RESEARCH
O&G Forum 2024; 34 – 2s: 37- 41

RUBIA CORDIFOLIA MEDIATED ZINC OXIDE NANOPARTICLES AND ITS ANTI-CARIOGENIC ACTIVITY

Hidhayathul Wajeeha [1], Dr.Abirami Arthanari [2*], Dr. Rajesh Kumar [3]
[1] Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077. 152101044.sdc@saveetha.com
[2] Senior lecturer, Department of Forensic Odontology, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences Chennai-600077. abiramia.sdc@saveetha.com
[3] Professor, Department of Pharmacology, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences Chennai- 600077. rajeshkumar.sdc@saveetha.com

Abstract
Introduction: Indian madder, also known as Manjistha, is a perennial climbing plant native to the Indian subcontinent and certain parts of Southeast Asia. It belongs to the Rubiaceae family and is renowned for its medicinal benefits. For centuries, it has been an essential component of traditional Ayurvedic medicine, with its roots being the main focus for therapeutic purposes. Manjistha is particularly valued for its ability to purify the blood, aiding liver health, ldetoxification, and enhancing skin complexion. Moreover, it finds use in treating various conditions like skin ailments, urinary disorders, arthritis, and menstrual problems
Materials and methods:
Rubia cordifolia is powdered and aqueous extract is prepared.
The extract is combined with zinc oxide nanoparticles.
Extract is concentrated through heating until the volume reduces to 3-5 millimoles, which typically takes about 20-30 minutes. After concentration, the mixture is transferred to a centrifuge tube.
The centrifuge tube is sealed with foil and placed in a stirring machine for continuous agitation over a period of 3 days.
Results: The graph shows that zinc oxide nanoparticle showed the highest anticariogenic activity against S.mutans as compared to the other microorganisms as it had the highest zone of inhibition. It also had significant amount of anticariogenic activity against S. aureus, E.faecalis, C.albicans.
Discussion: The focus of our research is on evaluating the anti-cariogenic potential of Rubia Cordifolia mediated ZnO NPs. We found that the synthesized zinc oxide nanoparticles exhibited significant anticariogenic activity against cariogenic microorganisms, such as Streptococcus mutans, which is a key bacterium involved in dental caries development. We also found that it exhibited anticariogenic activity against other microorganisms such as S.aureus, E.faecalis, C.albicans.
Conclusion: The research focused on investigating the potential of Rubia Cordifolia, a medicinal plant with various therapeutic properties, in synthesizing Zinc oxide nanoparticles. The study revealed promising outcomes, as the Zinc oxide nanoparticles exhibited noteworthy anti-cariogenic effects. This indicates that these nanoparticles have the capability to prevent or hinder the occurrence of dental caries, a prevalent oral health problem caused by bacterial activity and the formation of plaque.

INTRODUCTION

Indian madder, also known as Manjistha, is a perennial climbing plant native to the Indian subcontinent and certain parts of Southeast Asia. It belongs to the Rubiaceae family and is renowned for its medicinal benefits. For centuries, it has been an essential component of traditional Ayurvedic medicine, with its roots being the main focus for therapeutic purposes (1). Manjistha is particularly valued for its ability to purify the blood, aiding liver health, ldetoxification, and enhancing skin complexion. Moreover, it finds use in treating various conditions like skin ailments, urinary disorders, arthritis, and menstrual problems.

Rubia cordifolia contains key active components, such as rubiadin, purpurin, and alizarin, which are anthraquinones responsible for its red color(2). These compounds possess beneficial properties like antioxidants, anti-inflammatory agents, and antimicrobial capabilities (3). In Ayurvedic medicine, it is highly regarded as a potent blood purifier, capable of cleansing the blood, eliminating toxins, and supporting the liver's detoxification functions. By enhancing blood circulation, it is believed to contribute to overall well-being and vitality. Aside from its medicinal value, Rubia cordifolia has historical use as a natural dye. Its roots yield a red dye, and it has been traditionally utilized to color textiles like silk and wool (4). The plant is sometimes referred to as "Indian madder" due to its resemblance to Rubia tinctorum, a dye-producing plant known as "madder" in Europe (5).

Zinc oxide nanoparticles demonstrate remarkable optical characteristics, including high visibility in the visible spectrum and effective absorption of ultraviolet (UV) light (6). These

attributes make them ideal for UV-blocking coatings in sunscreens and optical filters. Additionally, they function as efficient photocatalysts, accelerating chemical reactions when exposed to light. This property finds utility in diverse applications like water purification, air pollution control, and creating self-cleaning surfaces. Furthermore, their antibacterial and antifungal properties have been under scrutiny, making them potentially valuable for medical uses such as wound dressings and antimicrobial coatings. (6,7) (8)

MATERIALS AND METHODS

-The process begins by taking 2 grams of Rubia cordifolia powder and mixing it accurately with 100ml of distilled water to create the Rubia cordifolia extract. The mixture is then heated in the range of 50-60 degrees Celsius for 15 to 20 minutes using a heating mantle.

-Next, the extract is filtered using either muslin cloth or filter paper to eliminate any solid particles, resulting in a clear liquid extract.

-In a separate flask, a solution containing 0.819 grams of zinc nitrate (30 millimolar zinc nitrate solution) is prepared, and 50ml of distilled water is added to it.

-The Rubia cordifolia extract (50ml) is combined with the zinc nitrate solution (50ml) in the second flask.

-The mixture in the second flask is concentrated through heating until the volume reduces to 3-5 millimoles, which typically takes about 20-30 minutes. After concentration, the mixture is transferred to a centrifuge tube.

-The centrifuge tube is sealed with foil and placed in a stirring machine for continuous agitation over a period of 3 days.

-This procedure details a green approach to synthesizing zinc oxide nanoparticles (ZnO NPs) using Rubia cordifolia extract. The interaction between the plant extract and zinc nitrate leads to the formation of ZnO nanoparticles, which may have potential applications in fields such as medicine and catalysis. Further analysis and characterization of the synthesized nanoparticles are required to assess their properties and potential uses.

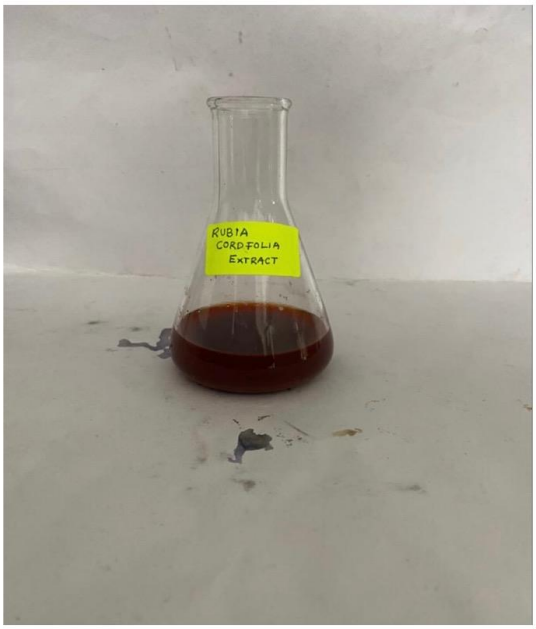


Fig 1: Rubia cordifolia extract after condensing

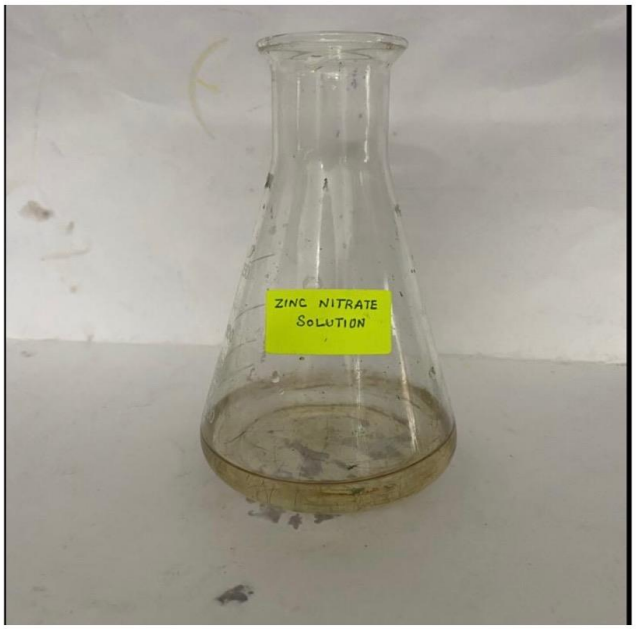


Fig 2: Zinc Nitrate solution

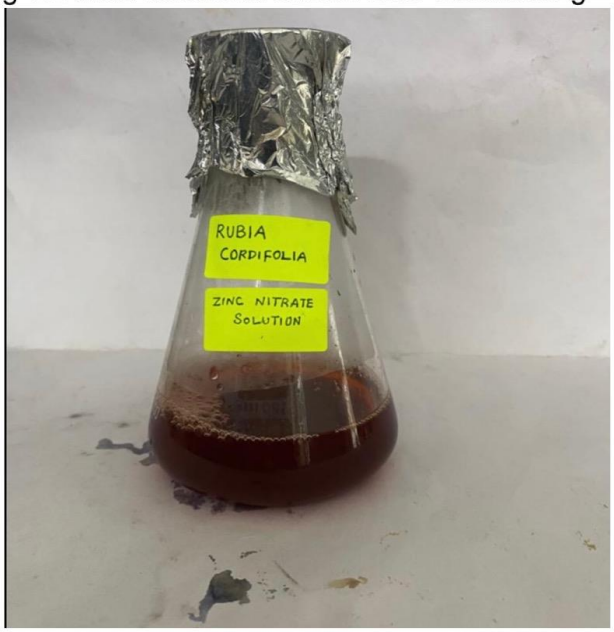


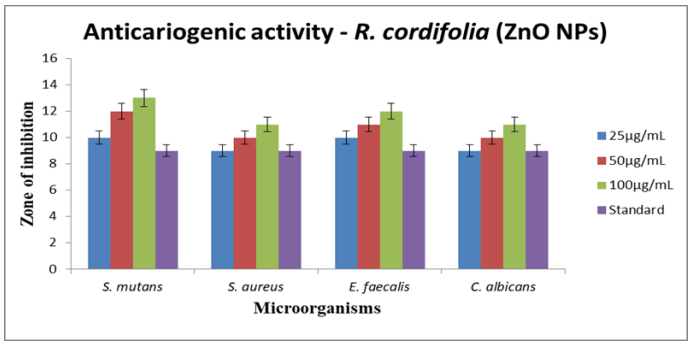
Fig 3: Rubia cordifolia zinc nitrate solution

Fig 4: Rubia cordifolia extract before condensing

RESULTS

TABLE 1:

Organism	25µg/mL	50µg/mL	100µg/mL	Standard
S. mutans	10	12	13	9
S. aureus	9	10	11	9
E. faecalis	10	11	12	9
C. albicans	9	10	11	9



GRAPH 1: ANTICARIOGENIC ACTIVITY OF *Rubia cordifolia* MEDIATED ZINC OXIDE NANOPARTICLES

The graph shows that zinc oxide nanoparticles showed the highest anticariogenic activity against *S.mutans* as compared to the other microorganisms as it had the highest zone of inhibition. It also had significant amount of anticariogenic activity against *S. aureus, E.faecalis, C.albicans.*

Fig 1: E. faecalis

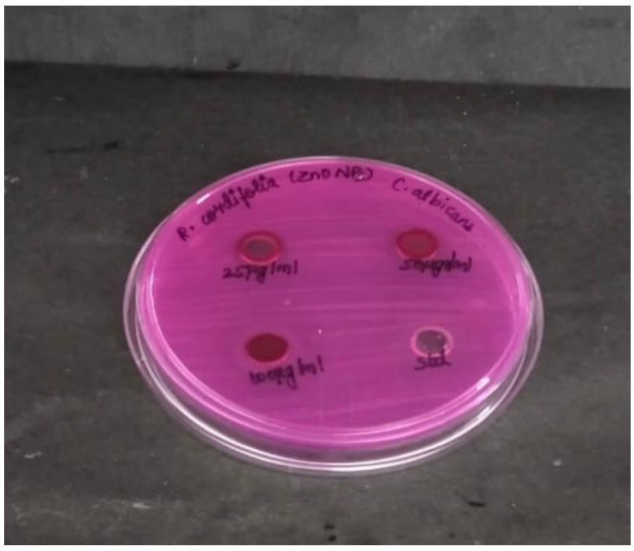


Fig 2: C.albicans

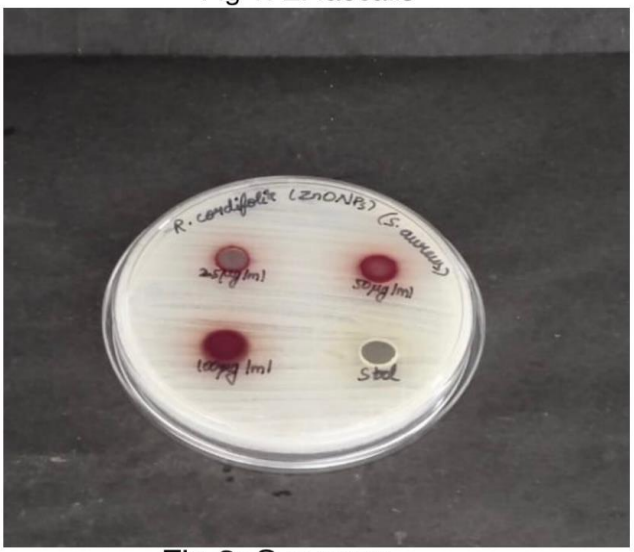


Fig 3: S.aureus

Fig 4: S.mutans

DISCUSSION

The focus of our research is on evaluating the anti-cariogenic potential of Rubia Cordifolia mediated ZnO NPs. We found that the synthesized zinc oxide nanoparticles exhibited significant anticariogenic activity against cariogenic microorganisms, such as *Streptococcus mutans,* which is a key bacterium involved in dental caries development. We also found that it exhibited anticariogenic activity against other microorganisms such as *S.aureus, E.faecalis, C.albicans.*

In a prior research conducted by Patil et al., they investigated the potential neuroprotective properties of R. cordifolia (9). Their study revealed that when rats were administered a methanol extract of R. cordifolia at doses of 100, 200, and 300 mg/kg body weight, in combination with vitamin E at a dosage of 10 mg/kg body weight, notable effects were observed in a reserpine-induced orofacial dyskinesia model (10). Specifically, this combined treatment significantly reduced cavitary chewing movements, tongue protrusion, and enhanced the rats' exercise capacity.Furthermore, the authors observed that the co-administration of R. cordifolia and vitamin E resulted in increased levels of key antioxidants such as SOD, CAT, and GSH in the forebrain region of the rats(11). This treatment also inhibited lipid peroxidation (LPO) and elevated the levels of dopamine. However, it's important to note that administering the highest dose of the methanol extract of R. cordifolia (300 mg/kg body weight) alone did not yield any significant changes in these parameters.These findings collectively suggest that R. cordifolia possesses neuroprotective properties, particularly when combined with vitamin E. This combination appears to be effective in shielding animals from the adverse effects of reserpine-induced orofacial dyskinesia.

In another research study, limited reports have been published regarding the potential of R. cordifolia in inhibiting cell proliferation (12). Notably, the cytotoxic effects of ZnO and CeO2 nanoparticles derived from R. cordifolia leaf extract have been documented against MG-63, a human osteosarcoma cell line (12,13). Furthermore, the methanolic extracts of R. cordifolia have exhibited cytotoxicity against Hela and Hep-2 cell lines, with respective IC50 values of 23.12 and 11.92 µg/mL.

R. cordifolia has demonstrated its ability to hinder the growth of various cancer cell types, including human colon carcinoma (HT-29), human breast carcinoma (MCF-7), human liver carcinoma (HepG2), and once again, human colon carcinoma (HT-29) cell lines (14). It is suggested that the mechanism responsible for this antiproliferative activity may involve the inhibition of DNA synthesis. R. cordifolia has been shown to impede the incorporation of [3H] thymidine and suppress the expression of the c-fos gene, which plays a pivotal role in regulating cell proliferation and differentiation (5,14)

Further, in a study done by Adwankar and Chitnis, they isolated a pure compound RC-18 from *R. cordifolia*, which was found to have antitumor activity against different types of in vivo solid tumor models (B16 melanoma) (15). The cyclic hexapeptides of *R. cordifolia* were also reported for their anticancer activity by inhibiting the process of protein synthesis. It does so by binding to the 80 s subunit of the ribosome and thus inhibiting the binding of aminoacyl-tRNA and translocation of peptidyl-tRNA. Secondary metabolites found in *R. cordifolia* L., such as purpurin and munjistin have antitumor activity. They may be contributing to the anticancer activity of *R. cordifolia* against different types of cancer. (16)

CONCLUSION

The research focused on investigating the potential of Rubia Cordifolia, a medicinal plant with various therapeutic properties, in synthesizing Zinc oxide nanoparticles. The study revealed promising outcomes, as the Zinc oxide nanoparticles exhibited noteworthy anti-cariogenic effects. This indicates that these nanoparticles have the capability to prevent or hinder the occurrence of dental caries, a prevalent oral health problem caused by bacterial activity and the formation of plaque.

LIMITATIONS

Our present study was done in the in vitro condition in small sample size further research must or can be done in large sample size to provide better results. Much more assays need to be checked for the anticariogenic activity.

FUTURE SCOPE

Rubia cordifolia is a medicinal plant rich in bioactive compounds such as polyphenols, flavonoids, and alkaloids.These compounds can be used as reducing agents and stabilizers in the synthesis of ZnO nanoparticles through green chemistry approaches.Green synthesis methods are eco-friendly and reduce the use of toxic chemicals.

ETHICAL CLEARANCE

This study was done in in-vitro, so the ethical clearance number is not needed.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING

Sri Sri dental specialities, Tadepalligudam.

AUTHOR CONTRIBUTION

All authors are equally contributed.

ACKNOWLEDGEMENT

We extend our sincere gratitude to the Saveetha Dental College and Hospital and Department of Forensic Odontology for their constant support and successful completion of this work.

References

1. *Insaf A, Parveen R, Srivastava V, Samal M, Khan M, Ahmad S. TLC-MS-Bioautographic identification of antityrosinase compounds and preparation of a topical gel formulation from bioactive fraction of RSM optimized alcoholic extract of Rubia cordifolia L. stem. J AOAC Int [Internet]. 2023 Jul 20; Available from: http://dx.doi.org/10.1093/jaoacint/qsad076*
2. *Park DY, Hwang J, Kim Y, Lee D, Kim YY, Kim HS, et al. Antimicrobial activity of Limosilactobacillus fermentum strains isolated from the human oral cavity against Streptococcus mutans. Sci Rep. 2023 May 17;13(1):7969.*
3. *Veremeichik GN, Gorpenchenko TY, Rusapetova TV, Brodovskaya EV, Tchernoded GK, Bulgakov DV, et al. Auxin-dependent regulation of growth via rolB-induced modulation of the ROS metabolism in the long-term cultivated pRiA4-transformed Rubiacordifolia L. calli. Plant Physiol Biochem. 2023 Aug 3; 202:107932.*
4. *Zhao SY, Muchuku JK, Liang HY, Wang QF. A complete chloroplast genome of a traditional Chinese medicine herb, and phylogenomics of Rubiaceae. Physiol Mol Biol Plants. 2023 Jun;29(6):843–53.*

5. *Sisubalan N, Ramkumar VS, Pugazhendhi A, Karthikeyan C, Indira K, Gopinath K, et al. ROS-mediated cytotoxic activity of ZnO and CeO nanoparticles synthesized using the Rubia cordifolia L. leaf extract on MG-63 human osteosarcoma cell lines. Environ Sci Pollut Res Int. 2018 Apr;25(11):10482–92.*
6. *Kaur J, Anwer MK, Sartaj A, Panda BP, Ali A, Zafar A, et al. ZnO Nanoparticles of Extract Formulation Developed and Optimized with QbD Application, Considering Ex Vivo Skin Permeation, Antimicrobial and Antioxidant Properties. Molecules [Internet]. 2022 Feb 21;27(4). Available from: http://dx.doi.org/10.3390/molecules27041450*
7. *Pourmoslemi S, Larki-Harchegani A, Daneshyar S, Dastan D, Nili-Ahmadabadi A, Jazaeri M. Antibacterial and Anti-Glucosyltransferase Activity of Against Cariogenic Streptococci. J Pharmacopuncture. 2023 Jun 30;26(2):139–46.*
8. *Lin H, Zhou R, Zhang M, Huang R, Fan C, Zhou S, et al. In vitro antibacterial activity of a novel acid-activated antimicrobial peptide against Streptococcus mutans. Curr Protein Pept Sci [Internet]. 2023 Aug 18; Available from: http://dx.doi.org/10.2174/1389203724666230818111515*
9. *Jeong GS, Lee DS, Kim DC, Jahng Y, Son JK, Lee SH, et al. Neuroprotective and anti-inflammatory effects of mollugin via up-regulation of heme oxygenase-1 in mouse hippocampal and microglial cells. Eur J Pharmacol. 2011 Mar 11;654(3):226–34.*
10. *Shilpa PN, Venkatabalasubramanian S, Devaraj SN. Ameliorative effect of methanol extract of Rubia cordifolia in N-nitrosodiethylamine-induced hepatocellular carcinoma. Pharm Biol. 2012 Mar;50(3):376–83.*
11. *Shilpa PN, Sivaramakrishnan V, Niranjali Devaraj S. Induction of apoptosis by methanolic extract of Rubia cordifolia Linn in HEp-2 cell line is mediated by reactive oxygen species. Asian Pac J Cancer Prev. 2012;13(6):2753–8.*
12. *Habib S, Rashid F, Tahir H, Liaqat I, Latif AA, Naseem S, et al. Antibacterial and Cytotoxic Effects of Biosynthesized Zinc Oxide and Titanium Dioxide Nanoparticles. Microorganisms [Internet]. 2023 May 23;11(6). Available from: http://dx.doi.org/10.3390/microorganisms11061363*
13. *Mozhiarasi V, Karunakaran R, Raja P, Radhakrishnan L. Effects of Zinc Oxide Nanoparticles Supplementation on Growth Performance, Meat Quality and Serum Biochemical Parameters in Broiler Chicks. Biol Trace Elem Res [Internet]. 2023 Jul 18; Available from: http://dx.doi.org/10.1007/s12011-023-03759-0*
14. *Ghasemian Yadegari J, Khudair Khalaf A, Ezzatkhah F, Shakibaie M, Mohammadi HR, Mahmoudvand H. Antileishmanial, cellular mechanisms, and cytotoxic effects of green synthesized zinc nanoparticles alone and in combined with glucantime against Leishmania major infection. Biomed Pharmacother. 2023 Aug; 164:114984.*
15. *Gong XP, Sun YY, Chen W, Guo X, Guan JK, Li DY, et al. Anti-diarrheal and anti-inflammatory activities of aqueous extract of the aerial part of Rubia cordifolia. BMC Complement Altern Med. 2017 Jan 5;17(1):20.*
16. *Xiong Y, Yang Y, Xiong W, Yao Y, Wu H, Zhang M. Network pharmacology-based research on the active component and mechanism of the antihepatoma effect of Rubia cordifolia L. J Cell Biochem. 2019 Aug;120(8):12461–72.*
17. *Sneka S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from Capparis decidua against Escherichia coli and Lactobacillus Species. Research Journal of Pharmacy and Technology. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773*
18. *Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2): S505-S509. doi: 10.4103/japtr.japtr_189_22.*
19. *Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.*
20. *Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.*