GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Research Article)

Check for updates

Antimicrobial activity of copper and silver metal nanoparticles synthesized from *Riccia fluitans*

Ganga G *

Department of Biochemistry and Industrial Microbiology Sree Ayyappa College Eramallikara Chengannur Alappuzha District Pin: 689109.

GSC Biological and Pharmaceutical Sciences, 2021, 18(01), 077–082

Publication history: Received on 25 May 2021; revised on 26 June 2021; accepted on 29 June 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.1.0183

Abstract

Riccia bryophyte, a genus of liverworts that comes under the family of Ricciaceae, order Marchantiales. The plants are primitive plant structure, that's not differentiated into root, stem, and leaf. The material utilized in this study was a Spp of a *Riccia fluitans*, that grows on damp soil or, less unremarkably, floating in ponds, and is usually utilized in aquariums. The nanoparticles utilized in this study exhibit potential medicinal drug activity. Advantages of its therapeutic potential can be utilized in a sizable number of fields like health care, cosmetics, biomedical, food and feed, drug-gene delivery, surroundings, health mechanics, optics, chemical industries, physical science, area industries, energy science, catalysis, lightweight emitters, single lepton transistors, nonlinear optical devices, and photo-electrochemical applications. This study aimed to gauge the medicinal drug activity of Riccian nanoparticles, copper-loaded nanoparticles, and silver-loaded nanoparticles against varied microorganisms. The result of the study showed that the Nanoparticle, Ag-NP and Cu-Np synthesized from Riccia is having good antimicrobial activity against tested organisms.

Keywords: *Riccia fluitans;* copper;nanoparticles; silver- nanoparticles; antimicrobial activity

1. Introduction

Green synthesis of metal nanoparticles utilizes bio-molecules present in plant extracts single-step process. Reductions of metal-ions to base metal can be enhanced by physical methods such as controlled temperature and pressure. This synthesis and scale-ups are driven by plant metabolites which are environmental friendly Reducing agents used in this study are water-soluble plant metabolites (example alkaloids, phenolic compounds, terpenoids) and coenzymes.

Synthesis of metal nanoparticles such as Gold (Au} Silver (Ag) Copper (Cu) Zinc (Zn) nanoparticles have gained particular attention for plant-based synthesis. There are many reports available on Plants based green synthesis of metal nanoparticles using a diverse range of plant species. Apart from plant extracts, live plants can be used for synthesis [1-2].

Metallic nanoparticles had received attention in the field of science due to their small size and tremendous potential in many areas of research and applications. Recent research explores its antimicrobial potential against many pathogens as there is an increase in demand in the treatment of drug-resistant bacteria. Amid the several potent noble metal nanoparticles, silver nanoparticles have attained significant attention due to their wide therapeutical application.

Nanoparticles are synthesized conventionally using chemical approaches by using toxic and hazardous chemicals. These methods involve various biological risks due to their general toxicity; engendering the serious concern to develop

* Corresponding author: GANGA G

Assistant Professor in Microbiology, Department of Biochemistry and Industrial Microbiology Sree Ayyappa College Eramallikara Chengannur Alappuzha District Pin: 689109.

environment-friendly processes. Green synthesis assisted by biological molecules derived from plant sources exhibiting superiority over chemical and/or biological methods [3].

Diverse plant species have diverse biologically active Plant-based compounds which endures highly controlled assembly for making them suitable for the metal nanoparticle synthesis Principles of green synthesis are advantageous over chemical mode which utilized synthesis rapid and single-step procedure [2-4].

Metallic nanoparticles are considered to have remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics, and the developments of resistant strains. Among metal nanoparticles, silver nanoparticles have notable potentials in the field of nanotechnology which has gained utmost interest due to their exceptional properties such as chemical stability, good conductivity, catalytic, and most important antibacterial, anti-viral, antifungal, and anti-inflammatory activities. They have widely employed the development of composite fibers, cryogenic superconducting, food packaging, fabrics, wound healing creams and pastes, cosmetic therapies, dental implants etc [4-9].

Copper oxide nanomaterials also are unique due to their optical, thermal, electrical, chemical and biological properties. [10, 2] These characteristics allow for a wide range of applications in a variety of domains, including biosensors, storage devices, supercapacitors, and infrared filters, as well as the health and environmental sectors [6] Recently a great interest in Cu Nps due to its antimicrobial properties. Synthesis of CuO nanomaterial is cost-effective as compared to other silver (Ag), gold (Au), and platinum (Pt) nanoparticles,

Liverworts (Marchantiophyta) are the second largest group of bryophytes Riccia is a bryophyte, a genus of liverworts belonging to the Ricciaceae family, and the order, Marchantiales *Riccia fluitans* is an amphibious liverwort of Ricciaceae family that can thrive in aquatic and terrestrial environments [11]. These are primitive plants having a thallus that does not distinguish between root, stem, and leaf. They can be strap-shaped and 0.5 to 4mm wide, with dichotomous branches, or they can form rosettes up to 3cm in diameter, that are gregarious and form intricate mat A mid-ventral ridge on the lower surface has multicellular scales that start as a single row but split into two as the thallus spreads. Multicellular and hyaline (glassy) in appearance, or violet due to the pigment anthocyanin The “slender riccia” (*Riccia fluitans*) is one of the more than 100 species in this genus, which grows on damp soil or, less commonly, on the ground [12]. Tosun et al 2013 [8] exhibiting promising pharmacological and biological activities. They have been used as remedies for cuts, fractures, burns, bruises, open wounds, inflammation, and pneumonia sesquiterpene an anti-inflammatory drug [8, 13].

By reducing Cu^{2+} ions in the copper sulphate solution using *Riccia fluitans* extract, an attempt was made to establish an eco-friendly, cost-effective technique for the synthesis of Ag nano and CuO nanoparticles. There are no many reports are available on the use of *Riccia fluitans* extract for Nanoparticle production. The present study comprises of synthesis of Ag and Cu0 nanoparticle and their evaluation of antimicrobial properties against Gram-positive and negative bacteria.

2. Material and methods

2.1. Collection of samples

Riccia fluitans samples were collected near the temple premises at Shornur Municipality of Palakkad district. Samples were cleaned and washed with hypochlorite and 70% Ethanol and kept in the fridge until processed. Bacterial pathogen used for understudies has been obtained from MTCC culture collection Chandigarh, India. They were subclustered and the purity of the cultures was identified by microscopic observation and biochemical characteristics. Pure cultures were obtained from them and they were maintained at 37°C in a nutrient agar medium.

2.2. Synthesis of Riccia-metal nanoparticles

Synthesis protocol in one step $CuSO_4$ was prepared at 25mM and 50mM concentrations in 50ml of 5g Riccia plant extract. It was then given 25mM and 50mM of $AgNO_3$ as well as a mixture of 25mM and 50mM of $CuSO_4$ and $AgNO_3$. The sample was then thoroughly blended. It was then incubated at 72°C for 12 hours. The colloid was centrifuged at 12,000rpm for 10 minutes after 12 hours to remove the particles from the suspension. The substance was finally resuspended in acetone (90%, v/v) followed by centrifugation at 6,000rpm, the acetone wash was repeated twice and a final wash with 70% ethanol was carried out to remove the traces of impurities. The precipitate obtained was dissolved in ultrapure water, vacuum dried, and stored at 4 ∘ C until use. U-V absorbance was observed confirm the identity of Nanoparticle using UV visible spectrophotometer.

2.3. Antibacterial assay

A well diffusion method was used to assay the antibacterial activity of synthesized Cu-Riccia NPs, Ag-Riccia NPs, and a combination of Cu and Ag Riccinia NPs against test strains in Muller-Hinton agar media. (Hi-Media, India) For control experiments, *Escherichia coli* and *Klebsiella pneumoniae* were used for gram-negative, and for gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* were used. Riccia was also used separately to check the antimicrobial activity. The plates were incubated for 24 hrs. at 37°C and inhibition zones were measured.

Agar well diffusion method was used to evaluate the antimicrobial activity of Cu.NP and Ag.NP nanoparticle. The agar plate surface is inoculated by spreading a volume of the microbial inoculation over the entire agar surface, then a hole with a diameter of 6-8mm is punched aseptically with a sterile corn borer or a tip, and a volume of 200µl of the antimicrobial agent is introduced into the well. Then agar plates were incubated under suitable conditions depending upon the test organisms. The antimicrobial agent diffused in the agar medium and inhibits the growth of microbial strain tested.

2.4. Antimicrobial activity of nanoparticles

Riccia bryophytes collected forms Shornur district of Kerala and identified as *Riccia fluitans* (Figure 1). Riccia Cu Riccia .NP and Ag Riccia .NP was Synthesised successfully and spectrometric analysis was carried out using a U-V spectrophotometer. (Figure2) The antimicrobial activity of different antimicrobial activity of Cu.NP and Ag.NP prepared was studied by the well diffusion method. Well diffusion method was followed.

Figure 1 *Riccia fluitans* collected from three different sites (A-C) at Shornur Municipality of Palakkad District

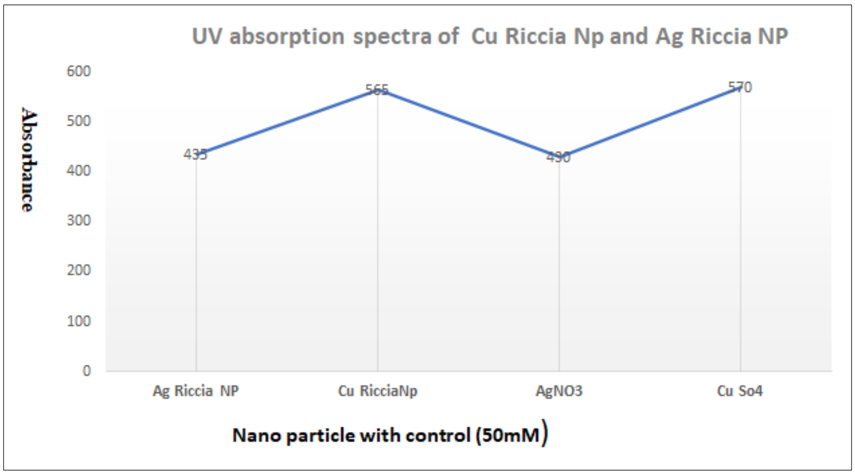

Figure 2 UV-Absorption spectra of Nano particle Cu Riccia Np Ag Riccia Np and control

- Control,2. Ag-Riccia-Nps 3, Ag-Riccia-Np+ Cu-Riccia-Np (25mM)
- Control,2. Ag-Riccia-Nps 3, Ag-Riccia-Np+ Cu-Riccia-Np (50mM)

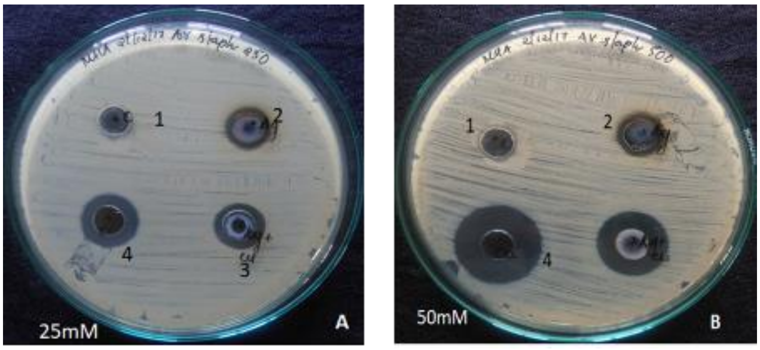

Figure 3 Antimicrobial activity of Cu Riccia Np and Ag Riccia Np against Staphylococcus aureus

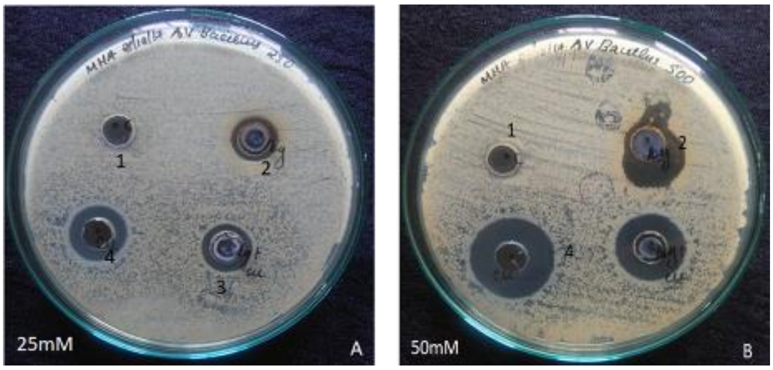

Figure 4 Antimicrobial activity of Cu Riccia Np and Ag Riccia Np against *Bacillus subtilis*

- Control,2. Ag-Riccia-Nps 3, Ag-Riccia-Np$_{+}$ Cu-Riccia-Np (25mM)
- Control,2. Ag-Riccia-Nps 3, Ag-Riccia-Np$_{+}$ Cu-Riccia-Np (50mM)

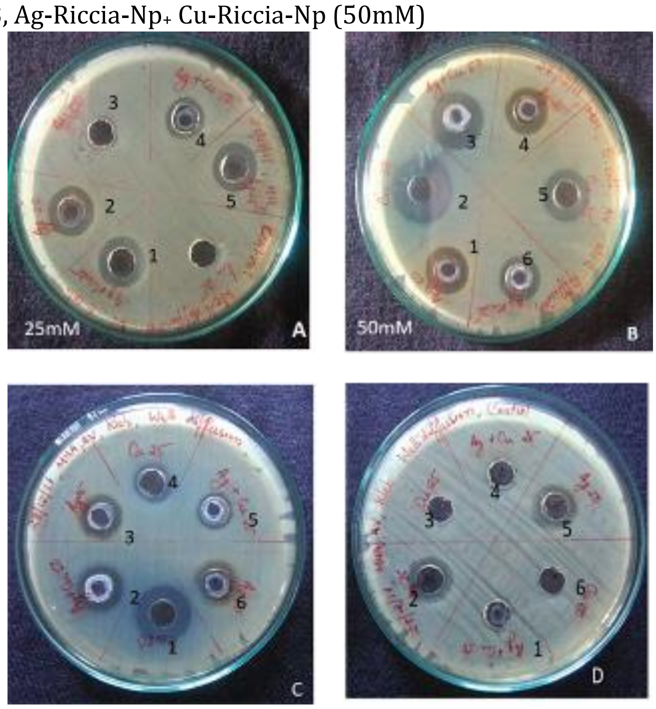

Figure 5 Antimicrobial activity of Cu Riccia Np and Ag Riccia Np against *Escherichia Coli and Klebsiella* pneumonia

- A.1. Ag-Riccia-Np$_{+}$ Cu-Riccia-Np, 2.Ag-Riccia-Nps 3, Control AgNo3 4. Ag**+**Cu 5. Cu-Riccia-Np 6 CuSo4-control(25mM)
- B. 1.Ag-Riccia-Nps 2. Cu-Riccia-Np 3.Ag-Riccia-Np$_{+}$ Cu-Riccia-Np 4. Ag-Riccia-Nps 5. Ag-Riccia-Np 6. Ag-Riccia-Np$_{+}$ Cu-Riccia-Np (50mM)

- C. C.1. Cu Riccia Np (50mm) 2.Ag RicciaNp+ Cu Riccia (50mM) Np 3. Ag Riccia Np (50mM) 4. Cu Riccia Np(25mM) 5 Ag Riccia Np + Cu Riccia (25mM) Np 6. Ag RicciaNp (25mM)
- D 1. AgNo3+CuSO4 control 2.CuNp (Chemically synthesised 3.Cuso4 control 4. AgNo3+CuSo4
- 5.AgNp(Chemically synthesised 6.CuSo4

It was observed that antimicrobial activity was more in gram-positive bacteria than in negative bacteria. Antibacterial activity was observed when treated with Riccia copper nanoparticles.

The highest antimicrobial activity was observed against Staphylococcus aureus against Cu Riccia) .NP (2.4 cm) and Ag Riccia .NP as well in a combination of both. Maximum antimicrobial activity was observed by Cu Riccia.NP combination Ag Riccia is with*. E.coli* which indicates that Ag Riccia Np and Cu Riccia Np have a cumulative effect on gram-negative organism Even though in NP the .least activity was observed against Klebsiella species combined application of both Nps are found to have some effect on Klebsiella pneumoniae. The comparative analysis of both Nps on Bacillus subtilis' showed that Ag Riccia.Np is equally good as Cu Np, the result of the study is given in figure 3-5.

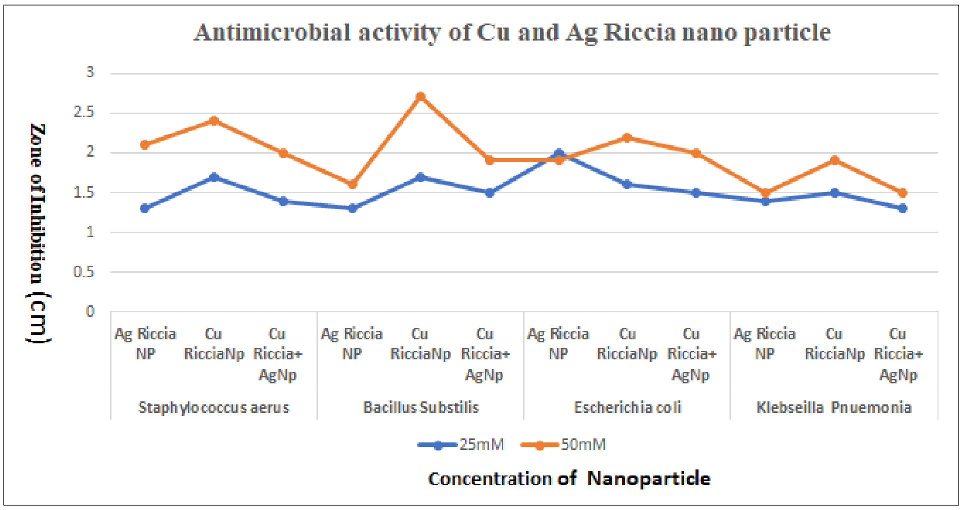

Figure 6 Comparative analysis synthesised NpS against various Staphylococcus aureus Bacillus subtilis Escherichia Coli and Klebsiella pneumonia

3. Discussion

Green synthesis of Nanoparticle from *Riccia fluitans* was done and the identity of the synthesized particle was analyzed by UV spectrophotometric analysis. Nanoparticle synthesized by using Riccia plant extract assisted method is found to be effective against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli* and are found to be effective against all the organism tested*.* Codiţă et al 2010 [5] reported that both Copper (Cu) and Silver (Ag) have efficient antimicrobial activities , this study also proved that antimicrobial activity was more in gram-positive bacteria than in negative bacteria. A study showed that more Antibacterial activity was observed when treated with.Cu Riccia Np than Ag Riccia Np .However, another study conducted by Mukherji et al 2012 [7] reported that Chemically synthesized Ag Nps have more activity than Cu-Nps. The study also showed that a cumulative effect of two nanoparticles when they mix can also effective against bacterial pathogens, in this study this cumulative effect *E.coli*. Codiţă et al 2010 {9] also reported the cumulative effect of Ag -Nps, and Cuo-Nps against biofilm-forming bacteria.

4. Conclusion

Antimicrobial activity was seen more in gram-positive bacteria than in gram-negative bacteria. Gram positive bacteria used are *Staphylococcus aureus* and *Bacillus subtilis.* Gram negative bacteria used are *Klebsiella pneumoniae* and *Escherichia coli.* Antibacterial activity was seen more with copper nanoparticles against gram-positive and gram-negative bacteria. The study also showed Green synthesized nanoparticles from *Riccia fluitans* bryophytes are effective against selected bacterial pathogen Study also can be extrapolated to identify its activity against biofilm and Drug-resistant pathogen, Antifungal and anti-cancerous effect of the Nps can also need to be screened.

Compliance with ethical standards

Acknowledgments

Author is thankful to Dr. Leslie Jose Selvaraj for extending his support for suugestions and Nexgene Lab for technical support.

Disclosure of conflict of interest

No conflict of interest.

References

[1] Ali DM, Thajuddina N, Jeganathanb K, Gunasekaranc M . Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. . *Colloid Surf B Biointerfaces* 2011; 85(2): 360–365.

[2] Bhattacharjee A, Ahmaruzzaman M. CuO nanostructures: facile synthesis and applications for enhanced photodegradation of organic compounds and reduction of pnitrophenol from aqueous phase. *RSC Adv.* 2016; 6(47): 41348–41363.

[3] lta-Videa, Yuxiong Huang, J. Parsons, Lijuan Zhao, L. López-Moreno, J. Hernandez-Viezcas, J. Gardea-Torresde. Plant-based green synthesis of metallic nanoparticles: scientific curiosity or a realistic alternative to chemical synthesis? . *Nanotechnol. Environ. Eng* 2016; 4(1): . DOI 10.1007/s41204-016-0004-5 (accessed).

[4] Ana Cláudia Paiva-Santos, Ana Margarida Herdade, Catarina Guerra, Diana Peixoto, Miguel Pereira-Silva, Mahdi Zeinali, Filipa Mascarenhas-Melo, António Paranhos, Francisco Veiga. Plant-mediated green synthesis of metal-based nanoparticles for dermopharmaceutical and cosmetic applications. *Int J Pharm* 2021; 59(7): 120311.

[5] Codiţă I, Caplan DM, Drăgulescu EC, Lixandru BE, Coldea I, Dragomirescu CC, Surdu-Bob C, Bădulescu M. . Antimicrobial activity of copper and silver nanofilms on nosocomial bacterial specie. *Roum Arch Microbiol Immunol.* 2010; 69(4): 204-12,

[6] Dagher S, Haik Y, Ayesh AI, Tit N. . Synthesis and optical properties of colloidal CuO nanoparticles. *J Lumin.* 2014; 151(): 149–154.

[7] Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles.. *Acta Biomater* 2008; 4(3): 707–716,

[8] Tosun A, Küpeli Akkol E, Süntar I, Özenoğlu Kiremit H, Asakawa Y.. Phytochemical investigations and bioactivity evaluation of liverworts as a function of anti-inflammatory and antinociceptive properties in animal models.. *Pharm Biol.* 2013; 51(8): 1008-13,

[9] Agnihotri S, Dhiman NK. Development of Nano-Antimicrobial Biomaterials for Biomedical Applications.. Anju Tripathi Jose savio Melo (ed). *Advances in Biomaterials for Biomedical Applications* , 66(doi:10.1007/978-981-10-3328-5 ed. Singapore: Springer; 2017. pp. 479-545.

[10] Devi HS, Singh TD. Synthesis of copper oxide nanoparticles by a novel method and its application in the degradation of methyl orange. . *Adv Electr Electron Eng* 2014; 4(1): : 83–88,

[11] Althoff F; Zachgo S. Transformation of Riccia fluitans, an Amphibious Liverwort Dynamically Responding to Environmental Changes. . *Int. J. Mol. Sci* 2020; 21(): 5410.

[12] Dinghy F. The morphology of Riccia F. Proceedings of Indiana Academy of Science (ed). , V25 ed. : WaynE Printing Company. ; 195. pp. 131-133.

[13] Türkoğlu S. Parlak, A.E. Determination of total phenolic and total flavonoid contents and antioxidant capacities of an aquatic plant (Riccia fluitans). *Ege J Fish Aqua Sci* 2014; 31(1): 35-40.