

Biological Synthesis of Copper Nanoparticles From green Apple (*Malus Pumila*) Extract

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Abstract- Nanotechnology is the art and science of manipulating matter at the nanoscale. Allows the placement of small structures placed with precision, simplicity and low cost. The understanding and control of matter at dimensions of roughly 1 to 100 nanometres. [1] Due to their relatively large surface area, nanoparticles often exist with distinctive size dependent properties. Nanoparticles are injected in matrix materials which are used as carriers for delivering drug molecules. At present time, various metallic nanomaterials are being engineered using copper, zinc, titanium, magnesium, gold, alginate and silver. We have discussed the different types of platforms of nanoparticles. General approaches to the synthesis of nanoparticles by using biological method. The present study reports biologically oriented process for green synthesis of CuO nanoparticles by using eco-friendly and non-toxic *Malus pumila* (Green apple) extract. Microscopic analysis revealed that synthesized CuO nanoparticles show the shape and morphology. The antibacterial activity of biosynthesized CuO nanoparticles was tested against four bacterial pathogens *Staphylococcus aureus*, *Lactobacillus casei*, *Salmonella sp.*, *Klebsiella sp.*, which are responsible for causing severe infectious diseases. CuO NPs exhibit enhanced antibacterial activity against all pathogens.

Keywords- Nanotechnology, Nanoparticles, Aloe Vera, Clove, Garlic, Green apple, Phytochemical test, Antibacterial activity.

I. INTRODUCTION

The term nanotechnology, derived from Greek word “nanos” means dwarf. The first ever concept was presented in 1959 by the famous prof. of physics Dr. Richard P. Feynman. The term nanotechnology had been coined by Norio Taniguchi in 1974. Dosages between 1 to 100 nm which can be increased up to 1000 nm. Main perspective is scientific & technological field. Application for drug delivery system, new herbal drug formulation, and nutraceutical like, polymeric nano particle, nano capsule, nanoemulsions, transferosomes, entosomes by using bio active plant extract and food materials & others (like pharma, food, print & ink, agriculture etc...). Nanoparticles, a ultrafine unit with dimensions measured in nanometers (nm; 1 nm = 10⁻⁹ meter) & exists in natural world, created as a result of human activities.

Classifications are nanoparticles zero-dimensional, one-dimension, two-dimension and three-dimensional. Properties of nanoparticles are three types. These are physical, chemical, biological. Physical properties of NPs include shape, size, specific area. In physical properties size & shape used more areas like, agglomeration/aggregation, surface morphology/topography. Chemical properties of NPs include in metallic nanoparticles, metal oxide NP, Quantum dots, polymeric NP & carbon NP. In chemical properties used in more areas like, medicine, tissue engineering, manufacturing & materials, environments, energy & electronics, drug delivery, cancer therapy, HIV&AIDS treatment etc. Biological properties of NPs is just like as chemical properties. In this properties basically most used in organic areas like, medicine, drug delivery, pharmaceuticals, cosmetics, foods etc..

In NPs can be divided in three groups like, physical method uses high energy ball milling, inert gas condensation, melt mixing, laser ablation, physical vapour deposition etc. Chemical method, we use sol-gel method, micro emulsion technique, hydro thermal synthesis, chemical vapour synthesis, plasma enhanced chemical vapour deposition technique. Biological method, we commonly use biogenic synthesis using micro organism, biogenic synthesis using bio molecules as the templates, biogenic synthesis using plant extract. In NPs are various types like, silver NPs, gold NPs, copper NPs, carbon NPs etc. In NPs I work with copper NPs. So I describe copper NPs which is round & appear as brown to black powder. Copper is too soft for some application, hence it is combined with other metals to form numerous alloys such as brass, which is a copper zinc alloy. It is highly flammable solids so they must be stored away from sources from ignition. Some applications in copper NPs which are 1) it is anti biotic, anti microbial, anti fungal agent when added to plastic, textile 2) copper diet supplement with efficient delivery characteristics 3) heat sink & highly thermal conductive materials 4) efficient catalyst for chemical reactions & for the synthesis of methanol & glycol etc.

Copper Nanoparticles and Its Antibacterial Activity:-

Nanomaterials are the leading in the field of nanomedicine, bio nanotechnology and in that respect

nanotoxicology research is gaining great importance. The US Environmental Protection Agency (EPA) has approved registration of copper as an antimicrobial agent which is able to reduce specific harmful bacteria linked to potentially deadly microbial infections. In addition, no research has discovered any bacteria able to develop immunity to copper as they often do with antibiotics. The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticle. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution.

Plant Description:-As the saying goes “An apple a day keeps the doctor away”, and it certainly holds true even for green apple. Apples are one of the most exotic and amazing fruits. They contain a lot of essential nutrients and vitamins which should form a part of each and every person’s daily diet. The red ones which are very common and the green ones which are sour and sweet to taste. Green apple (*Malus pumila*) has been recognized as one of the healthiest fruits. It is innately packed with a variety of essential nutrients such as proteins, vitamins (vitamin A, B and C), minerals (zinc, iron, copper, manganese, potassium), and fibres. They help prevent digestive problems, help regulate blood glucose and good cholesterol. Prominent health benefits include dietary fibre, which decreases the chance of colon cancer. Green apples also help lower cholesterol level, ease digestion, reduce liver and digestive tract issues. Green apples are rich in antioxidants, including flavonoids and polyphenol. Because of this, they can prevent various forms of cancer and DNA damage.[11]



Green apple

II. METHOD AND MATERIALS

Collection of extracts:-

1. Green apples were collected from the market. They were washed and cleaned with distilled water and dried with water absorbent paper. Using mortar and pestle the sample was crushed properly.
2. Then green apple extract was obtained they was filtered through cotton.
3. Then pH check. pH for green apple 3.7.

4. Then it was added with 50ml water 100 ml green apple juice.
5. the sample over under magnetic stirrer hot plate maintain the temperature at 80C for 30 minutes

synthesis of copper nanoparticles by using green apple:-

- (0.02M) copper sulphate was prepared in (100ml) distilled water and a blue solution was seen.
- Polyethylene glycol 200 (5ml) was added to the aqueous solution containing the copper sulphate with vigorous stirring.
- 50ml green apple extract was added to the copper sulphate containing PEG.
- Sodium hydroxide (2ml) was added drop wise to the solution under continuous rapid stirring. The colour of the aqueous phase changed from white to green .
- The appearance of this brownish colour indicates that the reduction has started. The formation of copper nanoparticles is confirmed by the colour change from green to greenish brown colour.

Screening of phytochemicals metabolites:-

Phytochemicals are the chemicals produces by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. These compounds have various activities such as antimicrobial and antibacterial some have been reported to exhibit haemolytic and foaming activity. Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings.

Primary screening (Antibacterial activity):-

Antibacterial activity that kill or inhibit the growth of bacteria and formed zone of inhibition. Bacteria used as test organism (staphylococcus aureus, Lactobacillus casei, salmonella sp. ,klebsiella sp.).

Copper ions released subsequently may bind with DNA molecules and lead to disordering of the helical structure by cross-linking within and between acid strands. Copper ions inside bacterial cells also disrupted the biochemical processes.

Procedure:-

1. prepare a (NAM) media and sterilized the media by autoclaved.

2. Pour the media on sterilized Petri plates and after solidification of media bacteria (staphylococcus aureus, Lactobacillus casei, salmonella sp., klebsiella sp.) was spread over the plates by used cotton swab.
3. By used Disc diffusion method the disc was deep in to a different copper nanoparticle sample and place into a plates.
4. Incubated the plate at 37C for 24hrs.
5. After incubated the plates was observed and formed zone of inhibition was measured.

The antibacterial activity of synthesized copper nanoparticle was treated against bacteria by zone of inhibition disc diffusion method.

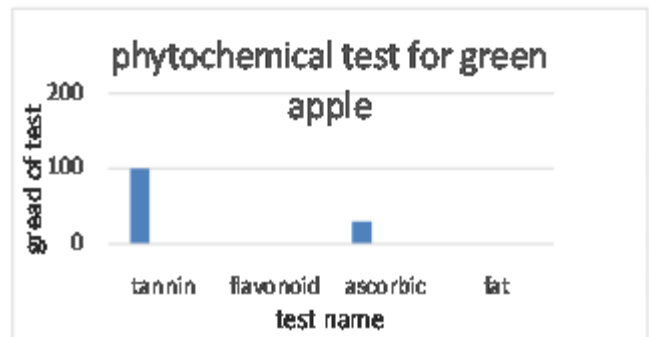
Antibacterial activity test against (Lactobacillus casei) bacteria and measure the zone of inhibition.

Sample	Zone of inhibition
Green apple	0.8mm

IV. RESULTS

Screening of phytochemicals metabolite

Phytochemical tests are tannin test, flavonoid test, ascorbic acid test, and fat test. Tannin test for green apple highly positive (100%). Flavonoid test for green apple is absent. Ascorbic acid test for green apple slightly position (30%). Fat test for green apple fat is absent.

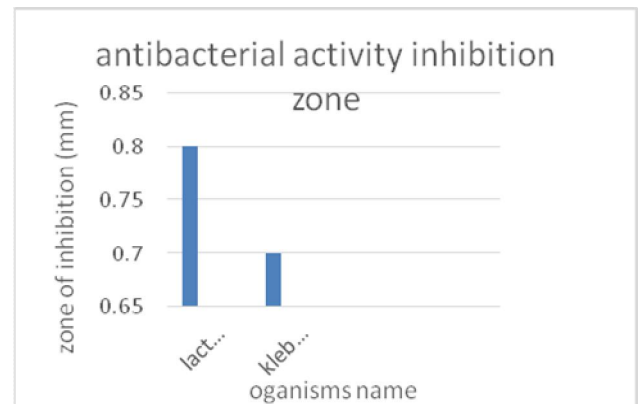


Test/sample	Green apple
Tannin test	+++
Flavonoid test	-
Ascorbic acid	+
Fat test	-

Antibacterial activity test against (Klebsiella sp.) bacteria and measure the zone of inhibition.

Sample	Zone of inhibition
Green apple	0.7mm

- (+++) highly positive, (++)moderate positive, (+)slightly positive. (-)absent.



Ascorbic acid test tannin test

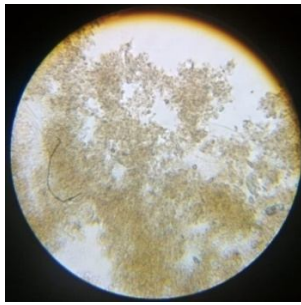
Microscopic examination result.

Primary screening (Antibacterial activity) result.

Antibacterial activity that kill or inhibit the growth of bacteria and formed zone of inhibition. Bacteria used as test organism (staphylococcus aureus, lactobacillus casei, salmonella sp. Klebsiella sp.). the zone of inhibition was measured in (mm).

Microscopic examination is the most common characterization technique to determine the shape and morphological characterisation under 100x.

Biological method



Microscopic examination for Green apple nanoparticle synthesis (*Malus pumila*).

[6] <http://shodhganga.inflibnet.ac.in/handle/10603/119298>

V. DISCUSSION

The copper nanoparticle was prepared by using biological and chemical method. But biological method is eco-friendly and cost-effective. characterization was done of prepared copper nanoparticles on the basis of microscopic analysis , and antimicrobial activity. we prepared copper nanoparticle's green apple as sample resultant in greenish brown colour (green apple) representing copper nanoparticle synthesis. characterization was done by compound microscopic study shape circular (green apple) , antimicrobial activity against (*klebsiella* sp.) was show green apple (0.7) and (*Lactobacillus casei*) was show green apple (0.7) very good result as compared to *klebsiella* sp. antibacterial activity. the characterization was done by compound microscope study circular shape. than we examine the qualitative test tannin test, flavonoid test, fat test, ascorbic acid test. the sample green apple fat content was absent, so it is very healthy food for human beings.

VI. CONCLUSION

In conclusion, here we report an eco-friendly preparation of copper nanoparticles by plants extracts (green apple). This method has an advantages over other reported methods as it is easily available starting materials, not costly and the procedure is easy to handle in the laboratory, no toxic agent is used and pollution free.

REFERENCE

- [1] Ipsa Subhankari and P.L. Nayak, synthesis of copper nanoparticle using plant extract ,World Journal of Nano Science and Technology 2(1): 14-17, 2013.
- [2] [https:// www.slideshare.net](https://www.slideshare.net)
- [3] <https://timesofindia.indiatimes.com/life-style/food-news/what-is-green-apple-is-it-healthier-than-red-apple/photostory/72056584.cms>
- [4] [https:// www. Reseachgate.net](https://www.Researchgate.net)
- [5] [https:// www.omicsonline.org/open-access](https://www.omicsonline.org/open-access)