

Antibacterial and Antifungal Activity of Cadmium Sulphide Nanoparticles

Sandhiyapriya T¹, Muneeswari K¹, Gomathi M^{1*}, Shanmugarathinam A², Akshay S³ and
M.I. Niyas Ahamed⁴

¹Department of Biotechnology, Marudhar Kesari Jain College for Women, Vaniyambadi- 635751, Tamilnadu.

²Department of Pharmaceutical Technology, University College of Engineering, Bharathidasan Institute of Technology (BIT) Campus, Anna University, Tiruchirappalli -620 024, Tamilnadu.

³Department of Physics, Maharani Lakshmi Ammanni College for Women Autonomous, Malleswaram, Bengaluru-560012, Karnataka.

⁴Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur- 635601, Tamilnadu.

*Corresponding Author: gomathi83ms@gmail.com

Abstract

The application of plants is well known to us and it is still espousing in the recent era. The plant kingdom is a treasure house for potential medicines thereby enhancing knowledge of medicinal plants in recent years. Cerium, an element in the sequence of lanthanides, shows oxidation state of Ce³⁺, Ce⁴⁺ and the ability to significantly change or quickly turn over its state. The methanolic extract from *Morus alba* leaves was prepared by using Soxhlet apparatus and the preliminary phytochemical investigation was conducted and confirmed the presence of carbohydrates, steroids, amino acids, anthocyanins, phenols, tannins, alkaloids, flavonoids, terpenoids and glycosides. By observing the XRD pattern, the CdS NPs was confirmed by its characteristic peak. Furthermore, by using CdS NPs the antibacterial and antifungal activity against *Salmonella typhi*, *Escherichia coli* and fungal isolate *Candida Albicans*, *A. Niger* has been carried out. Maximum zone of inhibition is observed in *Salmonella typhi* and *Candida albicans*.

Keywords: Phytochemical, Antibacterial activity, *Morus alba*, Medicinal plants,

1. Introducing

Nanotechnology is characterized by its naturally existing broad spectrum and application, nanotechnology, including number of emerging technologies [1]. Recently *Fusarium oxysporum* as a mycotoxic fungus has many advantages for the development of nanoparticles. In the Moraceae family, Mulberry, a genus of flowering plants, possesses 10-16 species of deciduous trees commonly known as Mulberries, which in many temperate world regions grow wild and under cultivation [2-4]. The closely related genus *Broussonetia*, especially the paper mulberry, is also commonly known as mulberry. Phytochemicals are

commonly known as study compounds rather than vital nutrients since there is still little evidence of their potential health effects [5]. Research-based phytochemicals may be grouped into major groups, such as carotenoids and polyphenols, including phenolic acids, flavonoids and stilbenes. Compounds which inhibit oxidation are antioxidants.

2. Materials and Methods

2.1 Collection of plant materials

Fresh parts of *Morus alba* were collected from Yelagiri hills, Tamil Nadu, India. Taxonomically it was classified and authenticated the plant materials by the Department of Botany, Loyola College (Chennai), Tamil Nadu, India. When all the aqueous molecules evaporated shade dry plant leaves were used for grinding and transferred to airtight containers with appropriate labeling for potential use.

2.2 Preparation of extracts from plants

Crude plant extract with updated available literature [6] was prepared by using Soxhlet extraction process. Approximately 10gm of plant material was equivalently packed into a thimble and separately extracted by using 100ml of various solvents. Methanol was used as solvent for extraction. It was permitted to run for 24 hours at 30-40⁰C it was incubated [7].

2.3 Assay for Phytochemical Screening

2.3.1 Carbohydrate Examination

2.3.1.1 Test Molish 's

With 2 ml of concentrated sulphuric acid, the 2 ml of plant extract was stirred, and then add 2 ml of Molish reagent, the existence of reddish ring indicates the presence of carbohydrate [8].

2.3.1.2 Check Benedict's

With 5ml of Benedict's reagent, the 0.5ml of plant extract was stirred, then boil for 5 minutes and allow the solution to cool, the appearance of Orange red precipitate it indicates the presence of carbohydrate [9].

2.3.2 Fehling Test Failing

2.3.2.1 Test Fehling's (A)

With 1ml of Fehling's (A) reagent, the bluish green colour appearance, indicates carbohydrate presence, the 1ml of plant extract was stirred [10].

2.3.2.2 Test for Fehling's (B)

The appearance of brown colour suggests the presence of carbohydrates in 1 ml of plant extract was combined with 1 ml of Fehling's (B) reagent

2.3.3 Amino Acids Test

2.3.3.1 Test Ninhydrin

With 1 ml of 10% Noah, the 1 ml of plant extract was stirred, then added a few drops of 1% CuSo₄, the appearance of violet colour indicates the presence of amino acid.

2.3.4 Phenol Test

The addition of 1 ml of plant extract combined with 1 ml of 5 percent ferric chloride (FeCl₃), the development of a bluish black colour represents the existing of phenol [11].

2.3.5 Flavonoids Screening

With 1 ml of 10 percent Noah the 1ml of plant extract was stirred, the appearance of yellow colour shows the existence of flavonoids.

2.3.6 Steroids Test

With 2 ml of chloroform and add 1 ml of concentrated H₂SO₄, the appearance of a red acidic layer signaling the presence of steroids, to the 5ml of plant extract [12].

2.3.7 Anthocyanins Test

With a few drops of concentrated sulfuric acid, the 1 ml of plant extract was stirred, the existence of the yellow orange colour suggests the presence of anthocyanins.

2.3.8 Alkaloids Test

2.3.8.1 Test of Dargendroff's

The 2 ml of the plant extract was combined with 0.2 ml of the diluted HCl and 1 ml of the dargendroff reagent was applied, the yellowish orange colour appearance suggesting the presence of alkaloids.

2.3.9 TERPINIDS TEST

With 2 ml of chloroform, the 1 ml of plant extract was stirred and added 3 ml of concentrated sulfuric acid, the appearance of a reddish-brown pigment, suggesting the presence of terpenoids [13].

2.3.10 Glycosides Examination

2.3.10.1 The H₂SO₄ Concentrated Test

The 2 ml of plant extract was continuously stirred with 2 ml of glacial acetic acid (GAA) and added a few drops of 5 % FeCl₃ solution, adding a few drops of concentrated sulfuric acid, reddish brown in colour, suggesting the presence of glycosides [14].

2.3.11 Tannins Test

With 1 ml of 5 percent FeCl₃ solution added, the appearance of brownish green colour was agitated with 3 ml of plant extract, suggesting the presence of tannins [15].

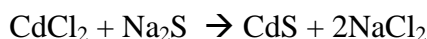
2.4 Assay of Antioxidant In Vitro Assays

2.4.1 Assay with 1,1-diphenyl-2-picrylhydrazyl (DPPH)

With some modifications, the DPPH radical scavenging behaviour of the phytochemicals was calculated by the Brand-Williams et al. procedure. The DPPH (250 µM) methanol solution was combined with an equal aliquot of varying concentrations (0.5-7µM) of methanol phytochemicals. The absorbance was spectrophotometrically estimated at 515 nm after 2 minutes of incubation in the dark [16].

2.5 CdS Nanoparticles synthesis

Synthesis of cadmium sulphide nanoparticles (CdS NPs) was achieved under the influence of obtained plant cure extract and the reaction of sodium sulphide and cadmium chloride. To synthesis of CdS, 0.1 M concentration of sodium sulphide and cadmium chloride was used for this experiment. In order to verify the impact of cadmium chloride (CdCl₂) on nanoparticle formation, four different ratios of sodium sulphide and cadmium chloride ranging from 1:1 to 4:1 were used respectively.



This experimental setup was confirmed created an orange-yellow color suspension of cadmium sulphide (CdS) to which the same amount of supernatant was applied to each tube and thoroughly mixed. The fusion was held for around 10-20 minutes in the water bath at 60 ° C until fluffy orange-yellow color was seen at the bottom, suggesting the development of nanoparticles. The suspension was left to cool and incubated at room temperature for 24 hours [17].

2.6 Antimicrobial Activity Determination:

The microbial cultures (*Escherichia coli*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans*) were obtained from Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur district, Tamil Nadu, India. By spreading the inoculum on the surface of Mueller Hinton Agar and Potato Dextrose Agar plates were prepared and inoculated with test bacterial and fungal isolates. In the medium, wells with the size of 6 mm in diameter were punched. 1 ml of Dimethyl sulfoxide (DMSO) was combined with plant samples with 20 µg/ml concentrations, mixed well and added into the well. Well, DMSO alone acts as a negative regulation. The plates have been incubated for bacterial isolates at 37° C and fungal isolates were incubated at room temperature for 24 hours. The antimicrobial activity was evaluated for both bacteria and fungus by measuring the diameter of the inhibition zone (in mm) [18-21].

3. Results and Discussion

The phytochemical properties of obtained *Morus alba* leaves extracts are summarized in Table-1. The findings indicated the existence of medically active compounds. It can be seen from the table that the leaves of *Morus alba* contains carbohydrates, steroids, amino acids, anthocyanins, phenols, tannins, alkaloids, flavonoids, terpenoids and glycosides. The concentration of *Morus alba* leaves extract activity increased steadily with increases in concentration relative to regular ascorbic acid. The results of the DPPH assay show that 70 µg/ml indicates higher free radical scavenging activity compared to normal and compared to available literature [22].

Table 1: Phytochemical components of the extract of *Morus alba* leaves.

S.No	Tests	Results
1	Carbohydrates	+
2	Steroids	+
3	Amino Acids	+
4	Anthocyanins	+
5	Phenols	+
6	Tannins	+
7	Alkaloids	+
8	Flavanoids	+
9	Terpinoids	+
10	Glycosides	+

***In Vitro* Antioxidant Assay**

The partially reduced oxygen and nitrogen metabolites of free radicals are extremely toxic and reactive. The following figure 1 shows that the plant leaves crude extract when tested with the DPPH assay, it shows a good *in vitro* antioxidant activity [23].

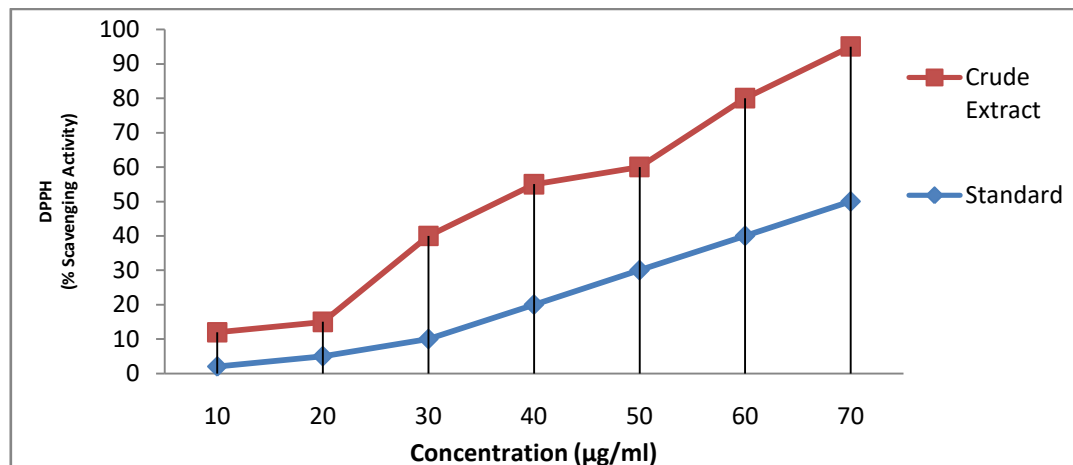


Figure 1: DPPH assay for *Morus alba* Leaves Extract

XRD pattern for CdS nanoparticles

The obtained CdS nanostructure's XRD peaks showed good absorptive pattern in Figure 2 at 2x ratio. The grain size was evaluated by using Scherrer equation with effective peak was expressed as follows,

$$D = (0.94 \lambda) / (\beta \cos \theta)$$

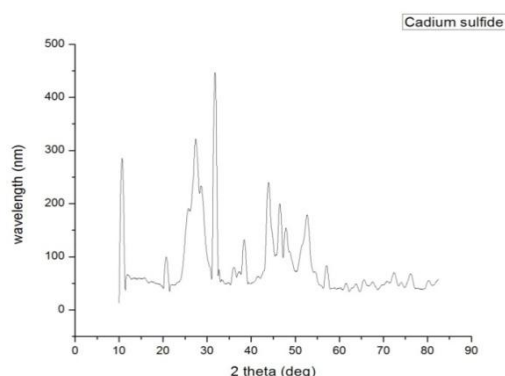


Figure 2: XRD Pattern for CdS NPs

In this β is the complete width of the line at half the maximum (FWHM) angle of diffraction, if λ is the wavelength ($\lambda = 1.542 \text{ \AA}$) (CuK α). The grain size measured for CdS nanoparticles using the comparative strength peak (220) was found to be 48 nm and the rise in

XRD peak sharpness suggests that the particles are crystalline in nature. The (111), (200), (220), (311) and (222) reflections are obviously seen and closely follow the CdO (Joint Committee for Powder Diffraction Studies (JCPDS) reference patterns. The sharp XRD pattern indicates the polycrystalline structure of the particles and the nanostructure grow with aindiscriminate orientation compared to the literature available [24].

Antimicrobial activity

With an increase in the concentration of extracts (25-100 µg/ml), the antibacterial and antifungal activities of the extracts increased linearly. The findings showed that *Salmonella typhi*, *Escherichia coli* and *A. niger*, *Candidaalbicans* in the crude extracts for opposed to standard medicines. As shown in figure 3, shows good results as opposed to negative DMSO. In comparison with *S. typhi* demonstrates excellent antibacterial activity and *C.albicans* shows increased activity is shown by. The findings obtained were interpreted with available literature [25].

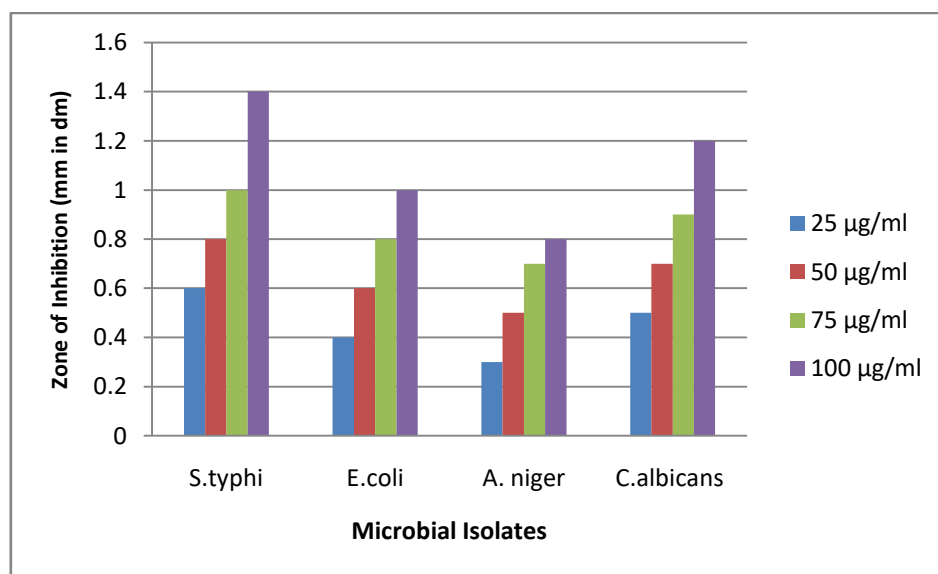


Figure 3: Antimicrobial activity of an extract of *Morus alba* leaves

Conclusion

Mulberry is a medicinal plant which is well-known to each and every one. The notable species of the genus *Morus* are the red mulberry, white mulberry and black mulberry. The existence of steroids, carbohydrates, amino acids, anthocyanins, phenols, tannins, alkaloids, flavonoids, terpenoids, and glycosides was verified in a phytochemical study of mulberry leaves were confirmed. The mulberry leaf extract, the cadmium sulphide nanoparticles (CdS NPs) were synthesized and the results showed orange to yellow color transformation. The characterization of nanoparticles of cadmium sulphide was observed by

XRD. The value of the peak has been calculated. In addition, the plant extract shows effective anti-oxidant activity. The antibacterial activity was performed in two separate bacteria using Cds nanoparticles and fungi. It shows maximum zone formation in *Salmonella typhi* and *Candida albicans*.

Acknowledgements

The authors would like to thank their respective management of their Institutes for rendering academic liberty for the successful completion of the research work.

Conflict of interest

The creators reported no irreconcilable situation. This report doesn't contain any investigations with human or creature subjects experienced by any of the authors.

References

1. M. I. NiyasAhamed, S. Sathya, Ragul. V. An in vitro study on Hexavalent Chromium [Cr (VI)] Remediation using Iron Oxide Nanoparticles Based Beads, Environmental Nanotechnology, Monitoring & Management. 2020;14:1-5.
2. Ramamurthy V, Sathiyadevi M. Preliminary phytochemical screening of methanol extract of Indigo feratritra Linn. J Mol Histol Med Physiol 2017;2:1-5.
3. Dileepkumar G, Tulasi CD, Ramakrishnaiah G. Phytochemical screening and evaluation of in vitro antimicrobial activity of Drosera spatulata Var. Bakoensis-an indigenous carnivorous plant against respiratory tract infectious microbes. Asian J Pharm Clin Res 2016;9:274-83.
4. Kripa KG, Sangeetha R, Chamundeeswari D. Pharmacognostical and physicochemical evaluation of the plant Leucas aspera. Asian J Pharm Clin Res 2016;9:263.
5. Hassan and H. Ullah, "Antibacterial and antifungal activities of the medicinal plant veronica biloba," Journal of Chemistry, 2019.
6. M. Labiad, H. Harhar, A. Ghanimi, and M. Tabyaoui, "Phytochemical screening and antioxidant activity of Moroccan thymus satureioides extracts," Journal of Materials and Environmental Sciences, 2017;8:6:2132–2139.
7. R. A. Hetty Manurung, R. A. Nugroho, Y. Puspita Sari, R. Chernovita, and Auliana, "Phytochemical analysis and antioxidant activity of leaves extracts of endemic plant jahebalikpapan (etlingera balikpapanensis A.D. Poulsen)," International Journal of Scientific & Technology Research, 2019;8;9.

8. Hassan, H. Ullah, and M. Israr, "The antioxidant activity and phytochemical analysis of medicinal plant veronica biloba," Letter In Applied NanoBioscience, 2019: 8:4: 732–738, 2019.
9. G. Rehman, M. Hamayun, A. Iqbal et al., "In vitro antidiabetic effects and antioxidant potential of Cassia nemophila pods," BioMed Research International, 2018: 1-6.
10. K. Chatoui, A. Talbaoui, M. Aneb et al., "Phytochemical screening, antioxidant and antibacterial activity of Lepidium sativum seeds from Morocco," Journal of Materials and Environmental Science, 2016:7:8: 2938–2946.
11. J. Sharifi-Rad, "Herbal antibiotics: moving back into the mainstream as an alternative for superbugs," Cellular and Molecular Biology, 2016:62:9:1-2.
12. F. Shahidi and P. Ambigaipalan, "Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects-a review," Journal of Functional Foods, 2015:18:820–897.
13. T. O. Elufioye, C. G. Chinaka, and A. O. Oyedeji, "Antioxidant and anticholinesterase activities of macrosphyralongistyla (DC) hiern relevant in the management of alzheimer's disease," Antioxidants, 2019:8:9;400-408.
14. W. Wan Rosli, S. N. Haffizah, and H. R. Nurraihana, "Antioxidative and scavenging Properties of polyphenolic rich-fraction of cornlettes (young Zea mays)," International Journal of Recent Technology and Engineering, 2018:7:51-8.
15. R. M. S. Al-Muniri and M. A. Hossain, "Evaluation of antioxidant and cytotoxic activities of different extracts of folk medicinal plant Haplophyllumtuberculatum," Egyptian Journal of Basic and Applied Sciences, 2017:4:2:101–106.
16. Z. S. S. Al-Hashemi and M. A. Hossain, "Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine," Pacific Science Review A: Natural Science and Engineering, 2016:18:2:128–131.
17. S. R. Kanatt, A. Siddiqui, and S. P. Chawla, "Antioxidant/antimicrobial potential of emblica officinalis gaertn and its application as a natural additive for shelf life extension of minced chicken meat," Biointerface Research in Applied Chemistry, 2018:8:4:3344–3350.
18. Z. Kanwal, T. U. Rahman, M. A. Zeb, and M. Sajid, "Antioxidant, α -amylase inhibitory and antiglycation activity of berberis royleana roots," Biointerface Research in Applied Chemistry, 2018:8:6:3725–3728.

19. G. Bahramian, L. Golestan, and K. Khosravi-Darani, "Antimicrobial and antioxidant effect of nanoliposomes containing zataria multiflora boiss essential oil on the rainbow trout fillets during refrigeration," *Biointerface Research in Applied Chemistry*, 2018;8:5:3505–3513.
20. . Hossain S T, Mukherjee S K, 'Toxicity of cadmium sulfide (CdS) nanoparticles against Escherichia coli and HeLa cells, *Journal of Hazardous Materials*, 2018;260:1073-1082.
21. Durga B, Raziya S, Rajamahanti SG, Govindh B, Raju KV, Annapurna N, 'Synthesis and Characterization of CdS Nanoparticles Using Artabotrys hexapetalus Leaf Extract as Capping Agent. *Der Pharma Chemica*, 2017;9:14:157-162.
22. El-Shanshoury AR, Elsilk SE, Ebeid ME, 'Rapid biosynthesis of cadmium sulfide (CdS) nanoparticles using culture supernatants of Escherichia coli ATCC 8739, Bacillus subtilis ATCC 6633 and Lactobacillus acidophilus DSMZ 20079T. *African Journal of Biotechnology*, 2012;11:31:7957-7965.
23. Seoudi R, El- Bailly AB, Eisa W, Shabaka AA, Soliman SI, Abd El Hamid RK, Ramadan RA, Synthesis, Optical and Dielectric Properties of (PVA/ CdS) Nanocomposites. *Journal of Applied Sciences Research*, 2012;8:2:658-667.
24. Shivashankarappa A, Sanjay KR, Study on Biological Synthesis of Cadmium Sulfide Nanoparticles by Bacillus licheniformis and Its Antimicrobial Properties against Food Borne Pathogens. *Nanoscience and Nanotechnology Research*, 2015;3:1:6-15.
25. G. Kalidasan, P. Saranraj, V. Ragul and S. Sivasakthi, "Antibacterial Activity of Natural and Commercial Honey-A Comparative Study" *Advances in Biological Research*, Vol-11, Issue-6 (2017), Pg.No - 365-372, ISSN - 1992-0067.