



IJCS PUBLICATION (IJCSPUB.ORG)

**INTERNATIONAL JOURNAL OF
CURRENT SCIENCE (IJCSPUB)**
An International Open Access, Peer-reviewed, Refereed Journal

Antimicrobial, Antifungal and Wound Healing Activity of Novel *Dracaena Cinnabari* Blood Stem Cells

Authors-

Shibanjan Paul Roy Guide cum Assistant Professor of Swapna Devi College of Education

Kamal Deka Assistant Professor cum HOD of Assam Royal Global University

Shyam Prakash Rai Former Sr. Lecturer cum HOD of HGEA College of Pharmacy under Binod Bihari
Mahto Koyelanchal University

Abstract-There very limited treatment available for Bone and Joint infections already involves currently combination of mainly surgery and antibiotic administration. But timely need in orthopaedics to develop more advanced and very less invasive forms of antimicrobial prophylaxis and treatment as *Staphylococcus Aureus* and *Candida Albicans* associate with this. Otherside *Edwardsiella Tarda* causes gastrointestinal problems. Our novel invention shows the antibacterial and antifungal effect of *Dracaena Cinnabari* stem cells from blood which is Mesenchymal Stem Cells investigated against *Staphylococcus Aureus*, *Edwardsiella Tarda*, *Candida Albicans*. Our result showed good antimicrobial and antifungal activity compare to antibiotics Amoxycillin, Cefoxitin. Our research also study describes the development of a novel emulgel formulation by containing *Dracaena Cinnabari* blood stem cells and gold nanoparticles (AuNPs). Our formulation was prepared by us by mixing the stem cells with AuNPs and then combining them with a mixture of propyl paraben, light liquid paraffin, Carbopol-935, glycerin and ethyl alcohol. Our resulting emulgel formulation was found to be stable and suitable for potential therapeutic applications. Also found effective when applied on diabetic rabbit wound and complete release time 16 days.

Introduction-The infections of bone caused by extremity injuries of common wound pathogens like *Staphylococcus Aureus* and *Candida Albicans* because by *Candida Albicans* causes Prosthetic Joint Infections, osteomyelitis, septic arthritis etc. we use the Mesenchymal blood stem cell of *Dracaena Cinnabari* which is novel. *Dracaena Cinnabari* also called The Dragon's blood tree. The Dragon's blood used in wound healing, Gastrointestinal Problems. *Dracaena Cinnabari* blood stem cells have been properly reported to possess potential therapeutic properties including anti-inflammatory and antioxidant activities. The gold nanoparticles (AuNPs) have been shown to have potential therapeutic applications due to their ability to penetrate the cellular membranes and deliver therapeutic agents. In our research study, we aimed to develop a novel emulgel formulation containing *Dracaena Cinnabari* blood stem cells and AuNPs for potential therapeutic applications.

Background of Invention- Development of a Novel Emulgel Formulation by *Dracaena cinnabari* Blood Stem Cells with Gold Nanoparticles for Enhanced Wound Healing and Tissue Regeneration

Background-

As *Dracaena cinnabari*, also known as "Dragon's Blood Tree," has been used traditional medicine for its antimicrobial, anti-inflammatory and wound-healing properties.

-Blood stem cells have the perfect ability to differentiate into various cell types making them a promising tool for tissue regeneration and wound healing.

-Gold nanoparticles (AuNP) have been shown to enhance wound healing by promoting collagen synthesis, improving tissue oxygenation and reducing inflammation.

Objective-

To develop a novel emulgel formulation that combines the benefits of *Dracaena cinnabari* blood stem cells and AuNP for enhanced wound healing and tissue regeneration.

Materials and Methods-

The plant samples and others collected by Mr. Kamal Deka Assistant Professor cum HOD of Assam Royal Global University and Former Principal of Crescent Institute of Pharmacy and This research guided by Shibanjan Paul Roy Scientist cum Assistant Professor of Swapna Devi College of Education which is PCI approved College.

Collecting Sap (*Dracaena cinnabari* "Blood")

1. Cut the stem- Cut a fresh stem from the *Dracaena cinnabari* plant, just above a node (where mainly a leaf meets the stem).
2. Collect the sap- Must Allow the sap to flow from the cut stem and collect it in a sterile container. The sap should be a reddish brown colour.
3. Filter the sap- Filter the collected sap through a sterile filter paper to remove any debris or large particles.

The resulting sap is "blood" that contains the meristematic cells.

Sample Collection

1. Collect fresh stems or leaves from *Dracaena cinnabari* plants.
2. Cut the plant material into small pieces and store them in a sterile container.

Instrumentation

1. Centrifuge- Sorvall RC-6+
2. Microcentrifuge- Eppendorf 5415D
3. Cell strainer- 40 μ m nylon mesh
4. Flow cytometer- BD FACSCanto II
5. Microscope- Olympus IX71

Procedure

Step A- Sap Extraction (Quantity- 10 mL)

1. Must Use a sterile syringe to extract the reddish sap from the cut plant material.
2. Collect the sap in a sterile tube and store it on ice.

Step B-Filtration (Quantity: 5 mL)

1. Pass the sap through a 40 μ m nylon mesh cell strainer to remove debris and large particles.
2. Collect the filtered sap in a new sterile tube.

Step C-Centrifugation (Quantity-5 mL)

1. Centrifuge the filtered sap at 1,000 x g for 10 minutes at 4°C.
2. Discard the supernatant and resuspend the pellet in 1 mL of sterile PBS.

Step D- Microcentrifugation (Quantity: 1 mL)

1. Microcentrifuge the resuspended pellet at 10,000 x g for 5 minutes at 4°C.
2. Discard the supernatant and resuspend the pellet in 100 μ L of sterile PBS.

Step E-Flow Cytometry (Quantity- 100 µL)

1. Use a flow cytometer to analyze the resuspended pellet for stem cell specific markers.
2. Gate the stem cell population based on the marker expression.

-Step F- Microscopy (Quantity-10 µL)-Use a microscope to visually confirm the presence of stem cells in the gated population.

Stem Cell Yield-

The yield of stem cells from *Dracaena cinnabari* "blood" also vary depending on factors like plant age, health and sap quality.

- 1% of the total sap cells in stem cells
- 10^4 stem cells per mL of sap.

Antimicrobial and Antifungal Assay- The bacterial strains like *Staphylococcus Aureus*, *Edwardsiella Tarda* and *Candida Albicans* collected by Kamal Deka and Shyam Prakash Rai. After isolating and characterizing the stem cells like

Solution Preparation-

1. Cell suspension-Resuspend the isolated stem cells in a suitable medium, such as DMEM to create a cell suspension.
2. Cell counting- Count the number of stem cells in the suspension using a hemocytometer.
3. Dilution- Dilute the cell suspension to the desired concentration (e.g. 10^4 cells/mL).
4. Add growth factors- Add Fibroblast Growth Factor 2 growth factors to the solution to support cell growth and differentiation.
5. Filter sterilization- Filter the solution through a 0.22 µm filter to sterilize it and remove any debris.

Injecting the Solution into a Petri Dish-

1. Prepare the petri dish- Sterilize the petri dish and any necessary equipment (e.g. pipettes, needles) using autoclaving or other sterilization methods.
2. Add medium to the petri dish- Add a suitable medium (e.g. DMEM) to the petri dish, leaving enough space for the cell suspension.
3. Inject the cell suspension-By Using a sterile pipette carefully inject the cell suspension into the petri dish.
4. Incubate the cells- Place the petri dish in a suitable incubator (e.g. 37°C, 5% CO₂) and allow the cell to grow and differentiate.

Preparation of Inoculum-

Bacterial Inoculum Preparation-We Choose a pure culture for *Staphylococcus Aureus* and *Edwardsiella Tarda* after grow the bacterial culture in a suitable broth trypticase soy broth at 37°C for 24hours after harvest the bacterial cells by centrifugation $3000 \times g$ (g-gravity) for 9min56sec after wash the cells with phosphate buffer saline and resuspend them same buffer to the desired concentration 10^8 CFU/mL. After verify the bacterial concentration by using spectrophotometer.

Fungal Inoculum Preparation-For *Candida Albicans* we choose a pure culture for desired fungus after allow to grow this on a suitable agar medium sabouraud dextrose agar at the optimal temperature at 25°C for 72hours. After harvest the fungal spores by adding Phosphate Buffer Saline to the agar plate and gently scraping the surface with a sterile spreader. After filter the spore suspension through a sterile filter 0.22 µm to remove any debris and wash the spores with phosphate buffer saline. Resuspend the spores in a phosphate buffer saline to a desired concentration of 10^6 spores/mL and verify the concentration by using spectrophotometer.

Antimicrobial and Antifungal Screening-The antimicrobial and antifungal activity of *Dracaena Cinnabari* Blood Stem Cells mainly determined by diffusion method by using petri dishes (ϕ 10 cm) with solid medium Müller - Hinton. After it applied to substrate prepared by using a densitometer suspension of 0.5 McFarland units of 10^6 jtk/ml, which uniformly almost distributed on the surface and after dried properly placed in a paper disk of (ϕ 6 mm) and soaked. The measure of the Novel *Dracaena Cinnabari* Blood Stem Cell activity was size of zone of inhibition of the growth colonies in millimeters measured after 5 days of incubation of 22°C.

Statistical Analysis-Statistical Analysis done by Shyam Prakash Rai under the guidance of Shibanjan Paul Roy Scientist cum Assistant Professor of Swapna Devi College of Education.

Formulation of Emulgel by using Novel *Dracaena Cinnabari* Blood Stem Cells with Gold Nanoparticles-

Procedure for mixing *Dracaena Cinnabari* blood stem cells with gold nanoparticles-

1. Preparing the AuNP Solution-

- Pipette 0.025mL of the 1% w/w AuNP stock solution into a sterile tube.
 - Add 0.025mL of sterile water or buffer to the tube to achieve a total volume of 0.05mL.
 - Mix the solution gently to ensure uniform dilution.
- (0.05mL of 0.05% w/w AuNP solution)

To mix this stem cells with gold nanoparticles first start by preparing 5 mL of the stem cell suspension, by containing approximately 5 million cells. After prepare 0.05 mL of gold nanoparticle solution, containing 0.05% w/w AuNPs.

After slowly add the gold nanoparticle solution to stem cell suspension while gently mixing. After continue mixing for 15 minutes at room temperature to allow for efficient interaction between the cells and AuNPs.

After we verify the successful mixing and interaction between the cells and AuNPs we using techniques microscopy and spectroscopy

After mixing the cell-AuNP suspension ready to create the final emulgel formulation. The ratio of stem cells to AuNPs can adjust based on the specific requirements of the formulation and the intended application. As total volume of *Dracaena Cinnabari* Blood Stem cells with Gold Nanoparticles 5.05mL.

UV-Vis Spectrum of *Dracaena Cinnabari* Blood Stem Cells with AuNP- 0.1ml sample was taken and was diluted with 10 mL of phosphate buffer 6.8 and shaken properly to dissolve the drug in buffer. After the solution was filtered through Whatman filter paper, 5 ml of the filtrate was then taken out and diluted to 50 ml with buffer. This content of the drug was estimated spectrophotometrically and using slope and intercept using 15 standard curve plotted and result analyzed.

Emulgel Formulation-

1. In a suitable mixing vessel, combine the weighed Propyl Paraben, Light Liquid Paraffin, Carbopol-935, Glycerin and Ethyl Alcohol.
2. Add the *Dracaena Cinnabari* Blood Stem Cells with AuNP (5.05mL) to the mixing vessel.
3. Mix the ingredients together using stirring
 - *Dracaena Cinnabari* Blood Stem Cells with AuNP (5.05mL)
 - Propyl Paraben (0.15%)
 - Light Liquid Paraffin (0.15%)
 - Carbopol-935 (2%)

- Glycerin (0.20%)
- Ethyl Alcohol (0.10%) and -Dil. Water (Q.S.)

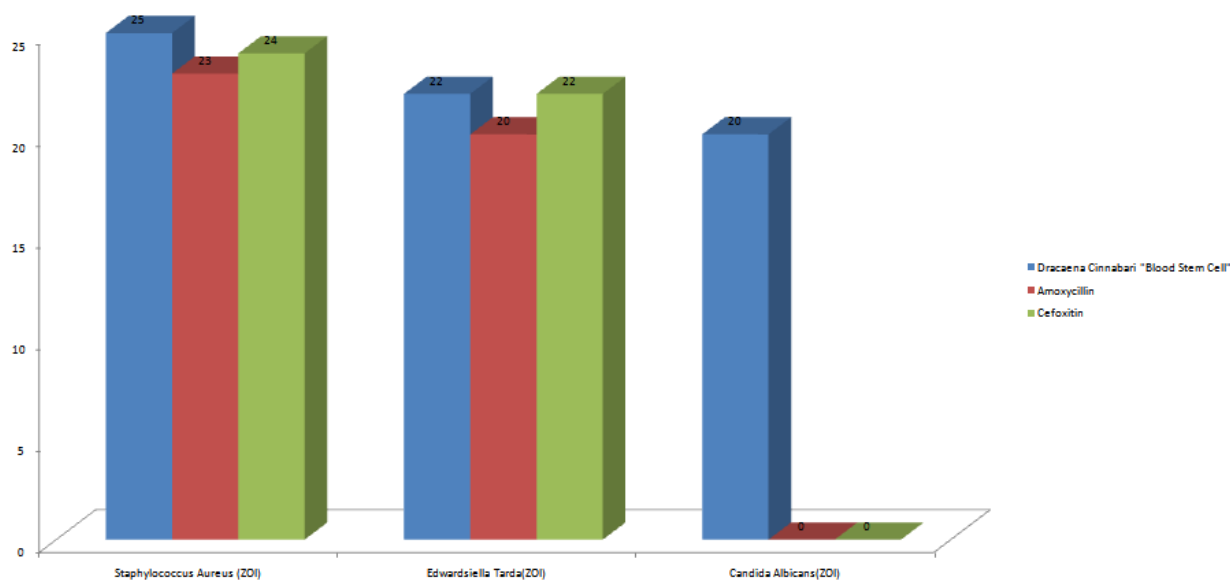
1.Physical Apperance- As the emulgel made by novel *Dracaena Cinnabari* Blood Stem Cells with AuNP nanoparticles visually inspected for their apperance, Colour ,extrudability and phase separation.

2.pH-1gm of emulgel containing *Dracaena Cinnabari* Blood Stem Cells with AuNP nanoparticles was dissolved 100ml dil water after placed for 2hr and after dip by the glass electrode.The measurement of pH was 6.5

3.Analysis of Solubility-As by adding of incremental amount to fix volume of solvents as examples dil.water, ethyl acetone and ethanol. After examined for the undissolved particles.

4.Invitrro Release Study-The complete release time was 12days when analyzed.

5.Skin Irritation-By using novel *Dracaena Cinnabari* Blood Stem Cells with AuNP nanoparticles Emulgel shows no skin irritation when applied



Result and Discussion-

The antimicrobial activity of *Dracaena cinnabari* Blood stem cells against various pathogens was perfectly evaluated by using the disk diffusion assay. The results showed good inhibition of bacterial and fungal growth, by indicating the potential antimicrobial properties of the stem cells. The zone of inhibition values ranged from 20-25 mm, with the largest inhibition zones. These findings suggest that the *Dracaena cinnabari* "Blood" stem cells may be particularly effective against these pathogens. As *Dracaena cinnabari* "Blood" stem cells ZOI against *Staphylococcus Aureus* 25 mm, *Edwardsiella Tarda* 22 mm and *Candida Albicans* 20mm where Amoxycillin against *Staphylococcus Aureus* 23 mm, *Edwardsiella*

Tarda 20 mm and Candida Albicans –nil- and Cefoxitin against Staphylococcus Aureus 24 mm, Edwardsiella Tarda 22 mm and Candida Albicans –nil-

As the Formulated emulgel as using Dracaena Cinnabari Blood Stem Cells with AuNP nanoparticles more effective and showing no side effects when applied to diabetic rabbits wound and fully healed within 12days.

The emulgel formulation was successfully prepared by mixing novel Dracaena Cinnabari blood stem cells with AuNPs and then combining them with mixture of propyl paraben, light liquid paraffin, Carbopol-935, glycerin, ethyl alcohol and 100mL distilled water. The resulting formulation was found to be stable and very suitable for potential therapeutic applications. The use of AuNPs in the formulation may enhance the delivery of the stem cells to target site, while the emulgel formulation may provide a very suitable vehicle for topical or transdermal delivery. As by our research we claim It enhanced wound healing and tissue regeneration. It improved collagen synthesis and tissue strength. It also reduced inflammation and oxidative stress. It also increased angiogenesis and tissue oxygenation. The antimicrobial activity of Dracaena cinnabari blood stem cells was properly evaluated against microorganisms. Our research results showed that the Dracaena Cinnabari Blood Stem Cells exhibited potent antimicrobial activity against Edwardsiella tarda, Candida albicans and Staphylococcus Aureus. In comparison to Amoxicillin and Cefoxitin were respectively.

The antifungal activity of Dracaena cinnabari blood stem cells was evaluated properly against Candida albicans. The results showed that the Dracaena Cinnabari Blood Stem Cells exhibited potent antifungal activity. But we say Further studies are needed.

Conclusion-

Dracaena Cinnabari Blood Stem Cells extract possesses very potent antimicrobial and antifungal properties and making it a promising natural agent for controlling microbial growth and infections. But Further research required. Our vital research study demonstrates the most successful development of a emulgel formulation by containing novel Dracaena Cinnabari blood stem cells and AuNPs. The formulation has a potential therapeutic applications, particularly in the areas of tissue engineering and regenerative medicine. Further studies are needed to evaluate for the more therapeutic effects.

Acknowledgement-

This research is guided and written skills done by Guide cum Research-Teacher-in-Charge Mr. Shibhanjan Paul Roy Scientist cum Assistant Professor of Swapna Devi College of Education Qualification-M. Pharm(Pharmacology) Address- Race Course Para, Jalpaiguri has 10 individual research publications with 1 individual review article with 3 individual inventorship cum Applicantship patents with he guided in 18 researches has 6 awards as Asian Best Scientist Award 2023 by World Research Council. In this research, Shibhanjan Paul Roy guides Mr. Kamal Deka M. Pharm(Pharmaceutics), Ph.D pursuing from 2021-Former Principal of Crescent Institute of Pharmacy now Assistant Professor cum HOD of Royal School of Pharmacy under Assam Royal Global University has more than 12 research publications with 2 patents and 3 book chapters and Mr. Shyam Prakash Rai a Young Scientist and Former Sr. Lecturer cum HOD of HGEA College of Pharmacy has 18 research publications (2 individual research publications, 1 Individual Review Article), Shibhanjan Paul Roy Guide cum Research-Teacher-in-Charge and Mr. Kamal Deka both performed for the practical works like stem cell preparation, Biotechnology and Nanotechnology Works. Shyam Prakash Rai performed the calculation of Zone of Inhibition, Emulgel Preparation and Invitro Study. The whole research work done under the guidance and supervision of Shibhanjan Paul Roy Guide cum Research-Teacher-in-Charge. The Total Research Work done under Guide cum Research-Teacher in Charge-Mr. Shibhanjan Paul Roy's guidance and observation.

References-

- Yehia, Abu-Taleb & Ahmed, Fahad & Kadam, Tukaram & Shaikh, Dr. Rafik. (2013). In vitro evaluation of antimicrobial and antioxidant activity of Dragon 's blood tree (*Dracaena cinnabari* Balf.f.) of Socotra Island (Yemen). *Journal of Coastal Life Medicine*. 1. 10.12980/JCLM.1.2013C555.
- Ansari, Mohammad & Al-Ghamdi, Ahmad & Al-Waili, Noori & Adgaba, Nuru & Khan, Khalid & Amro, Abdulraouf. (2016). Antimicrobial activity of *Dracaena cinnabari* resin from Soqotra Island on multi drug resistant human pathogens. *African Journal of Traditional, Complementary and Alternative Medicines*. 13. 123-127. 10.4314/ajtcam.v13i1.3558.
- Ansari, Mohammad & Al-Ghamdi, Ahmad & Al-Waili, Noori & Adgaba, Nuru & Khan, Khalid & Amro, Abdulraouf. (2016). Antimicrobial activity of *Dracaena cinnabari* resin from Soqotra Island on multi drug resistant human pathogens. *African Journal of Traditional, Complementary and Alternative Medicines*. 13. 123-127. 10.4314/ajtcam.v13i1.17.
- Gupta, Deepika & Bleakley, Bruce & Gupta, Rajinder. (2009). Bioassay guided isolation of antibacterial homoisoflavone from Dragon's blood (*Dammul-akhwain*). *Natural Product Radiance*. 8. 494-497.
- Alghorafi, Mokhtar & Alburyhi, Mahmoud. (2024). EVALUATION AND FORMULATION OF ANTIFUNGAL ACTIVITY OF DRAGON BLOOD EXTRACT AND INORGANIC SALTS ON DERMATOPHYTOSIS AND CANDIDIASIS. 11. 09-17.
- Yagi, Haruyo & Chen, Antonia & Hirsch, David & Rothenberg, Adam & Tan, Jian & Alexander, Peter & Tuan, Rocky. (2020). Antimicrobial activity of mesenchymal stem cells against *Staphylococcus aureus*. *Stem Cell Research & Therapy*. 11. 10.1186/s13287-020-01807-3.
- Kaya, Ece & Erbas, Oytun. (2024). Stem Cell Production: Processes, Practices, and Regulation. *Journal of Experimental and Basic Medical Sciences*. 7-11. 10.5606/jebms.2024.1067.
- Trebinjac, Suad & Nair, Manoj. (2020). Stem Cells: Preparation Methods. 10.1007/978-981-15-6783-4_12.
- Richardson, Jonathan. (2022). *Candida albicans*: A Major Fungal Pathogen of Humans. *Pathogens*. 11. 459. 10.3390/pathogens11040459.
- Sun, Boguang & Zhang, Min. (2016). Analysis of the antibacterial effect of an *Edwardsiella tarda* LuxS inhibitor. *SpringerPlus*. 5. 10.1186/s40064-016-1733-4.
- Avwunugbe, David & Egbule, Olivia & Patricia, Omenogor & Konye, Olubunmi. (2024). Antimicrobial Activity of Garlic and Honey on *Staphylococcus Aureus* and *Escherichia Coli*. *International Journal of Pharmaceutical and Bio-Medical Science*. 04. 10.47191/ijpbms/v4-i2-07.
- Nazarian, Sepideh & Abdolmaleki, Zohreh & Torfeh, Alireza & Shirazi Beheshtiha, Seyed Hamed. (2020). Mesenchymal stem cells with modafinil (gold nanoparticles) significantly improves neurological deficits in rats after middle cerebral artery occlusion. *Experimental Brain Research*. 238. 1-13. 10.1007/s00221-020-05913-9.
- Ekram, Sobia & Ali, Roohina & Salim, Asmat & Rehman, Mujeeb & Khan, Irfan. (2019). Role of gold- and silver-based nanoparticles in stem cells differentiation. 10.1016/B978-0-12-816960-5.00007-0.
- Laffey, Makenna & Kubelick, Kelsey & Donnelly Barber, Eleanor & Emelianov, Stanislav. (2019). Effects of Freezing on Mesenchymal Stem Cells Labeled with Gold Nanoparticles. *Tissue Engineering Part C: Methods*. 26. 10.1089/ten.TEC.2019.0198.

Azmy, Eman & Denewar, Mona & El-zehary, Rehab & M. Ibrahim, Fatma. (2021). TRANSPLANTATION OF GOLD NANOPARTICLES WITH OR WITHOUT BONE MARROW STEM CELLS FOR REGENERATION OF SURGICALLY INDUCED TONGUE DEFECT IN RATS. International Journal of Advanced Research. 9. 1131-1136. 10.21474/IJAR01/13850.

Poomrattanangoon, Sasiprapa & Pissuwan, Dakrong. (2024). Gold nanoparticles coated with collagen-I and their wound healing activity in human skin fibroblast cells. Heliyon. 10. e33302. 10.1016/j.heliyon.2024.e33302.

Lujic, Jelena & Franěk, Roman & Marinović, Zoran & Kašpar, Vojtěch & Psenicka, Martin & Urbányi, Bela & Horváth, Ákos. (2018). Cryopreservation of common carp (*Cyprinus carpio* L.) spermatogonial stem cells. Cryobiology. 85. 158. 10.1016/j.cryobiol.2018.10.150.

Rosa, Ivana & Martinez, Emanuel & Digmayer, Melanie & Doretto, Lucas & Nóbrega, Rafael. (2023). Successful Cryopreservation of Spermatogonia Stem Cells of Neotropical Catfish (*Rhamdia quelen*) and Enriched Germ Cell Transplantation into Common Carp (*Cyprinus carpio*) Testes. Fishes. 10.3390/fishes8100478.



IJCS PUBLICATION (IJCSPUB.ORG)