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Green Synthesis, UV Characterization and Antibacterial Activity of Copper Nanoparticles Derived from *Nigella sativa*

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A B S T R A C T

In recent ages, green nanotechnology has gained attraction in the synthesis of metallic nanoparticles due to their cost-effectiveness, simple preparation steps, and environmentally-friendly. In the present study, copper oxide nanoparticles (CuO NPs) were prepared using *Nigella sativa* leaf extract as a reducing, stabilizing, and capping agent. Bioactive copper nanomaterials are an emerging class of nano-antimicrobials providing complimentary effects and characteristics, as compared to other nano-sized copper oxide metal. The CuO NPs were characterized via UV–Vis Spectroscopy. The UV–Vis spectra of CuO NPs showed a surface plasmonic resonance band to occur at 851 nm. Furthermore, as-formed CuO NPs shown strong antibacterial activity against the Gram-positive bacteria (*Bacillus cereus*, *Bacillus substillus*), and Gram-negative bacteria (*Pseudomonas aureginosa, Salmonella typhimurium, Enterobacter aerogenus*). According to the results of this investigation, green synthesized CuO NPs with *Nigella sativa* leaf extract may be used in biomedicine as a replacement agent for biological applications.

Introduction

“Nanotechnology” is an emerging field of innovation and research that deals with manufacture and use of materials in the size range of atoms or molecules. Literal meaning of the word ‘nano’ is means billionth part of a metre or 10−9 m.

Richard Feynman (1960) described nanomaterials as molecular tools with atomic precision in his famous lecture ‘There's Plenty of Room at the Bottom’ and gave birth to the notion of nanotechnology. The name nanotechnology (NT) was framed by Professor Norio Taniguchi of Tokyo University of science in 1974 while demonstrating manufacturing of matter at the nanometre level with high accuracy (Taniguchi *et al.,* 1974). Small size particles (Nanoparticles) exhibits totally changed properties as compared to respective bulk materials.

This is because of the fact that nanoparticles typically have unusually small dimensions, high surface area to volume ratio that facilitates high heat and charge transfer and confers several other distinct properties, opening new avenues for their application in various fields of industry. Metallic nanoparticles find application in optical, thermal, magnetic, sensoric devices, catalysis, as antimicrobial agent and various other fields (Huang and sheen, 1997). Nanoparticles are more precise and economic means to check pathogen growth as well as disease spread, but the development of consistent and unfailing technology to produce nanoparticles is a vital aspect of nanotechnology (Huang *et al.,* 2011).

Till date, research in the area of biosynthesis of nanoparticles was by and large limited to noble metals like silver, gold, zinc and to some amount copper (Olesja *et al.,* 2013). Copper-based nanoparticles offer better advantages because of their minimal cost, convenient availability in comparison to other noble metals and other physical and chemical properties (Ruparelia, 2008). Hence additional research is being diverted towards ecofriendly and cost effective biosynthesis of copper nanoparticles.

Green Synthesis of Nanoparticles

Green or biological synthesis of nanoparticles uses biotechnological tools i.e. living organisms or their parts for nanomaterial fabrication (Mohanpuria *et al.,* 2008). These methods are safe and environmentally sound and is fast emerging as an alternative to conventional chemical and physical methods (Joerger *et al.,* 2000). A number of researchers have reported biological or green approach for nanoparticle synthesis. Such nanoparticles are low-cost, non toxic and true to task (Thakkar *et al.,* 2010). Green Engineering is the key weapon to tackle the need of the day (Garcıa-Serna *et al.,* 2007).

Synthesis of Nanoparticles Using Microbes

A microbial cell can prove to be an efficient bioreactor for nanoparticle synthesis. Microbes living in various habitats interact with metals quiet often. Sometimes these metals or minerals are a requirement for optimal growth for example as cofactors for various enzymes, whereas some other times microbes have to evolve mechanisms to do away with these heavy metal pollutants.

Microbes can neutralise, alter or trap and precipitate the metals to form nanoparticles so that their toxicity is reduced (Honary *et al.,* 2012). Microbes can also chelate with metals to form nanoparticles. It has been demonstrated that proteins like phytochelatins (PC) are over expressed in cells exposed to heavy metals such as copper. This process is easy to scale up and can be further extended to the manufacture of nanoparticles of different chemical compositions, sizes and morphology upon identification of required enzymes secreted by the microbe (Sastry *et al.,* 2003). Bukhari *et al.,* (2021) synthesied copper oxide nanoparticles utilising cell free extracts of a marine Streptomyces sp. MHM38 and studied their antimicrobial action against a number of bacteria and fungi. A gram negative bacterium from the genus Serratia was employed for synthesis of copper/ copper oxide nanoparticles. Nanoparticles were collected after cell lyses and characterized with different techniques (Hasan *et al.,* 2008). Other Examples of bacterial nanoparticle synthesis include magnetotactic bacteria for synthesis of magnetic nanoparticles (Alphandery, 2014), S-layer bacteria for calcium carbonate and Gypsum nanoparticles (Sleyter *et al.,* 2014) and Pseudomonas spp that inhabit siver mine, reduce anionic silver ions to uncharged silver in nanoparticulate form (Quinteros *et al.,* 2016).

Characterization of Copper Nanoparticle

The properties of nanoparticles such as shape, size, morphology, stability, dispersity, elemental composition, surface charge and other chemical and physical characteristics are crucial and indispensible in determining their potential future applications and biological function like cellular uptake, dissolution and toxicity (Ruparelia, 2008).

Thorough characterization of synthesised nanoparticles is thus necessary to determine their aptness for decided function. These properties principally rely on the materials, methods and reaction conditions employed for synthesis. Different techniques can be utilized to obtain details on structure and other such characteristics of nanoparticles such as UV–visible absorption spectroscopy.

UV-Visible Spectra Analysis

Nanoparticle formation, at preliminary level, is confirmed by studying their UV-Visible spectroscopy absorbance patterns. The characteristic absorption peak of Cu nanoparticles lies in the wavelength range of 560-590 nm (Prema, 2010). A progressive rise in absorbance with increasing reaction duration or concentration of reactant (biological extract and metal salt ions) is an indication of nanoparticle formation. The principal behind the construction of nanomaterials using herbal extracts is the reduction of the charged metal ions to pure metallic non-ionic nanoparticulate form which is indicated by colour change and thus UV-Vis spectrum

(Das *et al.,* 2010). UV-Vis absorption spectrum shows peak characteristics of the surface plasmon resonance of nanosized particles (Abboud *et al.,* 2013). The better the absorbance the higher is the concentration of nanoparticles. Shift in absorbance peak towards higher wavelength indicates larger size particles.

Antibacterial Activity of Nanoparticles

The multi drug resistant bacterial pathogens with the tools of genetic mutations, antigenic shifts and/or drifts are able to resist and win over current fungicides. This resistance to chemical fungicides by plant- pathogens has erupted as a major problem to farmers; therefore it is the need of the hour to develop new technology that can overcome the drawbacks of traditional methods. Nanoparticles (NPs) are being increasingly employed to target microbes as an substitute to antibiotics and hence nanotechnology may be particularly helpful in controlling bacterial destruction of crop plants too.

Of action making them suitable to target a specific organism. Some other advantages are short time persistence, shorter shelf life and no residual threats. Furthermore such compounds have natural origin, have minimal adverse effects on the physiological processes of plants and are easily transformed into common eco-friendly organic materials (Gnanamanickam, 2002).

The study on antibacterial effects of copper nanoparticles using *E. coli* and *Bacillus subtilis* revealed the fact that the Cu NPs exhibited superior antibacterial activity compared to the silver nanoparticles. Cu NPs demonstrated good antimicrobial influence on Bacillus spp. and prominent fungicidal influence on Penicillium spp. microorganisms. Cu NPs exhibited greater inhibition on Escherichia coli in comparison with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Propionibacterium acnes,* and *Salmonella typhi*. Cu NPs have been synthesized by using extracts of various plants found all over the globe. But no research has been conducted as far as green synthesis of Cu NPs is concerned using extracts of medicinal plants of Ethiopia. Therefore, the present research work was proposed to explore the synthesis of green Cu NPs (g-Cu NPs) using extracts of medicinal plant of *Nigella sativa.*

More than 95% of traditional medical preparations in Ethiopia are of plant origin. A medicinal plant species of Ethiopia identified for the biogenic synthesis of g-Cu NPs in aqueous media is *Nigella sativa.*

Future Perspective and Challenges

Nanomedicine is the science that is likely to play a significant role in overcoming the obstructions and challenges in the field of agriculture, cosmetics and healthcare. In the present situation of pandemic, nanotechnology has been utilized for developing antiviral paints, furnitures and even fabrics.

Remarkably, as compared to chemically synthesized nanoparticles the toxicity concerns for green synthesized nanoparticles have been found to be low. Several groups have reported the in vitro and in vivo biocompatibility of green synthesized nanoparticles (Mukherjee *et al.,* 2016). Furthermore, green nanoparticles adsorbed to a lesser extent on serum proteins present of the blood plasma, indicating their in vivo compatibility (Chanda *et al.,* 2011). Careful in vitro and in vivo studies accompanied by preclinical and medical safety analysis can ascertain biologically synthesized nanoparticles as proficient substitute toward cancer diagnosis and treatment procedures in the coming years.

The present investigation aims to produce copper nanoparticles from *Nigella sativa* and test their potential as Bactericidal. Then, Preparation of plant extracts and their screening for nanoparticle synthesis and Characterization of nanoparticles using UV Spectroscopy. Also Invitro study of Antibacterial activity against Gram positive and Gram negative organisms.

Medicinal Plant

Morphology of the plant

N. sativa is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds

Characteristics of the Black seeds and powder

Macroscopically, seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, 2- 3.5mm×1-2 mm, black externally and white inside, odor slightly aromatic and taste bitter. Microscopically, transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick

Chemical composition of seed

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) *α*-pinene and thymol *etc*. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; *i.e.* isoquinoline alkaloids *e.g*. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent.

Traditional Uses of Folk Remedies

N. sativa has been traditionally used for the treatment of a variety of disorders, diseases and conditions pertaining to respiratory system, digestive tract, kidney and liver function, cardio vascular system and immune system support, as well as for general well-being. Black seeds and their oil have a long history of folklore usage in Indian and Arabian civilization as food and medicine. Its many uses have earned *Nigella* the Arabic approbation 'Habbatul barakah', meaning the seed of blessing. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy, amenorrhoea and dysmenorrhoea and in the treatment of worms and skin eruptions. Externally the oil is used as an antiseptic and local anesthetic. Roasted black seeds are given internally to stop the vomiting.

Materials and Methods

All the chemicals ($Cu(NO_3)_2{\cdot}3H_2O$, ethanol, Muller agar solution, and dimethyl sulfoxide DMSO) used in the experiments were of analytical grade (purchased from Merck chemical Industrial company) and used without any further purification.

Collection and Authentication of Plant Materials

Nigella sativa leaves were collected from…, after conducting the field surveys.

Preparation of Plant Leaf Extract

The leaves of *Nigella sativa* were surface cleaned and washed repeatedly with tap water followed by distilled water to remove dust particles and then allowed to dry under shadow for 15 days to remove moisture contents from the leaves. The dried leaves were ground using a grinding machine followed by packing in a brown bottle. The extraction was carried out by taking 20 g of the powdered leaves of *Nigella sativa* in a 500 ml of conical flask containing 400 ml of deionized water. After that, the mixture was shaken using a mechanical shaker for 90 minutes and allowed to warm at 50°C for 1 hour on a magnetic stirrer; then it was allowed to cool down to room temperature overnight. The prepared solution was filtered through Whatman No.1 filter paper to get clear solution.

Green Synthesis of Cu NPs

A 0.2 M aqueous Copper sulphate solution was prepared and stored in brown bottles. 100 ml of plant leaf extract was mixed with 400 ml of 0.2 M Copper sulphate solution (1 : 4) slowly dropwise with constant stirring. The mixture has been incubated at room temperature for 24 hrs. The color change was checked periodically (after 30 minutes and 60 minutes). The change in color from blue to light brownish visually indicates the formation of Cu NPs, and then, the solution was centrifuged for 15 min at 10000 rpm. The obtained g-Cu NPs were washed by deionized water and ethanol to remove any impurities. Thereafter, the NPs were allowed to dry and ground so as to be used for further analysis.

Characterization Techniques

The UV-visible absorbance and reflectance spectra of the samples were recorded in the range of 200–800 nm using Shimadzu's UV-2600, UV-visible spectrophotometer. UV-visible spectra were measured by transmission, whereas UV-DRS specifically refers to diffuse reflection spectroscopy.

The Cu nanoparticle solution was prepared by mixing the freshly prepared plant extract solution with Cu solution after appropriate dilution. An absorbance spectrum was recorded by allowing the instrument to scan wavelengths ranging between 200 and 860 nm, and the absorbance of the nanoparticle solution against blank extract solution was collected.

Qualitative Phytochemical Screening of Aqueous Extract of *Nigella sativa*

Aqueous extract was done by the cold maceration method. The sample subjected to various standard phytochemical test procedures to detect the presence or absence of various active phytoconstituents present in the crude extracts.

Phytochemcial Screening

Small amount of the extract/fraction was dissolved in required solvent and filtered. The filtrate was subjected to the following tests:

Carbohydrate

Benedict's test

To 0.5 ml of filtrate 0.5ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A reddish brown precipitate indicates the presence of reducing sugar (Sawant and Godghate, 2013).

Protein

Millon's Test

To 2 ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Nilanjana *et al.,* 2013).

Alkaloid

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids (Sawant and Godghate, 2013).

Tannin

1 g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot.1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride were observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins (Sawant and Godghate, 2013).

Flavonoids

Shinoda Test

To the extract, add a few fragments of Magnesium ribbon and concentrated hydrochloric acid. After a few minutes appearance of red to pink colour presence of flavonoids (Nilanjana *et al.,* 2013).

Ferric Chloride test

To the extract, neutral ferric chloride solution was added to indicate blackish green colour for the presence of phenolic content.

Terpenoids/Triterpenoids

Salkowski Test

To the extract few drops of concentrated H_2SO_4 and 2ml chloroform and shaken then allow standing, appearance of golden yellow colour indicates the presence of triterpenes (Nilanjana *et al.,* 2013).

Steroid

Bubble test

To the 1ml extract, add 5 ml of distilled water shake vigorously. Formation of foam indicates presence of steroids (Ling *et al.,* 2011). To the extract, 10 ml chloroform and conc. H_2SO_4 added to form the upper layer as red colour and the bottom layer as yellow with green fluorescence for the presence of steroids (Sawant and Godghate, 2013).

Test for Glycosides Anthraquinone glycosides

Borntrager's test (Harborne, 1983)

To 2ml of filtrate, 3 ml of chloroform mixed and shaken well. Chloroform layer was separated. Add 10%

ammonia solution and followed by shaking. A rose pink to red colour changes in ammoniacal layer indicated the presence of anthraquinone glycosides.

Antibacterial Activity

The *in vitro* antibacterial activity of g-Cu NPs was evaluated using an Agar disc-diffusion method against selected two gram-positive bacteria (*Bacillus cerus and Bacillus subtilis*) and two gram-negative pathogenic bacteria (*Enterobacter aeroges, Salmonella typhimurium and Pseudomonas aeruginosa*). Microbial strains were obtained from pastor institute. Prior to an antibacterial activity test, the bacterial strains were cultured in nutrient broth for 24 hours to obtain logarithmic growth phase of the test bacteria. The actively growing bacterial cultures of 1.3 X 10^8 CFU/ml concentration were inoculated/spread into the Muller Hinton Agar (MHA) (Ananda Murthy *et al.,* 2020).

The extract was prepared at 1 mg/ml (dissolved in Dimethyl Sulfoxide). The Whatman filter paper disks were punched at 6 mm diameter and impregnated with the dissolved extract and then placed to the MHA surface. Ampicillin disc was used as a positive control while DMSO was taken as the negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated in terms of zone of inhibition, measured, and recorded in millimeters using a ruler.

Results and Discussion

Synthesis of g-Cu NPs

The g-Cu NPs were synthesized by using copper sulphate as a precursor and plant leaf extract as a reducing and capping agent. The change in color from blue to light brownish visually indicates the formation of copper NPs. The g-Cu NPs were washed by deionized water and ethanol to remove any unwanted particles. Thereafter, g-Cu NPs were dried and ground and later subjected to various characterization methods.

Characterization of g-Cu NPs

The g-Cu NPs were characterized using UV-visible UV-Visible Spectral Analysis

The UV-visible absorbance spectrum recorded for g-Cu NPs exhibited of 851 as shown in Figure 5.

UV–Vis spectral analysis. The formation of CuO NPs was followed through measurement of its SPR peak using a UV–Vis spectrophotometer, and the results are depicted in Fig 3, 4 & 5. As shown in Fig. 4, the maximum absorption peak occurred at 851 nm, attributed to the SPR absorption band of CuO NPs. Te SPR absorption band of CuO NPs at 851nm confirmed the formation of CuO NPs. As the wavelength increased, the absorbance intensity decreased, indicating that formation did not occur at a large wavelength. CuO NPs formed in agreement with studies that proposed that CuO NPs formed between 300 and 860 nm.

Phytochemical Analysis

The presence of Alkaloid compounds, flavonoid and d glycosides was confirmed during the phytochemical screening of *Nigella sativa* leaf extract. The details of the screening are as given in Table 1. It is possibly believed that the bioactive compounds such as alkaloid or flavonoid compounds act as a ligand and bind to metal ions and reduce them and cap them to form nanoparticles. These ligands also act as particle size controllers as reported by the earlier researcher.

Primary components of *Nigella sativa* extract are Alkaloid, Flavonoid compounds, tannins, and glycosides. The antioxidant properties of alkaloid compounds are primarily due to their high inclination towards chelating the metals. Flavonoi compounds contain very high tendency to bind metal ions. Metal ions in solution interact with polyphenolic compound and helps in the nucleation and formation of Cu NPs (Ananda Murthy *et al.,* 2020).

Flavonoids are effective metal ion chelators and play a key role in the initiation of free radical processes (via the Fenton reaction). Metal chelation is thought to be another mechanism of flavonoids antioxidant activity, which, along with some biological effects of flavonoids, can be altered by flavonoid–metal interaction

Most of flavonoids are good metal chelators which can chelate many metal ions to form different complexes due to their high super delocalizability and conjugated system. The interaction of metals with flavonoids contributes to the anti-oxidant activity.

Flavonoids easily chelate metal ions and create complex compounds. Their additional antioxidant activity results from binding of metal ions like Fe(II), Fe(III) and Cu(I), which participate in free radical-generating reactions. Therefore, they act on two antioxidant pathways: (1) direct reactions with free radicals, (2) chelating of metal ions involved in production of reactive oxygen species.

Their chelation depends on the presence of certain chelation sites in the structure of the flavonoid as well as the solvent type and pH conditions. Flavonoids act as weak polybasic acids, so pH plays an important role in complex formation.

The optimal pH for complex formation is around 6, although it strongly depends on the metal ion. At pH below 3.0, flavonoids remain undissociated, which is unfavourable for complex formation. At high pH values flavonoids are deprotonated and form more complex species.

Furthermore, at higher pH values metal ions cause side reactions (hydrolysis) and hydroxocomplexes are formed.

Chen et al has been found successful chelation with transition metal species such as Cu(II) and Ni(II) to biochanin A (4′-methoxy-5,7-dihydroxy-isoflavone) (Misiak and Lodyga- Chruscinska, 2010).

Antibacterial Activity

From the preliminary phytochemical tests, the existence of alkaloids and flavonoids was confirmed, which could be correlated with the effect of the extract on bacterial strains. In our study, agar well diffusion method of antibacterial screening of CuNPs against Gram Positive (*Bacillus cereus*, *Bacillus substillus*) and Gram Negative bacteria (*Pseudomonas aureginosa, Salmonella typhimurium, Enterobacter aerogenus*) was performed.

The results from our current study showed a concentration-dependent effectiveness which coincided with some of the earlier literary findings where the antibacterial activity was shown to have a direct relation with the increasing concentration of biosynthesized CuNPs (Phongpaichit, 2007).

Table.1 Preliminary qualitative phytochemical analysis of Nigella sativa aqueous Extract.

S.No.	Phytoconstituents	Aqueous Extract
1	Carbohydrate	+++
2	Protein	-
3	Alkaloid	++
4	Flavonoid	+++
5	Tannin	++
6	Steroid	-
7	Terpenoids	++
8	Glycosides	++

+++: Highly Present
++: Moderately Present
+: Low
-: Absent

Table.2 Zone of Inhibition of Aqueous Leaf Extract of *Nigella sativa.*

Name of the Microorganism	Zone of Inhibition(mm)				Standard Penicillin
	25(µl)	50(µl)	75(µl)	100(µl)	
Bacillus cerus	10	12	19	21	15
Bacillus subtilis	3	5	15	18	5
Enterobacter aeroges	2	10	15	22	8
Salmonella typhimurium	5	11	20	28	10
Pseudomonas aeruginosa	6	12	21	22	8

Figure.1 CuS04 stock solution in water.

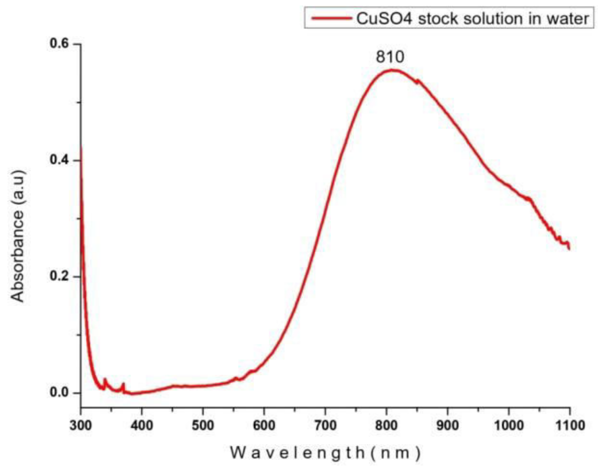


Figure.2 *Nigella sativa* Extract.

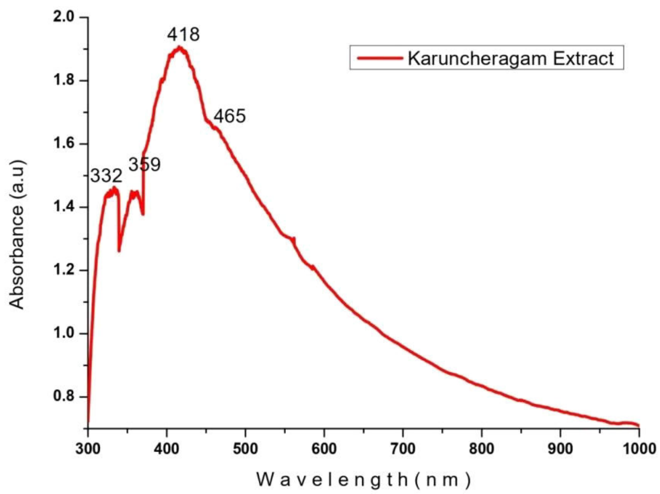


Figure.3 Copper Nanoparticle

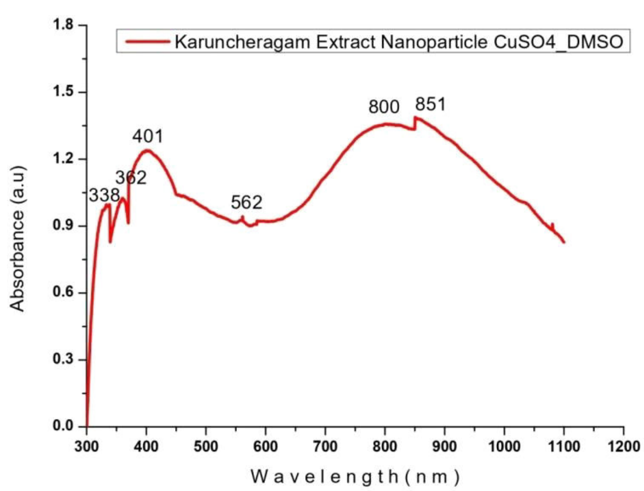


Figure.5 Phytochemical analysis of *Nigella sativa* aqueous Extract

Figure.6 Antibacterial Activity of Aqueous Leaf Extract of *Nigella sativa*

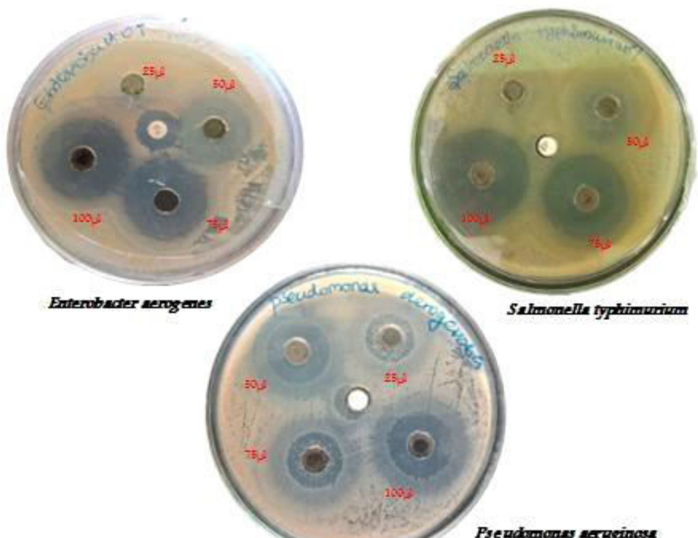
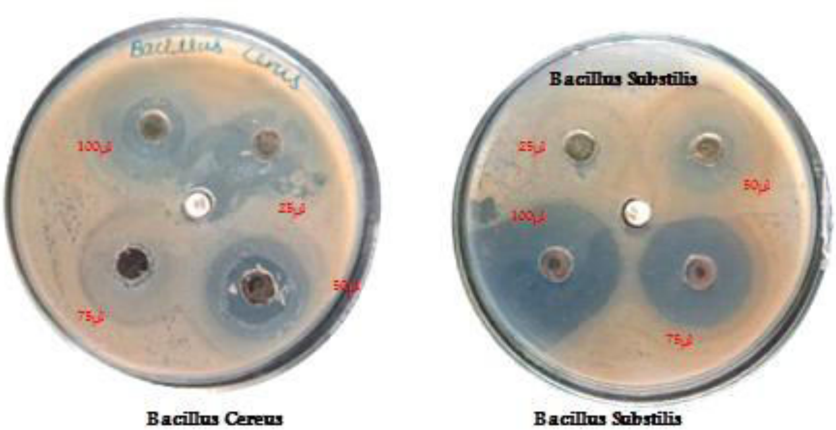


Figure.7 Gram positive Microorganisms



The g-Cu NPs synthesized by using the leaf extract of *Nigella sativa* showed a broad range of antibacterial activities against all tested pathogens: Gram Positive (*Bacillus cereus* – 19.0, and *Bacillus substillus* – 18.0) and Gram Negative bacteria (*Pseudomonas aureginosa* – 22.0, *Salmonella typhimurium* – 28.0, *Enterobacter aerogenus* – 22.0) respectively (Table 2).

The Cu NPs have proven record of an excellent antibacterial activity. Accordingly, this result showed that the g-CuO NPs are more active against gram-positive bacteria than gram- negative bacteria which is possibly due to the difference in the structure of cell walls of bacteria.

The antibacterial activity of NPs can be attributed to the presence of bioactive compounds on the surface of NPs as capping and stabilizing agents. In this regard, the activity was pronounceable against *Salmonella typhimurium* compared to penicillin, the standard antibiotic. The highest zone of inhibition (mm) recorded with g-Cu NPs against *Salmonella typhimurium* was 28.0 mm, and the lowest zone of inhibition (mm) recorded against *Bacillus substillus* bacteria was 18 mm. The wide zone of inhibitions of g-Cu NPs against pathogens confirms their great potential as a remedy for infectious diseases caused by the tested bacterial pathogens.

Additionally, the standard disc penicillin showed the comparable zone of inhibition with the g- Cu NPs, which is small, and it can be attributed to the development of resistance by bacteria against penicillin.

The antibacterial results obtained using g-Cu NPs were found to be better when compared with an earlier work reported by many researchers. The highest zone of inhibition (mm) recorded with g-Cu NPs against bacteria was 28.0 mm which is far above the zone of inhibition exhibited by Cu NPs synthesized by various plant extracts as mentioned in Table 2. The plant extracts were proved to exhibit a higher zone of inhibition which can be attributed to the presence of different type of phytoconstituents. Thus, it can be concluded that the synergistic effect of Cu NPs coupled with bioactive compounds such as alkaloid and flavonoid of *Nigella sativa* leaf extract was proved to be beneficial against pathogens.

Many mechanisms of antibacterial activity have been reported by the past researchers; accordingly, the action of g-Cu NPs on the bacteria is yet to be explored fully. It is assumed that the g-Cu NPs get adsorbed on to the cell wall of bacteria and interacts with the electronegative elements within the cell membrane. This results in failed metabolism thereby leading to interference and disruption of transcription in bacteria and hence causes antibacterial activity by g-Cu NPs. It is also believed that the synergistic effect of g-Cu NPs with bioactive compounds of extract would have played significant influence to inhibit the activity of pathogenic bacteria as suggested by the recent researcher.

It is possibly understood that the helical structure of DNA molecules would have been disrupted by the action of CuO NPs. In addition, electrochemical potential across the cell membrane decreases up on the interaction with the released Cu metal ion by Cu NPs affecting integrity of the membrane.

The green copper nanoparticles (g-Cu NPs) were successfully synthesized by using medicinal plant *Nigella sativa* leaf extract. The presence of phytoconstituents such as Alkaloid and Flavonoid played roles in reducing and capping agents during the formation of g-Cu NPs. The UV-visible absorbance and reflectance spectra showing of 851 nm, confirmed the formation of g-Cu NPs. The wide zone of inhibition of g-Cu NPs against pathogens confirms their great potential as a remedy for infectious diseases caused by the tested bacterial pathogens. Finally, it can be concluded that the synergistic effect of bioactive compounds from medicinal plant coupled with Cu NPs has been proved to be beneficial against pathogens.

Author Contributions

N. Ashwak: Investigation, formal analysis, writing—original draft. J. Prakash: Validation, methodology, writing—reviewing. K. Tamilarasan:—Formal analysis, writing—review and editing. N. V. Swathi: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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