parameter of copper-based catalyst (Umer *et al.*, 2012). As a substitute for the noble metals, copper based Nano-particles have numerous applications that includes heat transfer and micro-electronics (Eastman *et al.*, 2001).

Copper nanoparticles have some drawbacks such as quick oxidation on exposure to oxygen. It is challenging to prepare copper nanoparticles in a medium because it oxidizes to Cuprous oxide and cupric oxide in the time of storage and synthesis. Different routes are devised in order to create metal Nano-particles in the presence of stabilizer with the use of polymers such as poly vinyl pyridine, polyethylene-glycol and chitosan and with the help of surfactants such as Cetyl tri-methyl ammonium bromide to create coatings on the surface of particles of Nano-sizes. Latterly, green synthesis has employed the extracts of plants for the synthesis of metal Nano-particle (Shameli *et al.*, 2012).

Metal nanoparticles made by chemical method with the polymer like chitosan were examined for the characteristics such as antimicrobial and antibacterial. After cellulose, chitosan that occurs in nature mostly is the second most prevalent biopolymer and its isolated from the source chitin by disconnecting an acetyl group. Chitosan is a biologically compatible and biodegradable (Dash *et al.*, 2012) and comprised on the units of glucosamine and N-acetyl glucosamine (Usman *et al.*, 2012). It is a toxic free and finds novelty in many fields such as pharmaceutical and clinical (Dash *et al.*, 2012).

PRIMARY WAYS FOR SYNTHESIS OF NPS

There are two primary ways for creating Nano-particles.

- Top-down.
- Bottom-up.

i. TOP-DOWN METHOD

In this approach, material is being consideration for the size reduction by physio-chemical techniques which result as minuscule particles that are extremely small in size (Al-Haknni *et al.*, 2020). This method uses thermal, chemical and physical technique for the formation of nano-particle (Cerjak *et al.*, 2014). Physical methods like sputtering, laser ablation, ball-milling are used to split down the materials into manageable parts that are in bulk state (Alshammari *et al.*, 2016; Abdolhoseinzadeh *et al.*, 2020). This method is expensive to apply and don't provide flawless border and plane due to coarse and irregularity that develop in nano-materials (Makarov *et al.*, 2014).

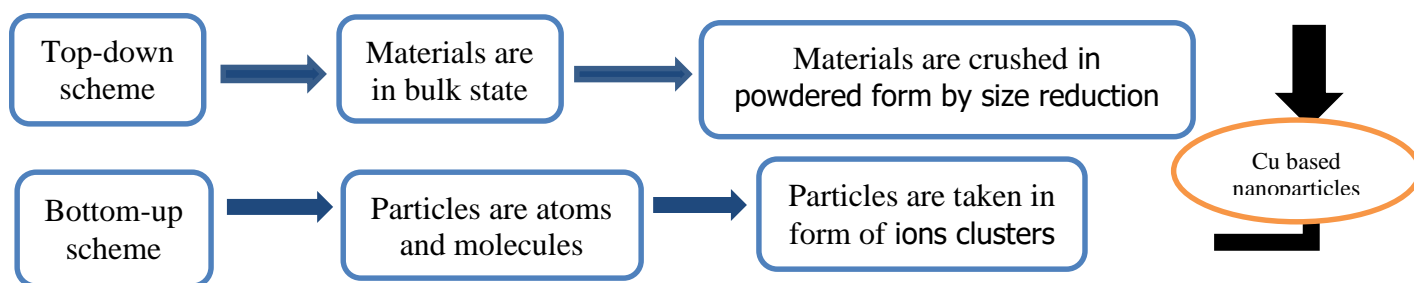


FIGURE 2.1: FRAMEWORK INVOLVES THE CONSTITUTION OF NANO-PARTICLE
(AL-HAKKANI *ET AL.*, 2020)

ii. BOTTOM-UP TECHNIQUE

In bottom-up method, atoms, molecules and small pieces of particles are the building blocks for the production of nano-particles (Al-Haknni *et al.*, 2020). In this method, biological and chemical procedures are applied to create nano-particles by the layer of atoms, molecules etc. Biological technique like condensation-precipitation, sol gel and micro-emulsions (Khodashenas *et al.*, 2014; Ben Hamida *et al.*, 2015; Hussein *et al.*, 2016).

Ecological and conventional procedures are referred to as following the bottom-up strategy (Mohamed *et al.*, 2020). The conventional method for producing nano-particles involves using hazardous substances as a reducing-agent (LIU *et al.*, 2012; Begletsova *et al.*, 2018) while green manner employs the natural substances as reducing-agent (Seetha *et al.*, 2020; Thiruvengadam *et al.*, 2019). By using a bottom-up strategy, good production of nanoparticles may be attained with no waste results that required to exclude. The nanoparticles of extremely small sizes may be produced due to control over the sizes of particles (Makarov *et al.*, 2014).

PHYSICAL TECHNIQUES

a) BALL-MILLING

It is the physical method used for the synthesis of nanoparticles. It is also considered the part of top-down strategy. When forming tiny particles, numerous components are grouped up in an environment that is inert using the milling process mechanically (Yadav *et al.*, 2012). In milling, plastic distortion that decides the particle form, breakage which results in the reduction of particle size then comes with the increase of size of particle. For synthesis of tiny particles, the technique of sputtering is carried out in which the nano-particles are embedded on the layer via emitting atoms through which they hit with the ions (Shah *et al.*, 2006).

b) ION SPUTTERING

In the process of sputtering, the electrodes are accelerated towards the transfer procedure and the material hits by the sputtered molecules which then detach to form the desired layer. Typically, annealing happens when thin film of nanoparticles is added via sputtering. The dimension as well as the form of tiny particles are dependent on the material type and its temperature (Saranyaadevi *et al.*, 2014). By changing the field of magnetism in accordance with the parameters of desired nano-particles, numerous alternatives of ion bombardment pathways can be utilized. Elements such as metal, semiconductor alloys and compounds such as iron oxide, nitric oxide can be sputtered.

c) LASER ABLATION TECHNIQUE

This technique is carried out with solvents and utilized different types of solvents for the formation of nanoparticles. A plasma that creates nanoparticles become condensed when the light from the laser hits on the metal which immersed in the liquid mixture (Amendola *et al.*, 2009). This process basically eliminates the material from the solid or liquid phase with the exposure of beam light. When there is insufficient light intensity, the matter gets hot by the energy of the laser that are collected and dries or dissolves while frequently it is altered to plasma under the high light intensity (Rane *et al.*, 2018).

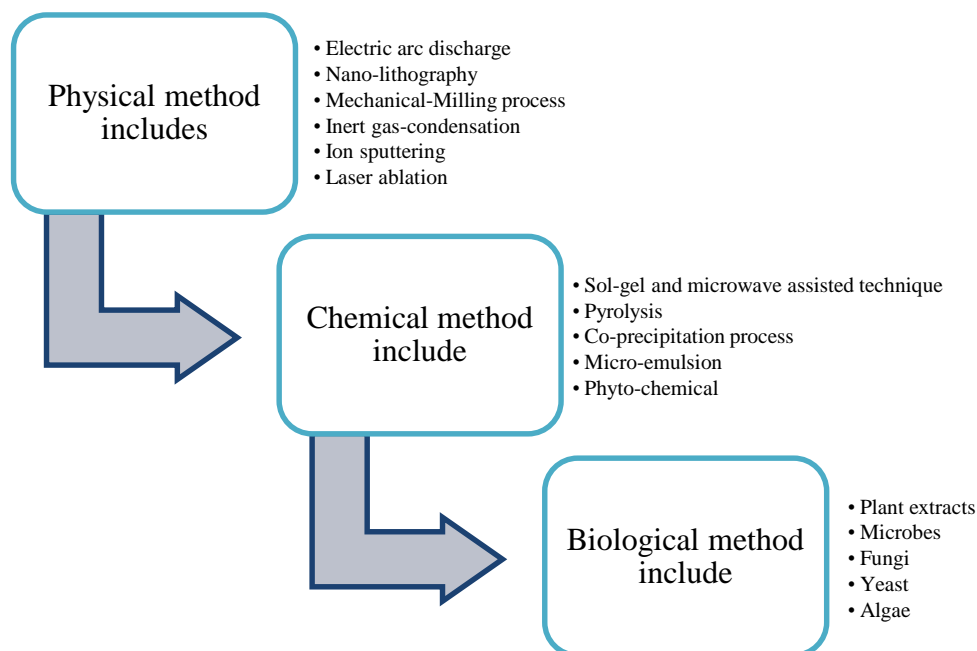


FIGURE 2.2: GRAPHICAL FRAMEWORK OF VARIOUS PROCEDURES FOR PREPARING NANOSCOPIC PARTICLES (DEVATHA *ET AL.*, 2018)

d) NANO-LITHOGRAPHY

It is the process of the study of nano-structures with minimum size of just one dimension like 1-100nm. There are distinct techniques are available for the synthesis of nano-lithography such as optical, electron-beam, multi-photon (Pimpin et al., 2012). One of the nano-lithography's key benefits is its ability to create bunches of specific form and dimension from the individual nanoparticle and its drawbacks include the cost and need of complex machinery (Saranyaadevi et al., 2014).

e) INERT GAS CONDENSATION

Inert gas condensation is another physical technique responsible for generating the nano-particles. In this technique, metals are vaporized under the extremely high pressure in a helium and argon filled vacuum chamber. After colliding with the gas molecules, the vaporized atoms of metals dissipate their energy of movement and compress into small fragments. These fragments eventually develop into crystals of nano-sizes with the process of Brownian fusion and aggregation.

f) PULSED-WIRE DISCHARGE

It is a physical technique utilized to create nanoparticles. This procedure is quite different from the others. A metal wire which is usually copper is vaporized by the pulsed current by forming

the vapor that then gets cooled by an atmospheric air to form nanoparticles. Metal, oxide and nitriles are formed by using this technique (Murai *et al.*, 2007). This process may have a high output rate and good energy consumption. Since this approach is not only exceedingly costly but also unsuitable to be used precisely for various metals, so it is rarely employed for common uses in industry. It works best with metals having high conductivity of electricity that are readily accessible in fine wire form (Umer *et al.*, 2012).

CHEMICAL TECHNIQUES

a. SOL GEL TECHNIQUE

Sol gel comprises of two words Sol and Gel. Sol means the materials are embedded with fluid in the solution containing gladiola particles and gel means the macro-molecule that is in solid form dissolved in liquid phase. Sol-gel technique is basically followed by the bottom-up strategy because of its ease of implementation and fact that the most nanoparticles are created from using this procedure. In this wet-chemical procedure, chemical solution is used that functions as a predecessor for the combined arrangement of individual particles. The sol-gel technique uses the oxides of and chlorides as precursor frequently (Ramesh *et al.*, 2013).

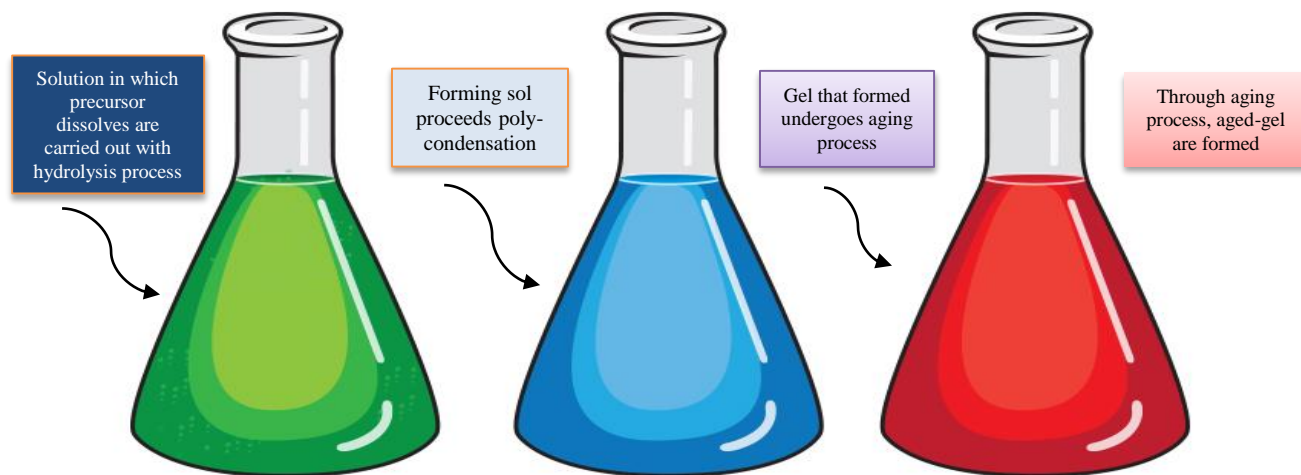


FIGURE 2.3: USING SOL-GEL TECHNIQUE, DEVELOPMENT OF NANOPARTICLES
(RANE *ET AL.*, 2018)

b. CHEMICAL REDUCTION METHOD

This method for the first time undergoes into examination by Michael Faraday, for creating the metallic gold that are in colloidal form with colors. The quickest and most common method for the copper-based nanoparticles synthesis is the reduction of copper salts chemically. It is feasible

to form copper nanoparticles with significant control over their shape and dimension by chemically reducing copper salts (Song *et al.*, 2004; Mott *et al.*, 2007). In this procedure, a copper salt reduces with the help of reducing substance like sodium boro-hydride (Aslam *et al.*, 2002), hydrazine (Zhu *et al.*, 2005). Suitable reaction conditions are required for the synthesis of copper nanoparticles.

c. MICROWAVE-ASSISTED TECHNIQUE

In this technique for the production of metallic nanoparticles, microwave radiations are employed in the process. In comparison to traditional technique, it has gained popularity for its ease and easy access of use, short duration of reaction and enhanced the output of the product (Komarneni *et al.*, 2003).

d. MICRO-EMULSION

One of the most effective technique for the synthesis of nanoparticles is micro-emulsion. Micro-emulsion is also referred to as colloidal method. A substance known as surfactant used in micro-emulsion used to transform two incompatible solvents into thermodynamically inert mixture. A basic emulsifier is made up of a single phase with the three ingredients such as oil, water or surfactant (Chen *et al.*, 2006). The most used process for producing nano-particle on a broad level in the industry is the pyrolysis. There is an ignition which is employed to ignite the precursor. The precursor used is generally a liquid or in vapor phase which enters the combustion chamber by the tiny opening at elevated pressure to ignition (Kammler *et al.*, 2001)

TABLE 2.1: REACTION CONDITIONS W.R.T REDUCING AND CAPPING AGENTS (UMER *ET AL.*, 2012)

Reducing substances		Capping substances	Conditions employed reaction	Rate affecting on reaction for
Sodium hydride (Aslam <i>et al.</i> , 2002)	boron-	Polyvinyl-pyrrolidine/ polyethylene-glycol	average	fast
Hydrazine (Aslam <i>et al.</i> , 2002)		Polyvinyl-pyrrolidine/ polyethylene-glycol	Less than 70.5	medium

Sugar Panigrahi et al., 2006)	Polyvinyl- pyrrolidine/ polyethylene-glycol	70.05to100	slow
Ascorbic-Acid (Xiong et al., 2011)	Ascorbic-acid	Approx..80	medium

It is manifested that metal Nano-particles has prospective to function in the future, as affordable and antiseptic, and have immense role in the medical issues, pollutant removal, and in field of industry, due to the attainment of distinct qualities like antibacterial, antifungal, antioxidant, exceptionally strong catalytic and photochemical activities (Elumalai & Velmurugan, 2015). Chemically, physically, biologically, Nano-particles can be generated (Hasan, 2015). Chemical and physical procedures include process utilizing microwave and sol-gel technique (Jia, Zhang, Shang, & Yang, 2008), combined-precipitation (Imran Din & Rani, 2016) and pulse wire electric arc, high-energy actinotherapy (Halevas & Pantazaki, 2018), litho-graphy (Zhang & Wang, 2008).

Chemically synthesis technique of Nano-particles has a numerous negative side effect because some harmful chemical chemicals are used in them. Chemical and physical methods have environmentally friendly substitute such as micro-organisms utilizing the microbes to create nanoparticles biologically (Hasan, 2015).

BIOLOGICAL TECHNIQUES

The processes used to synthesize the Nano-particles are carried out with the distinct biological methods that includes plants (Philip & Nanostructures, 2010); (Kumar, Singh, Kumari, Mozumdar, & Chandra, 2011), microbes, yeast (Kowshik *et al.*, 2002); (Mohanpuria, Rana, & Yadav, 2008) bacteria (Lengke & Southam, 2006); (Husseiny, Abd El-Aziz, Badr, Mahmoud, & Spectroscopy, 2007) and viruses (Merzlyak & Lee, 2006).

As previously mentioned, a diverse number of biological organisms like bacteria, yeast, microbes, fungi are utilized to generate the particles of nano-sizes. Every one of these biological organisms contains a different level of metabolism related processing abilities that might be manipulated for making distinct types of metallic nano-particles. Because of their inherent process of metabolism and activities involving enzymes, not every living organism are capable of creating nanoparticles. For the formation of NPs with obvious features like shape and length, an appropriate biological component has to be cautiously selected (Shah *et al.*, 2015).

BY USING MICROBES

Because of its ease of use, affordability and absence of dangerous compounds, microbes are employed for the formation of nano-particles. Furthermore, biological molecules that are involved not only reduce the levels of metal ions but they also stable the metal nano-particles and inhibiting the further oxidation of the earlier. Depending on where the nanoparticles formed, micro-organisms biosynthesis can be categorized into intra-cellular and extra-cellular strategy. The intra-cellular strategy relies on the enzymes existence that helps to carry the ions in the cells of microbes to create nanoparticles. Meanwhile in the extra-cellular strategy, metallic ions are accumulated in the outermost layer of the cell membrane and then break down by the enzymes to produce nanoparticles (Salunke *et al.*, 2016).

Due to the diversity of its metabolic activity and the ease of rapid and swift growth in lab environments, micro-organism can use for the formation of the nanoparticles. A number of strategies are utilized for the nanoparticles bio-synthesis. The first microbe described for the generation of copper nano-particles of size 10 to 30nm with using copper sulphate solution was a bacterium from the genus *Serratia* which is obtained from the insect species called *Stibara* (Saif Hasan *et al.*, 2008).

TABLE 2.2: CU-BASED NANOPARTICLES ARE COMPOSED BY UTILIZING DIFFERENT MICRO-ORGANISMS

Nanoparticles	Microorganism	Size
Copper (Saif Hasan et al., 2008).	Stibara	10.0-30.0nm
Copper (Varshney et al., 2010)	Pseudomonas stutzeri	8.0nm-15.0nm
Copper (V Singh et al., 2010)	Escherichia coli, Morganella morganii	10.0-14.0nm
Copper (Honary et al., 2012)	Penicillium auratiogriseum, P. waksmanii and P. citrinium	79.0-295.0nm
Copper (Salvadori et al., 2013); (Salvadori et al., 2014)	Hypocrea lixii and Trichoderma koningiopsis	24.05-87.05nm
Copper (Ghorbani et al., 2015)	Salmonella typhimurium	49.0nm

Copper (Cuevas et al., 2015)	<i>Stereum hirsutum</i>	5.0nm-20.0nm
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When exposed to metallic strain, microbes use a variety of strategies to get rid of harmful metals and live as an active discharge of ionized metals by cell membrane with the conversion of harmful ionized metals to harmless metals inside the cell with the collection of ionized metal. Ion pump and transport carrying the mediated ions and lipid penetration are responsible for this inflow in majority of the metals (Issazadeh *et al.*, 2013). The cultivation technique is crucial when dealing with micro-organisms. For the purpose to significantly enhance the activity of enzymes, cultivation factors like sunlight, moderate pH level, temperature, speed and nutrients can be optimized (Iravani *et al.*, 2011; Mukherjee *et al.*, 2001).

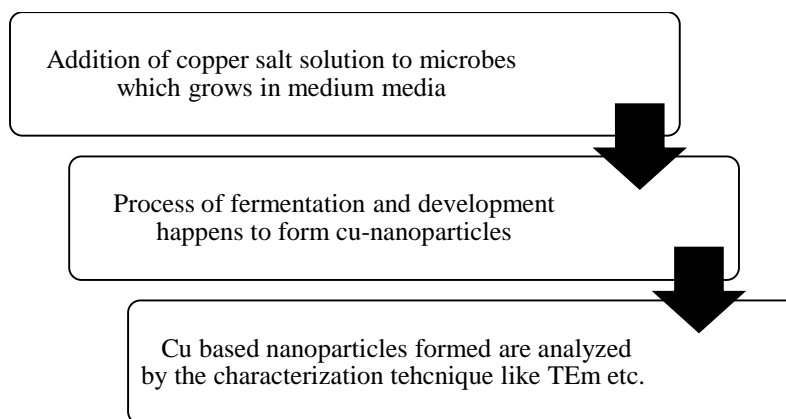


FIGURE 2.4: MICROBES ARE EMPLOYED IN THE PROGRESSION OF CU-NPS
(KASANA *ET AL.*, 2017).

BY USING FUNGI

Many laboratories around the world use fungi to produce nanoparticle through a process known as bio-synthesis, which can take place both inside and outside of cell (Philip *et al.*, 2009). Many different enzymes that are outside of the cells are produced by then to produce metallic particles. They are convenient to handle and offers great metallic durability. Due to its capacity to consume metallic substances, *Aspergillus niger* was employed to create copper-based nanoparticles. The widely distributed pathogenic fungus *Aspergillus niger* has a range of enzymes, including antioxidant and hydrolysis enzymes, which permit metallic ions to be collected from water-based solutions (Noor *et al.*, 2020).

As opposed to other microbes like bacteria, yeast and algae, filamentous fungi have distinct benefits including great metallic tolerance, potential for the bio-magnify, internal

metallic absorption and the capacity to endure the high flow rates and etc. (Salvadori *et al.*, 2014). As fungi have significant intracellular metal absorption rates so the nanoparticles forms are typically small in size (Mukherjee *et al.*, 2001). According to fluorescence spectrum research, the fungi form enzyme-stabilized nanoparticles as a consequence of the effects of activated reducing substances that form the membrane of the cell throughout extrinsic fabrication. The research succeeded in trying to demonstrate that the identical enzymes produced from the biomass were found in the solution itself and attached to the particle surfaces (Kumar *et al.*, 2003).

For producing metallic nanoparticles by using fungi, has been studied both extracellularly and intracellularly. The methods of extraction used in following steps for the synthesis of NPs, intracellularly have a problem of poor yields while in extracellular production, nanoparticles that are located at the cell membrane or at the boundaries of the cell, which makes ease for them in the following procedures to retrieve them (Dhillon *et al.*, 2012; Kathiresan *et al.*, 2009).

Over a period of seven days, *Talaromyces pinophilus*, cultures on media at 30°C and 150rpm. At the conclusion of the incubation stage, these fungi bio-mass isolated by the process of filtration. In order to get the molecule free filtrate, the process of filtration is carried out with the Whatman filter paper. The filtrate is utilized to form copper-based nanoparticles by adding the 0.1mm of copper solution to filtrate which is cell-free of fungus *T. pinophilus* and they incubated for 24 hours at 30°C. The synthesized copper nanoparticles isolated and dried about 48 hours at 80°C (Hasanin *et al.*, 2021).

BY USING ALGAE

Being aquatic organism, algae, they are capable for biological synthesis of metallic particles and researchers have found that they can consume metals (Luangpipat *et al.*, 2011). In the kingdom of plants, algae make up a different group. The nutritional significance of algae, their effectiveness in improving the bio-diesel and their enormous scope for the medicinal purposes are all under the investigation along with their production of nano-particles (Govindaraju *et al.*, 2009).

Using an algae method for the synthesis is accessible, harmless and inert. *Sargassum polycystum*, a brown sea-weed which is isolated from the marine sources are utilized for the copper-based nanoparticles synthesis which have potent anti-bacterial and anti-cancerous properties (Ramaswamy *et al.*, 2016). Silver nanomaterials can be produced at room temperature by using the extracts of *Chlorella vulgaris*. The results of the investigation showed that the extracts proteins have shaping, decreasing and stabilizing effects (Xie *et al.*, 2007).

BY USING BACTERIA

The major difficult problem facing nano-technology today is to economically manage their structure and arrangement in order to improve their properties like optical, electrical etc. To attain this purpose, bacterial species could be use in a systematic way (Gericke *et al.*, 2006). Bacteria are constantly subjected to nature to a variety of harsh environmental circumstances. Their capacity to withstand the adverse effects caused by environment will determine how well they survive in these challenging circumstances. To deal with different pressures, including toxicity caused by the excessive levels of metallic ions in the environment, bacteria have built in the protective system.

Changes of redox conditions, efflux infrastructure, precipitation intracellularly and deposition of metallic ions are some biological defensive systems to cope with the high levels of metal ions (Dhillon *et al.*, 2012). It is known that bacteria can synthesis metallic nanoparticles either extracellular or intracellular procedures (Ahmad *et al.*, 2003). *Actinobacter* sp., *Klebsiella pneumonia*, *Lacto-bacilli* spp., *Coryne-bacterium* sp., *Pseudomonas* sp., *Escherichia coli*. are the primary bacteria employed for the manufacture of nanoparticles (Mohanpuria *et al.*, 2008; Iravani *et al.*, 2014; Sunkar *et al.*, 2012).

Pseudomonas stutzeri, a bacterium which was isolated from the soil gave the initial study for the synthesis of copper-based nanoparticles by utilizing the strain of bacteria. The occurrences of bio-molecules in bio-mass aided in stabilization and the reduction of metallic ions in order to prevent them from additional oxidation (Varshney *et al.*, 2010). *Escherichia* sp., a bacterial strain that has been successful in treating the wastewater of textile, useful for the production of Cu-based nano-particles (Noman *et al.*, 2020). A bacterium *Morganella* taken in an aquatic physical atmosphere to form poly-dispersed nanoparticles in order to make copper NP_s (Din *et al.*, 2017).

BY USING YEAST

Like numerous other microbes, yeasts are capable of absorbing and storing the large number of harmful metals from the environment (Bhattacharya *et al.*, 2005; Mandal *et al.*, 2006). A range of decontamination approaches, including chelation, microbial precipitation, and microbial-sorption have been used by yeast cells as an outcome of adaptability to the toxic effects of metals. The various techniques applied by yeast organisms to create and maintain the nanoparticles during synthesis are responsible for the difference in the size of particles, setting, and granular attributes

(Hulkoti *et al.*, 2014).

Yeasts are also called semi-conductor crystals and Quantum semi-conductor crystals which are primarily investigated for the formation of cadmium nanoparticles (Varshney *et al.*, 2012). The dimensions and form of the nanomaterials may be readily manipulated during the process of synthesis step by modulating P. jadinii's cellular development and growth processes (Gericke *et al.*, 2006).

BY USING VIRUSES

In-organic compounds, like oxides of silicon, cadmium sulfide, ferrous oxide, and zinc sulphate have been given out by an innovative technique that involves the use of viruses in the generation of nanomaterial and the field of electronics has been especially fascinated with semiconductor materials like Cadmium sulphate and zinc sulfide and processes based on environmentally friendly chemistry have been extensively studied for their production.

Over the past ten years, research on using viruses to create quantum dots has been conducted (Lee *et al.*, 2002; Mao *et al.*, 2003). A desirable characteristic of viruses is their rich protein content in the capsid coating on the exterior, leading to an incredibly reactive interface that is capable of interaction with ions of metal (Makarov *et al.*, 2014). As opposed to the infect-free solutions, the virus presence not only greatly increased the number of nanoparticles but also reduced their size (Love *et al.*, 2014).

BY USING PLANTS EXTRACT

Living or decaying extracts from the plants can be used for the green synthesis of metallic nanoparticles. The stem, leaves, root, bark, peels and the other components of the plants can be used for the synthesis (Chandra *et al.*, 2020). The reduction of metals depends heavily on the biomolecules that are present in plants including proteins, terpenoids, amino-acids, ketones, aldehydes, vitamins, alkaloids, saponins, carbohydrates and phenolic substances (Nath *et al.*, 2013). Plants biomass can be used in synthesis in the form of extract or powder and then it is mixed with the metallic solution of choice (Pavithran *et al.*, 2020).

The quest for ecologically sustainable ways of making nanoparticles has been influenced by the enormous prospective of nano-particles. The development of secure and harmless substances along with environmentally acceptable and sustainable resources has drawn an abundance of attention to the creation of organic nanoparticles. According to studies, copper-based nanoparticles that are biologically active and teal-mediated have better environmental

accessibility and have the capacity to interact closely with other molecules (Wu *et al.*, 2020).

Fruit peels has become an increasingly efficient and affordable resource for the synthesis of the nano-particles, replacing the utilization of plants, bacteria, yeasts, viruses, actinomycetes, and fungi (Suhag *et al.*, 2022). Many investigations are carried out to synthesized metals NPs by using banana peel (Ibrahim *et al.*, 2015). dragon-fruit (Phongtongpasuk *et al.*, 2016), by using orange (Kaviya *et al.*, 2011), pomegranate (Fernandas *et al.*, 2018). Fruit peels have significant advantages related to health, anti-microbial and anti-inflammatory properties and have a wide range of biologically active compounds like steroids, phenols, glycosides, tannins, carotenoids, anthocyanins, triterpenoids, essential oils and vitamin. They are usually discarded by food industry as waste product. Future-oriented applications for a huge market for nutritional and medicinal delivery are made achievable using nanoparticles produced by applying biologically active elements from fruit peel (Suhag *et al.*, 2022).

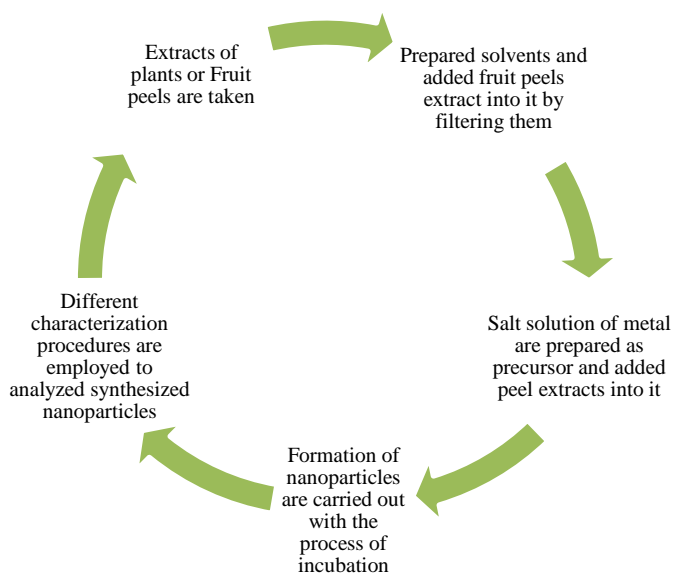


FIGURE 2.5: PLANT BASED GREEN SYNTHESIS OF METALLICS NANOPARTICLES
(PAVITHRAN *ET AL.*, 2020)

Malus domestica, the scientific name of apple is consumed globally and widely available. They contain a large amount of phenolic chemicals (Wojdył o *et al.*, 2008). Due to the presence of high levels of poly-phenols and phyto-chemicals, it is considered extremely important (Shehzadi *et al.*, 2022). There are five primary groups of poly-phenolic chemicals, and they have been found in many apple species. They consist of dihydro-chalcones, hydroxyl-cinnamic acids, anthocyanins, flavanoids and flavonols which comprise of pro-cyanidins, (2-*R*,3-*R*)-2-(3,4-dihydroxy-phenyl)-3,4-

di-hydro-2H-chromene-3,5,7-tri-ol and *(2-R,3-S)-2-(3,4-dihydroxy-phenyl)-3,4-di-hydro-2H-chromene-3,5,7-tri-ol* (Wojdył o *et al.*, 2008). Due to their antioxidant functions and high redox capacity, flavonoids are very essential (Ignat *et al.*, 2011).

As a result of their antioxidant properties, the polyphenols extracted from apple peel have been observed to be good for health of living organisms and for the prevention and treatment from disease (Kschonsek *et al.*, 2018). The bio-active compounds present in apple peels are *(2-E)-3-(3,4-dihydroxy-phenol) prop-2-enoic-acid*, chlorogenic acid, *2-R,3-S,4-S-2,2-Bis(3,4-dihydroxy-phenol)-3,3-4,4-tetrahydro-2-H-(4,8-bi-1-benzo-pyran)-3,5,7-hexol*, are the components of phytochemicals and consists of flavanoids that includes quercetin and cyanin glycosides (van der Sluis *et al.*, 2001).

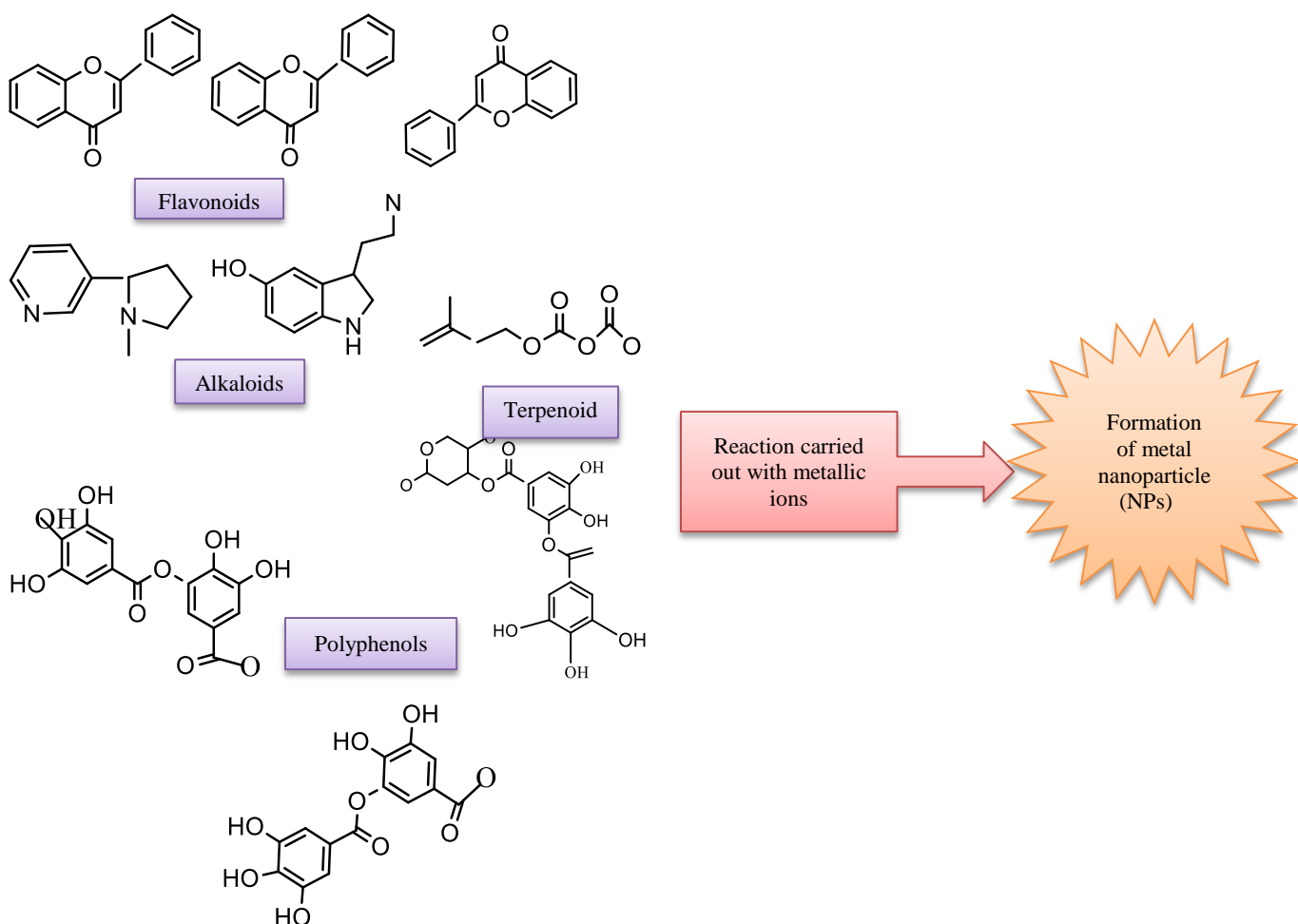


FIGURE 2.6: APPLE PEEL CHEMICAL CONSTITUENTS FOR PRODUCING METALLIC PARTICLES (KUMAR *ET AL.*, 2013).

TABLE 2.3: CHEMISTRY OF PHENOLIC COMPOUNDS PRESENT IN APPLE PEELS (SHEHZADI ET AL., 2020)

Flavanols have

molecular formula

C₁₅H₁₄O₂ with

molecular weight

226.026g/mol.

Its IUPAC name is

2-phenyl-3,4-dihydro-

2H-chromen-3-ol.

Xing *et al.*, 2015);

(Zanwar *et al.*, 2014);

(Vasconcelos *et al.*,

2012)

Dihydro-chalcones have

molecular formula

C₁₅H₁₄O with molecular

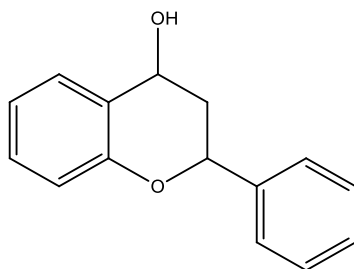
weight 210.07g/mol.

Its IUPAC name is

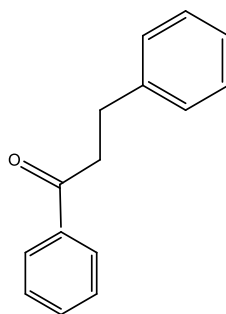
1,3-diphenyl-propan-1-

ol. (Najafian *et al.*,

2012)



It has phenolic compounds like pro-cyanins, catechin and epi-catechin which shows benefits like anti-inflammatory substances, controls the cell death as well as treat the cancer or its associated disorders and prevent from the inflammation of digestive tract.



It has phenolic compounds like phloridzin which shows favorable benefits in the cure of diabetes associated illness.

Phenolic acids have molecular formula

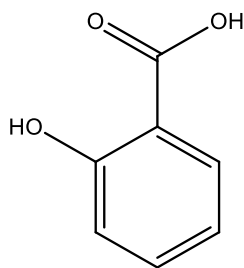
$C_7H_6O_3$ with molecular weight 139.12g/mol.

Its IUPAC name is 2-hydroxy-benzoic acid.

(Hwang *et al.*, 2014);

(Huang *et al.*, 2013);

(Faried *et al.*, 2007)



It has phenolic compounds like chlorogenic acid, caffeic acid and gallic acid which shows benefits includes anti-oxidant and anti-obesity substances, possess neurologically protective qualities and number of cancer cells reduced them to

The amount of poly-phenolic chemicals and their anti-oxidant power vary in several apple cultivars due to numerous factors such as environmental factors, the composition of the soil, the timing of harvest and storage methods. The concentration of these methods changing when placed in the apples (Tsao *et al.*, 2003).

The level of concentration of different phytochemicals, that are also found in apples and which is dependent on a number of different factors including apple growing and harvesting conditions, is also very high. In peels of apple are found catechin, glycosides, chloro-genic acids, quercetin and etc. Additionally, peel of apple has more quercetin than flesh does. Therefore, the apple's antioxidant capacity will be higher in the peel and lesser in the flesh of the apple (Shehzadi *et al.*, 2022).

Unlike the apple flesh, the peel of apples is effective in reducing the cancer of intestines (Miller *et al.*, 2000). The anti-oxidant activities of epi-catechin, catechin assist in lowering the Low-density lipo-protein cholesterol (da Silva Porto *et al.*, 2003). Likewise, chlorogenic acid possess a potent potential to eliminate the radicals of methyl per-oxides. Chlorogenic acid serves an essential part in reducing the risk of cancer development as radicals may boost the growth of cancer and malignancies (Kasai *et al.*, 2000).

ANTI-OXIDANT ACTIVITY OF APPLE PEEL

Heart disorders and cancer disorders are the leading cause of death all over the world. An apple has various benefits and is recommended for curing illnesses involving coronary artery disease and other ailments (Rop *et al.*, 2011). Since the effects of oxidative stress is linked to heart disease and ageing, particularly because of the presence of free radicals and other oxygen species that are reactive, it possesses a variety of antioxidant qualities that lower the risks of these conditions (Kschonsek *et al.*, 2018). Aside from reducing the risk of heart attacks and strokes, apples are also

known to reduce the risk of insulin resistance (Boyer *et al.*, 2004).

ANTI-HYPERTENSIVE PROPERTY OF APPLE PEEL

Apple peel shows anti-hypertensive property as apples have substantial amount of flavanoids that can improve health. Apples supply 22 percent of the nutritional phenolic compounds that are consumed in North America, such as flavonoids and phenolic acid (He *et al.*, 2008). In terms of health, hyper-tension is an alarming problem that is getting worse on a daily basis in the entire world. Angiotensin transforming enzyme is considered an essential therapeutic target for reducing overbearing arterial pressure.

The contents of apple peel extract which is high in flavanoids act as potent inhibitor of Angiotensin transforming enzyme (KAUR *et al.*, 2013). Due to its antioxidant qualities, quercetin provides anti-inflammatory qualities and the chemicals like Leuko-trienes and prostaglandins, as well as their inhibitor influence on enzymes and collaborate in order to terminate the process of inflammation. Quercetin, a flavonoid present in apples, red-grapes, and blueberries, is characterized to have anti-inflammatory actions that have been demonstrated in chronic illnesses such as arthritis (Martin *et al.*, 2010).

Metal nanoparticles are prepared by using plant extracts or fruit extracts on the base of its properties (Hamad *et al.*, 2023). Metal nanoparticles can be produced from several plants such as Aloe Vera, Strawberry leaves, Carica papaya, Bryonia laciniosa L., Arnabia Nobili, Azadirachta Indica, Orange reticulate, Curcuma longa, Piper nigrum, Lansium domesticum Correa and etc. (Hajialyani *et al.*, 2018).

The *Allium saralicum* is a medicinal plant used for the copper-based nanoparticles. Linolenic acid and methyl-ester are plentiful in the extract of Allium Saralicum leaves (Moradi *et al.*, 2019). Copper Nano-particles can also be synthesized by using leaf extracts of Euphorbia esula L., in absence of surfactants as they own reducing and stabilizing agents. In order to reduce 4-Nitro-phenol, these copper-based nanoparticles are chosen as they are versatile and reusable. It carries out by Ullmann-coupling reaction and as a result, substantial yield of products is obtained.

TABLE 2.4: SYNTHESIS OF CU-NPS FROM EXTRACTS OF DIVERGENT PLANTS

Plant extracts	Salt of Cu solution	Size of Cu-NPs (nm)
Henna	CuSO ₄ .5H ₂ O	84.0

(Cheirmadurai *et al.*, 2014)

Tea leaf	Cu (NO ₃) ₂	55-100
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(Sutradhar *et al.*, 2014)

Citrus grandis	CuSO ₄ .5H ₂ O	23-28
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(Sinha *et al.*, 2015)

Orchis mascula L.	CuCl ₂ .2H ₂ O	5.5
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(Nasrollahzadeh *et al.*, 2016)

Punica granatum	CuSO ₄ .5H ₂ O	25
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(Vidovix *et al.*, 2019)

Morus alba	Cu (CH ₃ COO) 2.H ₂ O	22-99
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(Singh *et al.*, 2017)

Copper based nanoparticles made by using five essential plant extracts in a green, cost-effective, and environmentally friendly approach includes Azadirachta-indica, Tamarindus- indica, Murraya-koenigii, Hibiscus-rosasinensis, and Moringa-oleifera. Various spectral analysis methods were used to investigate the framework, shape, and optical properties of the material. 0.2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid), 2,20-diphenyl-1-picrylhydrazyl and H₂O₂ removing free radical's experiments were employed to measure the antioxidant activity (Abboud *et al.*, 2014); (Katwat *et al.*, 2015). Cu-based nano-particles cytotoxicity was assessed against four cancerous human cancer cells (MCF-7, HeLa, Hep-2, and A549), as well as a normal human epidermal fibroblasts cell (Rehana *et al.*, 2017).

Copper nanoparticles anti-chain reactions, peroxide decomposition and elimination of radicals, have all been proposed as the processes explaining their anti-oxidant properties (Rajeshkumar *et al.*, 2019). By producing reactive oxygen species, that interact with other particles, the unstable condition of the oxygen-rich radicals of ionized copper destroys cells (Rehana *et al.*, 2017). The presence of different chemical groups that exists in peels of apple functioned as biologically reduced substance are contributed to improve the anti-oxidant activity of Cu-NPs (Nunes *et al.*, 2018).

ANTI-BACTERIAL ACTIVITY OF CU-NPS

Copper nanoparticles have potent anti-bacterial properties that work well on both plants and agricultural animals (Banik *et al.*, 2017). Therefore, both agricultural and industrial firms and organizations have encouraged the use of Cu-NPs as herbicides (Mastin *et al.*, 2000), fungicides

(Garcia *et al.*, 2003), pesticides (Mortazavi *et al.*, 2010), and algacides (de Oliveira-Filho *et al.*, 2004). Fruits subjected to produce NPs exhibits enhanced stiffness, high level of Vitamin C and lycopene-carotenoid. Furthermore, tomato fruits treated with Cu-NPs develops more bioactive substances, improved fruit quality, and greater capacity for antioxidants (González-García *et al.*, 2022).

CHARACTERIZATION TECHNIQUES FOR THE ANALYSIS OF SYNTHESIZED NPS

There are several techniques of characterization are employed for analysis of copper-based nanoparticles. XRD Technique which is known as X-Ray Diffraction technique is based on the orientations of the diffraction peak levels, used to figure out the atomic structure of unknown substances and to predict the quantity and form of cells in units. SEM technique is utilized for providing characteristics regarding the sample, that include its size, chemical material, the structure of crystals, and material alignment. TEM used to serve characteristics about the weight of the sample, shape, layout, and crystal arrangement. The purpose of FTIR is used for measuring an infrared spectrum's emission and absorption characteristics and also give data on chemical categories. UV-Visible used to measure the quantities of colored solutions and to identify components of the sample (Tamilvanan *et al.*, 2014).

LPSA is the particle sizing method in which substances with dimensions vary from hundreds of the nano-meters to several milli-meters. DLS also called as QELS refers to determine the dimensions of the particle and the arrangement of the molecules. AFM used for analyzing the surface's shape and features and creating a three-dimensional (3D) photograph of the outermost layer. It is also a type of scanning probe microscope (SPM). XPS used to measure the power of electrons emitted in the context of X-rays that have various frequencies with the objective to determine the composition of elements at a surface that is solid.

TGA also known as Thermogravimetric analysis used to quantify modifications to both chemical and physical characteristics as a function of passage of time or higher temperatures. Raman spectroscopy gives information regarding the structure and composition of chemicals. EDX is used to determine the chemical composition of the substance. It is also known as EDS or EDAX (Tamilvanan *et al.*, 2014).

METHODS

Nano-sciences and its related fields offer essential consideration to methodologies and processes that are used for the formation of metal nanoparticles. Physical, chemical and biological methods are utilized for this purpose (Shende *et al.*, 2015). The factors needed for the generation of copper

nanoparticles are the substrates that creates Cu-ions and reducing chemicals capable of releasing electrons by reducing the ions of copper.

Highly efficient technique for the synthesis of copper nanoparticles in physical approach has been described are atomization, annealing and sputtering of metals (Tsuji *et al.*, 2003). Other metallic nanoparticles are also prepared by these approaches besides copper-based nanoparticles. Physical rather than chemical approaches considered more favorable due to homogenous dispersion of nano-particles in absence of chemical materials (Hornyak *et al.*, 2008). In order to obtain pure, unpolluted nanoparticles, the laser ablation process is distinctive in contrast to other physical technique (Husseiny *et al.*, 2007).

Chemical method employs the usage of chemical compounds that acts as a source of charged particles to reduce the metallic ions in its basic state of size 1.0 to 1000nm (Hussain *et al.*, 2016). The two main groups of chemical methods include traditional and green- chemical techniques. As a reducing substance, the green approach uses naturally existing compounds such as citric acid (Venkatesha *et al.*, 2018). Whereas, some potentially hazardous chemicals that made from synthetic substances, including sodium-borohydride and hypo-phosphite, are employed as reducing substances in traditional process (Zhu *et al.*, 2004). The main drawbacks of this method are its harmful effects, degradation of the environment, and complexity of operation (Akintelu *et al.*, 2021).

Biological method uses the living-organism as carrier of negatively charged particles to reduce the salt of copper (Ahmed *et al.*, 2019). In addition, compared to other ways, biological is quick, simple, inexpensive and manageable. As plants can be used as reducing and stabilizing substances, copper-based nanoparticles by green process has made a significant impact in nano-technology (Jaswanth *et al.*, 2020).

PREPARATION OF FRUIT EXTRACT

Firstly, washed the apples three times with distilled water (Umoren *et al.*, 2014) then apple fruit are peeled with the help of peeler. By using the mortar and pestle, apple peel is crumbled and mashed in extremely small size. After that sample of peel put in de-ionized water of 100cm³ and at 60°C for 15 minutes in a vessel filled with water. The obtaining peel extract are then allowed to cool down and filtered the peel extract with Whatman filter paper. The filtered sample are allowed to synthesized copper nanoparticle (MUHAMMAD *et al.*, 2023). Copper sulphate pentahydrate is utilized without further filtration (Amer *et al.*, 2020). Copper sulphate solution of 0.05

M are prepared by dissolving 3.5 g salt in deionized water of 10.0cm³.

SYNTHESIS OF CU-NANOPARTICLES BY GREEN APPROACH

100ml aqueous solution containing 0.05 M copper sulfate penta-hydrate (CuSO₄·5H₂O) are mixed well with the 10.0cm³ of peel extract. Using a magnetic stirrer, mixture was stirred continuously as being heated to 85°C for 8 hours (MUHAMMAD *et al.*, 2023). During the 16 min, the initially blue hue of the copper sulfate penta-hydrate turned red-brown signifying the emergence of copper-based NPs by the reduction of ions of copper salt from the Cu (2) to copper metal (Amer *et al.*, 2020). The resulting suspension was centrifuged at a speed of 3000 rpm for ten minutes to separate the liquid residue. 10.0cm³ of de-ionized water are used to rinse the residue multiple times. To clean out the contaminants from the Cu-nanoparticles surface, five cycles of centrifugation, decantation process and washing process are used. The resulting precipitates are then dried out for one or two days in an oven at 50°C. The created nanoparticles are preserved for characterization and anti-oxidant research (MUHAMMAD *et al.*, 2023).

The first indication of formation of copper-based nanoparticles is the color shift that may be seen with the naked human eye (Thiruvengadam *et al.*, 2019). For several hours at room temperature, the hue shift of the copper salt and peel extract are observed in glass tube. From pale yellow in the first hour to brown in the eighth, the color shifts are highly apparent (Hamad *et al.*, 2023).

CHARACTERIZATION TECHNIQUES

The determination of copper nano-particles was made after visual observation by analysis techniques. Cu based nanoparticles are defined to observe entire elimination, to find groups with functional properties that took part in the process of biological reduction, to evaluate the levels of purity, and to quantify their nature both geographical and structural. The following strategies that frequently employed are described below;

UV-VISIBLE SPECTROMETRY

It is used to examine the reduction of copper metal between 200nm to 700nm. When copper-based nanoparticles are purified, the absorption spectrum revealed that ions of copper turned to metal nanoparticles by the reduction of ions. Every component of the reaction shows a peak in absorbance at 578nm that's the characteristic of copper nanoparticles (Amer *et al.*, 2020).

The idea for getting peak is that there must be electrons that are free on the outside of metal nanoparticles and that those free electrons must highly attracted to radiation possess certain wavelength (Ashtaputrey *et al.*, 2017). The absorbance will increase with the amount of

the copper sulphate penta-hydrate solution that converts to the Cu-NPs, which is a measure of concentration of copper particles (Parthasarathy et al., 2020).

TABLE 4.1: ANALYSIS OF FORMED COPPER-BASED NANOPARTICLES THROUGH PLANTS BY OPERATING UV-VISIBLE SPECTROPHOTOMETER

Salt of copper	Plants	Size of particle	Wavelength
Copper sulphate penta-hydrate (Lee <i>et al.</i> , 2011)	Magnolia extract	Kobus 50-100	570
Copper sulfate penta-hydrate (Kaur <i>et al.</i> , 2016)	Punica granatum peel	16-22	590
Copper sulfate pentahydrate (Ismail <i>et al.</i> , 2019)	Ripened erecta fruit	Duranta 80	585

XRD ANALYSIS:

It is a technique used for figuring out the dimension and structural composition of the nanoparticles (Arya *et al.*, 2019). Peak intensity are the measures of the degree of the nanoparticle's crystal structure (Wani *et al.* 2011). By using Scherrer formula, size of copper nanoparticles is determined.

$$D_p = K\lambda / \beta \cos\theta$$

Where, "D" indicates the dimensions and magnitude of copper nanoparticles, "K" is the proportionality constant of Scherrer equation, " λ " shows wavelength, " β " indicates the diffraction peak of full width which is maximum at half, " θ " is Bragg angle (Venkateswara Rao *et al.*, 2019).

The Cu-NPs were estimated to have an average size 22.5nm while in XRD, Cu-NPs instinct peaks revealed at 45, 55 and 70 respectively (Parthasarathy *et al.*, 2020). The exceptionally pure, impurity free copper nanoparticles are produced (Amer *et al.*, 2020).

FOURIER TRANSFORM INFRARED SPECTROSCOPY

It is used in order to determine and obtain an estimated description of the molecules in fruit extract and demonstrate its complexity by showing a lot of peaks. It varied from 4000-400 cm⁻¹ (Umoren *et al.*, 2014).

FTIR-spectrophotometer measures the intensity of infrared spectrum versus the wavelength. By using this technique, functional groups can generate electrons by reduction of copper salt (Sebeia *et al.*, 2019). To identify the functional group regulating the reduction of metal ions, the spectrum of both plant extracts and produced copper nanoparticles are analyzed (Akintelu *et al.*, 2020; Fathima *et al.*, 2018).

SCANNING ELECTRON MICROSCOPE

Copper nanoparticle's structural and graphical features are studied using SEM (Prabhu *et al.*, 2017). It is used to identify the geometric shape of produced nanoparticles. The NPs are identified spherical with the typical diameter of 28.0 nm.

TRANSMISSION ELECTRON MICROSCOPY

Because of TEM superior magnitude, image-quality, as well as the capacity to provide greater accuracy on form, crystallization, dimensions than SEM does. Many scientists choose to employ transmission electron microscopy rather than scanning electron microscope for visual assessment and shape identification (Akintelu *et al.*, 2021). TEM is more favorable when pictures from TEM are applied to determine the difference between crystallographic and porous objects with an area that are chosen for the diffraction of electrons (Caroling *et al.*, 2015). The typical size of particles obtained from this technique is 17nm. The synthesized copper nanoparticles have a 5.0 to 28.0nm wide size of particle dispersion and the limited size dispersion of nanoparticles have a range of 28.5nm.

TABLE 4.3: SIZE, SHAPE AND APPLICATION OF CU-NPS FROM PLANT AND FRUIT EXTRACTS WITH THEIR CHARACTERIZATION TECHNIQUES

Plants and Fruits extract	Shape of NPs	Size of NPs	Characterization techniques	Applications
Cassytha filiformis (Nasrollahzadeh <i>et al.</i> , 2018)	Shape like sheet	20nm	UV-Visible, FTIR, EDS, XRD	It exhibits Catalytic behavior
Strawberry (Hemmati <i>et al.</i> , 2020)	Spheroidal	15nm-31nm	UV, TEM, FTIR, EDS	It exhibits anti-bacterial activity

Okra (Ghazal <i>et al.</i> , 2021)	Spheroidal	50nm-80nm	FTIR, UV-Visible, EDAX, VSM	It shows cyto-toxicity and photo-catalytic properties
Walnut (Mehdizadeh <i>et al.</i> , 2020)	Spheroidal	16nm-20nm	TEM, FTIR, EDS	It shows anti-oxidant activity
Rhus Coriana (Er <i>et al.</i> , 2020)	Spheroidal	8nm-12nm	UV-Visible, FTIR, XRD, TEM	It exhibits anti-fungal behavior

ENERGY DISPERSIVE X-RAY SPECTROSCOPY

It's a method used to figure out the composition of elements of copper-based nanoparticles. Interpretation of the components is assisted by the distinctive groups of peaks on the by the element's distinct atomic arrangement (Noruzi *et al.*, 2015). EDX is employed to examine the level of purity in metallic nanoparticles (Devipriya *et al.*, 2017). Since, they are mostly derived from the composition of the plants extract are utilized as a reducing substance so the occurrence of elements like carbon and oxygen on the EDX spectrum is not considered to be an impurity (Valodkar *et al.*, 2011).

ATOMIC FORCE MICROSCOPY

It is employed to evaluate the shape and geometry of nanoparticles. Fruit Punica granatum used to form nanoparticles, sphere like shapes is characterized through atomic force microscopy (Chidanandappa *et al.*, 2020). Citrus medica L. juice utilized to create Cu-NPs by using AFM technique, the particle sizes varied between 5.27 and 2.96 nm (Shende *et al.*, 2015). Cu-NPs with a spherical shape are approved by AFM technique (Zhou *et al.*, 2016).

CONCULSION

The creation of nanoparticles with diameters ranging from 1nm-100nm is the primary goal of nanotechnology. According to their size, source and material characteristics, nanoparticles are divided into several categories. Metal nanoparticles are producing by variety of techniques. It includes top down and bottom-up methods. In top-down technique, Physical methods are utilized such as laser ablation, mechanically ball-milling process, Ion-sputtering and electric arch discharge methods to form nanoparticles. This route requires high temperature, pressure and expense. Bottom-up technique are classified by chemical and biological processes. Chemical

method is carried out with Sol-gel, co-precipitation, pyrolysis and micro-emulsions. Hazardous chemicals are used in this technique that harms the environment and produce unsafe products. Biological route is carried out with microbes, algae, yeast, virus and plant extracts. In comparison to chemical and physical approach, it is considered safe, quick, inexpensive, efficient and eco-friendly. Green synthesis is highly specified to form nanoparticles by using the plant and fruit extracts that own a remarkable impact due to their effectiveness and high-yielding features. Copper is a chemical element and considered an essential element in humans as a role of enzymes. Cu-NPs is harmless, efficient and economical material among the others nanoparticles. It exhibits activities like anti-oxidant, anti-fungal, anti-microbial and anti-bacterial. Fruit peels are more effective and reasonably price resource for the Cu-NPs synthesis. Fruit peels provide numerous health benefits, including anti-bacterial, anti-inflammatory, and physiologically active chemicals. *Malus domestica* that is the scientific name of apple, is consumed all over the world. Anthocyanins, flavonoids, and flavanols, which include pro-cyanidins, epicatechin, and catechin, as well as dihydro-chalcones, hydroxyl-cinnamic acids are the main phenolic compounds that can be found in peels of apple. These phenolic compounds help to prevent from diseases, so it is considered useful for human health. Cu-NPs are synthesized by using apple peel extract with copper sulfate solution that acts as reducing agents. The conversion of color from blue to red or dark brown indicates the visual confirmation of synthesis. The analysis of synthesized NPs is characterized by UV-visible, XRD, SEM, TEM, FTIR, EDRX, LPSA techniques.

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