

INVESTIGATING THE PHYTOCOMPOUNDS OF *Artocarpus hirsutus* AND *Cardiospermum halicacabum* AND THEIR EFFICACY FOR WOUND HEALING AND BONE DEVELOPMENT

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A++” by NAAC

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This is to certify that this Project Report is the bonafide work of **RIZWAN AHAMED N (40230055)** who carried out Project Work entitled "**Investigating the Phytocompounds of *Artocarpus hirsutus* and *Cardiospermum halicacabum* and their efficacy for Wound Healing and Bone Development**" under my supervision from October 2023 to March 2024.

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I, **RIZWAN AHAMED N (40230055)**, hereby declare that the Project Report entitled “Investigating the Phytocompounds of *Artocarpus hirsutus* and *Cardiospermum halicacabum* and their Efficacy for Wound Healing and Bone Development” done by me under the guidance of **Dr. P. PRAKASH** (Internal) and **Dr. K. GOBI SARAVANAN** (External) at **Sathyabama Institute of Science and Technology** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

This study delves into the multifaceted roles of the phytochemical composition of two medicinal plants, *Artocarpus hirsutus* and *Cardiospermum halicacabum*, in the realms of wound healing and bone fracture repair. This study aims to validate the efficacy of phytochemicals in the treatment of wound healing and bone fracture repair utilizing databases and bioinformatics tools to establish a comprehensive model to scrutinize the intricate relationship between phytochemicals and pivotal biological targets affiliated with the process of wound healing and bone fracture. Information regarding diverse phytochemicals and their respective target areas was collected from an array of sources. Subsequently, we thoroughly examined potential mechanisms by scrutinizing pathway enrichment analysis and GO biological processes. After in-silico validation, those plants, *Artocarpus hirsutus* and *Cardiospermum halicacabum*, are collected and dried for aqueous extraction using Soxhlation. Those plant extracts were characterized with FTIR. Furthermore, we investigated the cytotoxicity effects of those plants with 3T3 cell lines. Then, In-vitro scratch wound assay were assessed to determine the wound healing capacity of those plant extracts. Finally, In vivo studies were conducted using zebrafish models to assess the toxicity and regenerative capacity of the plant extracts. Zebrafish embryos were exposed to different concentrations of the extracts, and survival rates, morphological changes were evaluated. The regenerative capabilities are assessed by the rate of caudal fin regeneration. The findings contribute valuable insights into the therapeutic potential of these plant-derived compounds, shedding light on their applications in promoting wound healing, and bone regeneration. Further research is essential to unravel specific mechanisms and determine optimal concentrations for effective wound and fracture management.

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CHAPTER 1

INTRODUCTION

The utilization of plants in traditional medicine has been a longstanding practice, offering a more economical and environmentally friendly substitute to synthetic medications and serving as a noteworthy reservoir in pharmaceuticals. These botanical concoctions, which draw upon centuries of belief in our customs and are used in traditional medicine, are implemented over time by indigenous communities and commoners. Traumatic events, such as accidents, falls, sports injuries, and medical conditions, can occur at any time and lead to wounds or fractures which require extensive medical attention for recuperation. Wound healing and bone development are critical processes in the maintenance of overall health and well-being. (Yang et al., 2021, Brunetti et al., 2018) The ability to effectively regenerate tissues and repair injuries is paramount for the restoration of normal physiological function and the prevention of complications such as chronic wounds and bone disorders. (DiPietro, 2016)

The exploration of natural compounds for medicinal purposes has been a longstanding tradition, and in our pursuit, we turn our attention to two promising plant species: *Artocarpus hirsutus* and *Cardiospermum halicacabum*. These plants have been known for their unique phytochemical compositions and traditional medicinal uses, providing a rich avenue for investigation in the realms of wound healing and bone development. *Artocarpus hirsutus*, commonly known as wild jack, is indigenous to tropical regions and has been employed in various traditional healing practices for its purported therapeutic properties. Similarly, *Cardiospermum halicacabum*, also known as balloon vine, has been utilized in folk medicine across different cultures for its diverse pharmacological activities. (Karthik et al., 2022, Zalke et al., 2013)

Despite advancements in medical science, there remains a need to explore alternative and complementary approaches to enhance these biological phenomena. Nevertheless, the scientific evaluation of the utilization of *Artocarpus hirsutus* and *Cardiospermum halicacabum* in the healing of wounds and fractures remains unexplored. Consequently, taking into consideration various ethnobotanical and phytochemical substantiation, we intend to substantiate the effectiveness of *Artocarpus hirsutus* and *Cardiospermum halicacabum* in the healing of wounds and fractures. (Swargiary et al., 2020)

The phytochemical constituents of these plants have been of particular interest due to their potential bioactive properties. Phytochemicals are secondary metabolites synthesized by plants that often exhibit biological activities beneficial to human health. Research has indicated that these compounds possess antioxidant, anti-inflammatory, antimicrobial, and wound healing properties, among others, making them promising candidates for therapeutic applications. (Bansal et al., 2018)

The aim of this study is to investigate the phytochemical composition of *Artocarpus hirsutus* and *Cardiospermum halicacabum* and evaluate their efficacy in promoting wound healing and bone development. By delving into the bioactive constituents of these plants and their potential mechanisms of action, this study seeks to contribute to the growing body of knowledge on natural products with therapeutic implications.

CHAPTER 2

LITERATURE SURVEY

Amudha et al., 2024 - Facile synthesis and optical characterization of selenium nanoparticles synthesized using *Clitoria ternatea* and *Zingiber officinale* : in vitro biomedical evaluation of antioxidant potential and antibacterial activity against caries-causing microbes.

The synthesis of selenium nanoparticles using *Clitoria ternatea* and *Zingiber officinale* and their potential as a natural anti-cariogenic agent. Various analysis and evaluation techniques were used, such as UV-Vis spectroscopy, XRD, and SEM, to characterize the synthesized selenium nanoparticles. The antibacterial activity of the nanoparticles against dental caries-causing bacteria was assessed using the zone of inhibition and cytoplasmic leakage assays. The study also evaluated the time taken by the synthesized selenium nanoparticles to kill the cariogenic bacteria using a time-kill curve assay. The presence of functional groups in the synthesized nanoparticles was identified using FTIR spectroscopy, indicating the attachment of gelatin and glucose species to the nanoparticle surface. The synthesized selenium nanoparticles exhibited broad-spectrum antibacterial activity and low cytotoxicity, making them potentially useful as therapeutic agents and in preventing oxidative stress-related diseases.

Shanmugam et al., 2022 - Efficacy of graphene-based nanocomposite gels as a promising wound healing biomaterial.

The development of biocompatible nanocomposite hydrogels with effective wound healing and microbicidal properties. The study focuses on the synthesis, characterization, and angiogenic properties of graphene-based nanocomposite gels for wound healing. Graphene oxide (GO) and reduced graphene oxide (rGO) were incorporated into aloe vera gel and hydrogel, respectively, as wound healing materials. In-vitro biochemical techniques were used to characterize the formulations, which showed suitable cell viability, attachment, and proliferation.

Zainol Abidin et al., 2020 - The effects of *Piper sarmentosum* aqueous extracts on zebrafish (*Danio rerio*) embryos and caudal fin tissue regeneration.

The study aims to determine the toxicity of *P. sarmentosum* extracts on zebrafish embryos and its potential for tissue regeneration. The extract contains phytochemicals with antioxidant activity and has been found to exhibit DPPH scavenging activity. The embryotoxicity tests showed that higher concentrations of the extract led to decreased survival and slower embryogenesis. The extract also showed significant differences in tissue regeneration compared to non-treated samples. *P. sarmentosum* extracts have been reported to have medicinal effects, including anti-cancer and fracture healing properties.

Lin et al., 2019 - Identified the synergistic mechanism of drynariae rhizoma for treating fracture based on network pharmacology.

Drynariae Rhizoma (DR) is a plant from Davalliaceae and Davallia Sm. that has been widely used in the prevention and treatment of various fractures. The specific mechanisms of DR's active ingredients for treating fractures have not been elucidated. Network pharmacology, a systems biology and polypharmacology approach, can be used to explore the potential synergistic mechanisms of DR for treating fractures. The study aims to identify the active ingredients of DR, their gene targets, protein targets, and the signaling pathways involved in the treatment of fractures.

Sampath et al., 2023 - Facile green synthesis of zinc oxide nanoparticles using *Artocarpus hirsutus* seed extract: spectral characterization and in vitro evaluation of their potential antibacterial-anticancer activity

Nanoparticles were synthesized using the seed extract of *Artocarpus hirsutus*, which contains flavonoids and proteins as confirmed by a phytochemical study. The synthesized nanoparticles were characterized using various techniques such as XRD, SEM, EDAX, UV-visible spectroscopy, and FTIR. XRD analysis revealed that the particles are crystalline in nature with an average crystal size of

25 nm. SEM studies showed that the particles were rod- and spherical-shaped, and EDAX confirmed the presence of zinc and oxygen in the nanoparticles. The UV-vis spectrum confirmed the presence of ZnO nanoparticles with absorption peaks at 266 nm and 365 nm. The presence of proteins and amide groups in the seed extract acted as reducing and stabilizing agents, as confirmed by the FTIR spectrum. Antibacterial studies were conducted using the well-diffusion method and scanning electron microscopy against *Enterobacter aerogenes* and *Listeria monocytogenes*. The impact of zinc oxide nanoparticles on the HT-29 colon cancer cell line was investigated. Exposure to silver nanoparticles has been shown to induce oxidative stress and cytotoxicity in human colon carcinoma cells.

Kanimozhi et al., 2022 - Biogenic synthesis of silver nanoparticle using *Cissus quadrangularis* extract and its invitro study.

Green synthesis of metallic and metal oxide nanomaterials using plant extracts has gained significant importance in various fields. Aqueous extracts of *Cissus quadrangularis* have been used as reducing and stabilizing agents for the synthesis of silver nanoparticles. Silver nanoparticles synthesized from *Cissus quadrangularis* extract have exhibited anti-arthritis activity, as demonstrated by the inhibition of egg albumin denaturation and bovine serum albumin denaturation. The average size of the silver nanoparticles synthesized using *Cissus quadrangularis* extract was 24 nm, and their spherical shape was confirmed by SEM images. Green synthesis methods using plant extracts have advantages such as eco-friendliness and compatibility for drug and biomedical applications. Nanoparticles synthesized from *Cissus quadrangularis* extract have shown potential for arthritis applications and the development of new agents. Synthesis of silver nanoparticles using plant extracts has been successfully carried out with various plants, including *Ficus carica*, *Cocous nucifera*, *Moringa oleifera*, *Carica papaya*, *Eucalyptus hybrid*, *Allium cepa*, *Aloe vera*, and *Basella alba*, which have exhibited significant biological activities.

Sojan et al., 2022 - Zebrafish caudal fin as a model to investigate the role of probiotics in bone regeneration.

Probiotics are live microorganisms that enhance bone mineralization and have beneficial effects on the host. The role of probiotics in bone regeneration is not well studied. This study aims to investigate the effects of probiotic treatment on the caudal fin regeneration of zebrafish. The study analyzes the morphological changes, gene expression, and mineralization of the regenerated fins after probiotic treatment. Probiotics were found to increase the regenerated area, shorten and thicken lepidotrichia segments, and enhance the well-crystallized hydroxyapatite content in the fins. Probiotics also modulate various stages of osteoblast differentiation, suppress osteoclast activity, regulate cell cycle and phosphate homeostasis, and activate Wnt/ β -catenin and Retinoic acid signaling pathways. These findings provide new insights into the potential use of probiotics as a prophylactic treatment for accelerating bone regeneration and improving skeletal health.

CHAPTER 3

AIM AND SCOPE

3.1 AIM:

The aim of this study is to investigate the phytochemicals of *Artocarpus hirsutus* & *Cardiospermum halicacabum* and determine their efficacy in promoting wound healing and bone development, contributing to the advancement of natural-based therapeutic interventions.

3.2 SCOPE:

- To predict the targets of the *Artocarpus hirsutus* and *Cardiospermum halicacabum* for wound healing and bone development utilizing the methodology of network pharmacology and validate through Molecular docking
- To collect the plant *Artocarpus hirsutus* and *Cardiospermum halicacabum*, prepare extraction and characterize their phytochemical composition.
- To investigate the potential effects of plant extract on the healing of wounds and fractures by In-vitro experiments.
- To examine the potential effects of plant extract on the healing of wounds and fractures on bone healing by In-vivo zebrafish model.
- This study focuses on the **PO6** and **PSO2** of our department and **SDG3** and **SDG17** of United Nations as we contribute towards societal well-being by addressing health-related issues and understanding the responsibilities relevant to engineering practice on a global aspect.



Figure 3.1 SDG focused on this study

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS REQUIRED:

4.1.1 Plant material

- Fresh or dried plant samples of *Artocarpus hirsutus* (Wild Jack) collected from Aranmula, Kerala.
- Fresh or dried plant samples of *Cardiospermum halicacabum* (Balloon vine) collected from local markets in Chennai, Tamil Nadu.

4.1.2 Chemical Compounds

- Ethanol
- FTIR-grade potassium bromide (KBr) pellets
- Nutrient Agar medium
- Agar
- Sterile distilled water
- Antibiotic discs

- Dulbecco's Modified Eagle Medium (DMEM)
- Fetal bovine serum (FBS)
- Penicillin-Streptomycin solution
- Phosphate-buffered saline (PBS)
- MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
- E3 medium
- Tricaine (anesthetic)
- Personal protective equipment (lab coat, gloves and etc.,)

4.1.3 Apparatus

- 6-well culture plates
- P10 pipette tip
- Microscope slides

4.1.4 Instruments

- Inverted microscope
- Digital camera or imaging system
- Stereo microscope
- Biosafety cabinet
- Freeze-dryer

4.1.5 Microbial and Animal Model

- Gram-positive bacteria (e.g., Staphylococcus)
- Gram-negative bacteria (e.g., Pseudomonas)
- 3T3 cell line
- Zebrafish embryos
- Adult zebrafish

4.2 METHODOLOGY:

4.2.1 In-Silico Studies:

4.2.1.1 Retrieval of Bioactive Compounds in *Artocarpus hirsutus* & *Cardiospermum halicacabum*:

The bioactive phytochemicals of *Artocarpus hirsutus* & *Cardiospermum halicacabum* were retrieved from the database known as Indian Medicinal Plants, Phytochemistry, and Therapeutics 2.0 (IMPPAT 2.0) [<https://cb.imsc.res.in/imppat/>]. IMPPAT 2.0 is a comprehensive, publicly available database that contains meticulously curated data on more than 4010 Indian medicinal plants, 17,967 phytochemicals, and 1095 therapeutic applications. PubChem ID and SMILES (Simplified Molecular Input Line Entry System) from each Phytocompound retrieved from the PubChem database for further In-silico analyses. (Vivek-Ananth et al., 2023)

4.2.1.2 Computational Profiling of Drug-Likeness, Molecular Properties, and ADME Properties:

The SMILES of the phytochemicals were given as entries in Online tools such as SwissADME [<http://www.swissadme.ch/>]. The distinct ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics of the phytochemicals were profiled using Swiss ADME. The Lipophilicity, Water solubility, Pharmacokinetic parameters, Drug likeness, and Medical chemistry properties were tabulated separately for analyzing complex biological structures, predicting protein-ligand interactions and optimizing phytochemicals to enhance therapeutic effectiveness for wound and bone healing. (Vamshidhar reddy, 2023)

4.2.1.3 Computational Target Fishing of the Phytochemicals:

Computational target fishing is an in silico method that can accurately predict potential targets of given compounds in treating fractures and wounds. Based on the chemical structures of the given compounds, computational tools predict potential drug targets and off-targets. Target prediction by linking structures to biological activity spectra is a proven approach given one has in hand the experimental data to carry out this analysis through models built with machine learning methods. (Jenkins et al., 2006) One such tool is the SwissTargetPrediction tool [<http://www.swisstargetprediction.ch/>], an Online tool that probable macromolecular targets of assumed bioactive small molecules. The prediction is founded on a combination of 2D and 3D similarity with a library of 370,000 known actives on more than 3000 proteins from three different species (Homo sapiens, Mus musculus & Rattus norvegicus). (Daina et al., 2019) GeneCards is a human gene database that integrates data from various sources. It offers comprehensive information on annotated and predicted genes. The database includes genomic, transcriptomic, proteomic, genetic, clinical, and functional data. From the Genecards [<https://www.genecards.org/>], gene targets of both wound healing and bone fracture are retrieved. Those gene targets provide significant insights into the potential interactions of compounds with biological targets for treating fractures and wounds by using the Venny 2.1.0 tool [<https://bioinfogp.cnb.csic.es/tools/venny/index.html>] to acquire common intersecting genes with the Venn diagram. Venny 2.1.0 is an online tool for creating Venn diagrams, which help illustrate the relationships and intersections between different sets or groups. Here, a Venn diagram is taken for gene targets of the fractures and wounds and phytocompounds to determine the common intersecting genes.

4.2.1.4 Network Construction and Analysis:

Cytoscape is a widely used software for displaying biological pathways and molecular interaction networks. It has features for integrating, analyzing, and visualizing data to create network models including Compound-Target (C-T) and Protein-Protein Interaction (PPI) which investigates the molecular mechanisms underlying the effects of phytochemicals on fracture bone and wound healing. The PPI Network is where multiple protein molecules join together to form complexes using noncovalent bonds. The STRING database [<https://string-db.org/>] contains known and predicted PPIs, with a large number of protein interactions. It includes experimental and prediction data. The common intersecting genes of the wounds and fractures and phytochemicals were imported into the database and the species were set to humans. Within the STRING Database, "Homo sapiens" is confined to species, with a minimum interaction level of 0.4, with nodes lacking network connections being concealed. The PPI obtained in the STRING is transferred to Cytoscape. The results were updated and downloaded in TSV format and imported into Cytoscape for constructing the PPI network. The Network Analyzer plugin within Cytoscape computes the network topology parameters such as Degree, Node betweenness, and Closeness to illustrate the topological significance of nodes. These indices help quantify the significance of nodes in the network, with higher values indicating greater significance. The significance of a node in the network increases as its degree, node betweenness, and closeness increase. Subsequently, the Cytohubba plugin was utilized to identify potential hub targets and key sub-networks based on the Clustering Coefficient algorithm. (Lin et al., 2019)

4.2.1.5 Gene ontology (GO) and Pathway analysis:

To examine the functional association of targets, we performed gene ontology (GO) and pathway enrichment analysis using PANTHER gene list analysis, which is used to evaluate the characteristics of genes associated with osteogenesis [<http://www.pantherdb.org/>]. PANTHER (Protein ANalysis THrough Evolutionary Relationships) is a comprehensive resource for protein classification and analysis of gene functions, pathways, and biological processes. PANTHER will provide statistical enrichment analysis for biological processes, molecular functions, and cellular components associated with the hub genes. In addition to GO analysis, PANTHER also offers pathway analysis based on the PANTHER Pathways database. The top hub genes of the PPI Network were uploaded and the Gene Ontology (GO) analysis and pathway analysis were performed. Examine the enriched GO terms and pathways for significant functional associations and biological pathways with bone fracture and wound healing. The enriched GO terms and pathways are visualised using built-in visualization tools. The biological significance of the enriched GO terms and pathways is to gain insights into the molecular mechanisms underlying the effects of phytochemicals on bone fracture and wound healing.

4.2.2 Extraction and Characterisation:

4.2.2.1 Collection of *Artocarpus hirsutus* and *Cardiospermum halicacabum*:

Artocarpus hirsutus is known as 'Wild Jack', which is a tropical evergreen tree species that is native to India and endemic to the Western Ghats where it is a common tree in evergreen and semi-evergreen forests from South Maharashtra to Kanyakumari. The plant *Artocarpus hirsutus* has been collected from the regions of Aranmula, a village in Pathanamthitta District, Kerala.

Cardiospermum halicacabum known as 'Balloon vine', is a climbing plant often found as a weed along roads and rivers which is widely distributed across tropical and subtropical areas of Africa, Australia, and North America. The plant *Cardiospermum halicacabum* has been collected from the local market in Chennai, Tamil Nadu. (Karthik et al., 2022, Zalke et al., 2013)

4.2.2.2 Plant Extraction - Soxhlation:

The impurities of the plants were eliminated through the process of rinsing and cleaning. Then, the samples were dried in a hot air oven at 40°C overnight, after which they were pulverized to a fine powder utilizing a mortar and pestle. To acquire the plant powder, the sample was further ground. The extraction of bioactive compounds from the plant samples is obtained using Soxhlation. The round bottom flask was equipped with a Soxhlet extractor and condenser on an isomantle heater, into which 125 ml of distilled water (solvent) was introduced. The pulverized plant powder was loaded into the thimble and introduced within the Soxhlet extractor. Subsequently, the solvent was heated via the isomantle, causing it to evaporate and traverse the apparatus towards the condenser. After numerous cycles, the suspensions were filtered. After filtration, the extracted samples were lyophilized using a freeze-dryer to eliminate the solvent present in them. (Lopez-Bascon & Luque de Castro, 2020)

4.2.2.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The Fourier transform infrared spectrophotometer (FTIR) is a highly effective tool utilized for the identification of the functional groups that are present within compounds. To conduct the analysis, the powdered plant extract was diluted using distilled water. These samples were then loaded into the FTIR spectroscopy instrument, which had a range spanning from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} . The sample itself was placed directly onto the platform and subjected to uniform pressure, resulting in the recording of spectra as the average of 24 scans at a resolution of 4 cm^{-1} . The obtained spectrum was analyzed to determine the functional groups that were present. (M. et al., 2018)

4.2.3 In-Vitro Studies:

4.2.3.1 Anti-Microbial Assay:

The antimicrobial activity of the powdered plant extract was ascertained via the well diffusion method on the Nutrient Agar medium. The medium was quantified at 2.8 g and dissolved in 100 mL of distilled water, with the addition of 1 g of agar. Subsequently, the medium was subjected to sterilization. Following sterilization, the media was poured into sterile petri plates and allowed to solidify for 1 hour. Once the medium had solidified, the inoculums were spread onto the solid plates using a sterile swab moistened with the bacterial suspension. Gram-positive species *Staphylococcus* and Gram-negative species *Pseudomonas* were used for the study. Cork borers were used to create wells. In these wells, plant extract of varying concentrations (50, 100, 250 µg/mL), a negative control (20 µL ethanol), and Antibiotic discs were loaded. Subsequently, these plates were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured to ascertain microbial growth. (Amudha et al., 2024)

4.2.3.2 Cytotoxicity Assay:

The cytotoxic behaviour of the plant extracts can be determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) against the 3T3 cell line. Each cell line was initially seeded in a 96-well microplate separately, with a density of 1×10^6 cells/mL. Then, the microplates were subjected to incubation at a temperature of 37 °C for 24 hours, in an environment containing 5% CO₂. During this period, the cells were permitted to grow until they reached a state of 90% confluence.

After the incubation period, the culture medium was replaced, and the cells were subjected to treatments with plant extracts at a concentration of 100 µg/mL. After that, the samples were incubated for a duration of 24 hours. Following a gentle wash with PBS solution at a pH of 7.4, each well of the cells was supplemented with 20 µL of MTT solution (5 mg/mL). The cells were then left to incubate at a temperature of 37 °C in a dark. (Shanmugam et al., 2022)

$$\text{Cell Viability} = \left[\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \right] \times 100$$

4.2.3.3 Wound Scratch Assay:

In 6-well plates (8×10^5 cells/well), the 3T3 cells were seeded and cultured in optimal growth conditions until they reached a 90% confluence. To induce a wound, a scratch was created at the centre of the cell monolayer using a P10 pipette tip, and any cellular debris was eliminated by washing with fresh media. Over a period of 48 hours at 37 °C, the untreated cells were exposed to 100 µg/mL of the plant extracts and 50 µg/mL of a commercial medication as a positive control. Throughout the analysis of the closure of the scratch wound, four digital photographs were captured at various time points using an inverted microscope. (Shanmugam et al., 2022)

4.2.4 In-Vivo Studies:

4.2.4.1 Zebrafish Embryo Toxicity Test:

Zebrafish embryos were supplied by the Zebrafish Facility, Centre for Molecular and Nanomedical Sciences, Sathyabama Institute of Science and Technology. Zebrafish eggs were collected and selected under a stereo microscope at 1 h post fertilisation (hpf). Fertilised eggs were cleaned using sterile distilled water and incubated at 28 °C in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄ and 0.1% (w/v) methylene blue). Maintenance and breeding procedures for zebrafish were performed as per the Organisation for Economic Co-operation and Development (OECD) guidelines. The breeding process began with the separation of male and female zebrafish into different tanks for six days. During this period, they were fed with high-protein pellets. A high-protein diet promotes health and increases embryo production. During this time, all zebrafish were exposed to light for 14 h, followed by 10 h of darkness. On the sixth day, male and female zebrafish in a ratio of 3:1 were placed in the dark for 10 h, followed by exposure to 3 h of light to induce mating. After mating,

zebrafish embryos were collected and rinsed three times in sterile distilled water. After six hpf, ten embryos were transferred to each well of 24-well plates in the presence of 2–720 µg/mL of Plant extracts. Untreated embryos (0 µg/mL extract) were used as a control group. Embryos were observed under a stereo microscope. The survivability, heartbeat, and morphological changes were recorded at 24, 48, and 72 hpf. Since the heart is the first internal organ to form and function during zebrafish development, at approximately 24–48 hpf, heartbeats can reflect toxicological impact. The percentage of surviving embryos and associated heartbeats, along with embryonic morphological analyses, were used to assess the impact of extracts on embryos. (Zainol Abidin et al., 2020)

4.2.4.2 Caudal Fin Regenerative Capacity Test:

For regenerative capacity studies, two male and one female zebrafish, aged two months old, were used for each extract concentration. Zebrafish were anesthetised using 0.01% tricaine, and amputations of the caudal fin were performed using a sterile razor blade. Translucent sections of caudal fin regeneration tissues can be observed by the naked eye. Thin epidermal layers were formed after amputation. These layers were important for the formation of a blastema, which affects the regeneration of the caudal fin. During regenerative outgrowth, blastema which is identified as mesenchymal stem cells will proliferate and differentiate to replace amputated structures. All procedures were performed according to animal guidelines described in Muniandy et al.

The caudal fin of the zebrafish was amputated approximately 5 mm proximal to the first branch point of the lepidotrichia. Concentrations of extracts at 100 µg/mL per gram of fish body weight in 5 µL, were administered to zebrafish by oral gavage, twice daily for 10 days. Untreated male and female zebrafish were used as a control group and received 5 µL distilled water. Caudal fin regeneration was measured and recorded on days 0, 3, 4, 6, 8, and 10 using microscopy. The caudal fin regeneration rate was calculated as the regeneration of fin areas (mm²) grown over the number of days. (Zainol Abidin et al., 2020)

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 IN-SILICO STUDIES:

5.1.1 Retrieval of Bioactive Compounds in *Artocarpus hirsutus* & *Cardiospermum halicacabum*:

There are only 2 similar phytochemicals in *Artocarpus hirsutus*. There are 25 phytochemicals in *Cardiospermum halicacabum*. Tables 5.1 and 5.2 present the retrieved bioactive compounds from *Artocarpus hirsutus* and *Cardiospermum halicacabum*, along with their respective plant parts, IMPPAT ID, PubChem ID, SMILES notation, phytochemical names, and associated references.

5.1.2 Computational Profiling of Drug-Likeness, Molecular Properties, and ADME Properties:

5.1.2.1 General Physicochemical Properties

Tables 5.3 and 5.4 present the general physicochemical properties of the investigated phytochemicals, including their molecular formula, molecular weight, number of heavy atoms, number of aromatic heavy atoms, fraction Csp3, number of rotatable bonds, number of hydrogen bond acceptors, number of hydrogen bond donors, molar refractivity, and total polar surface area (TPSA). These physicochemical properties provide essential insights into the molecular characteristics and behaviour of the investigated phytochemicals, aiding in the assessment of their potential pharmacological activities and suitability for therapeutic applications.

Table. 5.1 Phytochemicals in *Artocarpus hirsutus* retrieved from IMPPAT database

Plant part	IMPPAT ID	PUBCHEM ID	SMILES	Phytochemical name	Reference
seed	IMPHY006383	5458461	<chem>CC(C)C=CC1=C(C=C2C(=C1O)C(=O)C(=C(O2)C3=C(C=C(C=C3)O)O)CC=C(C)C)OC</chem>	Artocarpin	ISBN:9788172362089
wood	IMPHY006382	5458462	<chem>CC(C)C=CC1=C(C=C2C(=C1O)C(=O)C3=C(O2)C4=C(C=C(C=C4)O)OC3C=C(C)C)OC</chem>	3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	ISBN:9770972795006

Table. 5.2 Phytochemicals in *Cardiospermum halicacabum* retrieved from IMPPAT database

Plant part	IMPPAT ID	PUBCHEM ID	SMILES	Phytochemical name	Reference
leaf	IMPHY001947	118701750	<chem>CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)OC(=O)CN(C6C(C(C(C(O6)CO)O)O)O)OC)C(=O)O</chem>	(3S)-O-(N-Methoxy-N-D-glucosylglycyl)butulinic acid	ISBN:9789327275590
leaf	IMPHY004661	5280443	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O</chem>	Apigenin	ISBN:9788172362089, ISBN:9770972795006, ISBN:9788172360818, ISBN:9788185042145
leaf	IMPHY011710	12912214	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O</chem>	Apigenin-7-o-glucuronide	ISBN:9788172362089, ISBN:9788185042138

leaf	IMPHY004433	5280666	<chem>COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O</chem>	Chrysoeriol	ISBN:9788172362089, ISBN:9788172360818
leaf	IMPHY015039	164619	<chem>COC1C(C(C(C(C1O)O)O)O)O</chem>	D-Pinitol	ISBN:9788172360818, ISBN:9788172362089, ISBN:9788185042114
leaf	IMPHY004660	5280445	<chem>C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O</chem>	Luteolin	ISBN:9788172362089, ISBN:9788172360818
leaf	IMPHY007450	971	<chem>C(=O)(C(=O)O)O</chem>	Oxalic acid	ISBN:9780387706375, ISBN:9789327275590
leaf, root	IMPHY014836	222284	<chem>CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4O)C)C)C(C)C</chem>	beta-Sitosterol	ISBN:9788172360818, ISBN:9780387706375, ISBN:9789327275590
seed	IMPHY011394	10467	<chem>CCCCCCCCCCCCCCCCCCCC(=O)O</chem>	Arachidic acid	ISBN:9788172362089, ISBN:9788172360818, ISBN:9788185042145
seed	IMPHY015072	12309055	<chem>CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)OC5C(C(C(C(O5)CO)O)O)O)C)C(C)C</chem>	beta-Sitosterol-beta-D-glucoside	ISBN:9770972795006
seed	IMPHY003104	2969	<chem>CCCCCCCCCCC(=O)O</chem>	Decanoic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY003016	3893	<chem>CCCCCCCCCCCCC(=O)O</chem>	Lauric acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY014990	5280450	<chem>CCCCCC=CCC=CCCCCCCCC(=O)O</chem>	Linoleic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY012723	5280934	<chem>CCC=CCC=CCC=CCCCCCCCC(=O)O</chem>	Linolenic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY000060	11005	<chem>CCCCCCCCCCCCCCC(=O)O</chem>	Myristic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY011797	445639	<chem>CCCCCCCCC=CCCCCCCCC(=O)O</chem>	Oleic acid	ISBN:9770972795006, ISBN:9788172362089

seed	IMPHY007327	985	<chem>CCCCCCCCCCCCCCCC(=O)O</chem>	Palmitic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY011666	445638	<chem>CCCCCCC=CCCCCCCC(=O)O</chem>	Palmitoleic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY004631	5281	<chem>CCCCCCCCCCCCCCCC(=O)O</chem>	Stearic acid	ISBN:9770972795006, ISBN:9788172362089
seed	IMPHY000165	11197	<chem>CCCCCCCCCCCCCCCCCCCCCCCC(=O)O</chem>	Tetracosanoic acid	ISBN:9770972795006, ISBN:9788172362089
Whole plant	IMPHY011711	5319484	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O</chem>	Apigenin 7-glucuronide	ISBN:9788172360818
Whole plant	IMPHY014972	151108	<chem>COC1C(C(C(C(C1O)O)O)O)O</chem>	L-Quebrachitol	ISBN:9780387706375, ISBN:9788172360818
Whole plant	IMPHY000622	108065	<chem>COC1=C(C=C(C=C1O)C2C(CC3=C(O2)C(=C(C=C3O)O)C4C(C(OC5=CC(=CC(=C45)O)O)C6=CC=C(C=C6)O)O)O)O</chem>	Proanthocyanidin	ISBN:9788172360818
Whole plant	IMPHY015047	5280805	<chem>CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O</chem>	Rutin	PMID:22557189
Whole plant	IMPHY014842	5280794	<chem>CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C</chem>	Stigmasterol	ISBN:9770972795006

Table. 5.3 General Physicochemical Properties of *Artocarpus hirsutus*

Phytochemical name	Formula	Molecular Weight	No. of heavy atoms	No. of Aromatic heavy atoms	Fraction Csp3	No. of Rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity	TPSA (Å²)
Artocarpin	C ₂₆ H ₂₈ O ₆	436.5	32	16	0.27	6	6	3	128.71	100.13
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	C ₂₆ H ₂₆ O ₆	434.48	32	16	0.27	4	6	2	126.26	89.13

Table. 5.4 General Physicochemical Properties of *Cardiospermum halicacabum*

Phytochemical name	Formula	Molecular Weight	No. of heavy atoms	No. of Aromatic heavy atoms	Fraction Csp3	No. of Rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity	TPSA (Å ²)
(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid	C ₃₉ H ₆₃ N ₁ O ₁₀	705.92	50	0	0.9	9	11	5	187.9	166.22
Apigenin	C ₁₅ H ₁₀ O ₅	270.24	20	16	0	1	5	3	73.99	90.9
Apigenin-7-o-glucuronide	C ₂₁ H ₁₈ O ₁₁	446.36	32	16	0.24	4	11	6	106.72	187.12
Chrysoeriol	C ₁₆ H ₁₂ O ₆	300.26	22	16	0.06	2	6	3	80.48	100.13

D-Pinitol	C7H14O 6	194.18	13	0	1	1	6	5	40.54	110.38
Luteolin	C15H10 O6	286.24	21	16	0	1	6	4	76.01	111.13
Oxalic acid	C2H2O4	90.03	6	0	0	1	4	2	15.27	74.6
beta-Sitosterol	C29H50 O	414.71	30	0	0.93	6	1	1	133.23	20.23
Arachidic acid	C20H40 O2	312.53	22	0	0.95	18	2	1	100.03	37.3
beta-Sitosterol-b eta-D-glucoside	C35H60 O6	576.85	41	0	0.94	9	6	4	165.61	99.38

Decanoic acid	C10H20 O2	172.26	12	0	0.9	8	2	1	51.96	37.3
Lauric acid	C12H24 O2	200.32	14	0	0.92	10	2	1	61.57	37.3
Linoleic acid	C18H32 O2	280.45	20	0	0.72	14	2	1	89.46	37.3
Linolenic acid	C18H30 O2	278.43	20	0	0.61	13	2	1	88.99	37.3
Myristic acid	C14H28 O2	228.37	16	0	0.93	12	2	1	71.18	37.3
Oleic acid	C18H34 O2	282.46	20	0	0.83	15	2	1	89.94	37.3

Palmitic acid	C16H32 O2	256.42	18	0	0.94	14	2	1	80.8	37.3
Palmitoleic acid	C16H30 O2	254.41	18	0	0.81	13	2	1	80.32	37.3
Stearic acid	C18H36 O2	284.48	20	0	0.94	16	2	1	90.41	37.3
Tetracosanoic acid	C24H48 O2	368.64	26	0	0.96	22	2	1	119.25	37.3
Apigenin 7-glucuronide	C21H18 O11	446.36	32	16	0.24	4	11	6	106.72	187.1 2
L-Quebrachitol	C7H14O 6	194.18	13	0	1	1	6	5	40.54	110.38

Proanthocyanidin	C ₃₁ H ₂₈ O ₁₂	592.55	43	24	0.23	4	12	9	151.18	209.76
Rutin	C ₂₇ H ₃₀ O ₁₆	610.52	43	16	0.44	6	16	10	141.38	269.43
Stigmasterol	C ₂₉ H ₄₈ O	412.69	30	0	0.86	5	1	1	132.75	20.23

5.1.2.2 Lipophilicity Properties

Tables 5.5 and 5.6 present the lipophilicity properties of the investigated phytochemicals, which were assessed using various logP values, including iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT, and Consensus Log P_{e/w}. These lipophilicity properties provide valuable insights into the hydrophobicity of the investigated phytochemicals, which play a crucial role in their absorption, distribution, metabolism, and excretion within the body thereby influencing their pharmacokinetic and pharmacodynamic profiles. Compounds with higher Log P values tend to be more lipophilic, which can affect their bioavailability and pharmacokinetic properties.

For instance, *Artocarpus hirsutus* exhibits relatively high lipophilicity. Conversely, in *Cardiospermum halicacabum*, compounds like Decanoic acid and Lauric acid have lower Log P values, indicating lower lipophilicity. Similarly, compounds like D-Pinitol and L-Quebrachitol demonstrate very low lipophilicity, with negative Log P values, suggesting a hydrophilic nature. On the other hand, compounds such as beta-sitosterol and Arachidic acid exhibit higher lipophilicity.

5.1.2.3 Water Solubility Properties

Tables 5.7 and 5.8 provide information on the water solubility properties of various phytochemicals derived from *Artocarpus hirsutus* and *Cardiospermum halicacabum*. Solubility is indicated in terms of Log S values, solubility class, and solubility in both mg/mL and mol/L. The solubility classification is based on Log S values, with values closer to 0 indicating higher solubility. *Artocarpus hirsutus* demonstrates poor solubility, conversely, *Cardiospermum halicacabum* exhibits high solubility.

Table. 5.5 Lipophilicity Properties of *Artocarpus hirsutus*

Phytochemical name	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log P e/w
Artocarpin	4.14	5.72	5.65	2.3	6.08	4.78
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	4.31	6.02	5.52	2.3	5.76	4.78

Table. 5.6 Lipophilicity Properties of *Cardiospermum halicacabum*

Phytochemical name	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log P e/w
(3S)-O-(N-Methoxy-N-D-glucosylglycyloxy)betulinic acid	4.46	4.48	4.3	3	2.84	3.82
Apigenin	1.89	3.02	2.58	0.52	2.52	2.11
Apigenin-7-o-glucuronide	1.53	1.46	0.14	-1.63	-0.1	0.28
Chrysoeriol	2.44	3.1	2.59	0.22	2.55	2.18
D-Pinitol	0.36	-3.17	-3.18	-2.81	-2.5	-2.26
Luteolin	1.86	2.53	2.28	-0.03	2.03	1.73
Oxalic acid	-0.35	-0.25	-0.84	-1.51	-0.97	-0.79
beta-Sitosterol	4.79	9.34	8.02	6.73	7.04	7.19
Arachidic acid	4.56	9.29	7.11	5.13	7.01	6.62
beta-Sitosterol-beta-D-glucoside	4.98	7.74	5.85	3.96	5.02	5.51
Decanoic acid	2.5	4.09	3.21	2.58	2.63	3

Lauric acid	2.7	4.2	3.99	3.15	3.5	3.51
Linoleic acid	4.14	6.98	5.88	4.47	5.77	5.45
Linolenic acid	3.36	6.46	5.66	4.38	5.59	5.09
Myristic acid	3.32	6.11	4.77	3.69	4.37	4.45
Oleic acid	4.27	7.64	6.11	4.57	5.95	5.71
Palmitic acid	3.85	7.17	5.55	4.19	5.25	5.2
Palmitoleic acid	3.53	6.58	5.33	4.09	5.07	4.92
Stearic acid	4.3	8.23	6.33	4.67	6.13	5.93
Tetracosanoic acid	5.62	11.46	8.67	6	8.77	8.1
Apigenin 7-glucuronide	1.53	1.46	0.14	-1.63	-0.1	0.28
L-Quebrachitol	0.36	-3.17	-3.18	-2.81	-2.5	-2.26
Proanthocyanidin	2.29	2.69	2.65	-0.07	1.7	1.85
Rutin	1.58	-0.33	-1.69	-3.89	-2.11	-1.29
Stigmasterol	5.01	8.56	7.8	6.62	6.86	6.97

Table. 5.7 Water Solubility Properties of *Artocarpus hirsutus*

Phytochemical name	ESOL				Ali				SILICOS-IT			
	Log S	Solubility		Class	Log S	Solubility		Class	Log S	Solubility		Class
		mg/ mL	mol /L			mg/ mL	mol/ L			mg/ mL	mol /L	
Artocarpin	-6.12	3.28 E-04	7.52 E-07	Poorly soluble	-7.59	1.12 E-05	2.57 E-08	Poorly soluble	-6.6	1.09 E-04	2.50 E-07	Poorly soluble
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	-6.43	1.61 E-04	3.70 E-07	Poorly soluble	-7.67	9.29 E-06	2.14 E-08	Poorly soluble	-6.56	1.21 E-04	2.78 E-07	Poorly soluble

Table. 5.8 Water Solubility Properties of *Cardiospermum halicacabum*

Phytochemical name	ESOL				Ali				SILICOS-IT			
	Log S	Solubility		Class	Log S	Solubility		Class	Log S	Solubility		Class
		mg/ mL	mol /L			mg/ mL	mol/ L			mg/ mL	mol/L	
(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid	-6.45	2.53E-04	3.59E-07	Poorly soluble	-7.69	1.44E-05	2.04E-08	Poorly soluble	-3.59	1.80E-01	2.55E-04	Soluble

Apigenin	-3.94	3.07E-02	1.14E-04	Soluble	-4.59	6.88E-03	2.55E-05	Moderately soluble	-4.4	1.07E-02	3.94E-05	Moderately soluble
Apigenin-7-o-glucuronide	-3.63	1.04E-01	2.33E-04	Soluble	-5	4.51E-03	1.01E-05	Moderately soluble	-2.22	2.67E+00	5.98E-03	Soluble
Chrysoeriol	-4.06	2.61E-02	8.69E-05	Moderately soluble	-4.87	4.04E-03	1.35E-05	Moderately soluble	-4.52	9.07E-03	3.02E-05	Moderately soluble
D-Pinitol	1.02	2.03E+03	1.05E+01	Highly soluble	1.42	5.11E+03	2.63E+01	Highly soluble	2.58	7.37E+04	3.79E+02	Soluble
Luteolin	-3.71	5.63E-02	1.97E-04	Soluble	-4.51	8.84E-03	3.09E-05	Moderately soluble	-3.82	4.29E-02	1.50E-04	Soluble
Oxalic acid	-0.17	6.02E+01	6.69E-01	Very soluble	-0.86	1.25E+01	1.39E-01	Very soluble	1.47	2.66E+03	2.95E+01	Soluble
beta-Sitosterol	-7.9	5.23E-06	1.26E-08	Poorly soluble	-9.67	8.90E-08	2.15E-10	Poorly soluble	-6.19	2.69E-04	6.49E-07	Poorly soluble
Arachidic acid	-6.44	1.13E-04	3.61E-07	Poorly soluble	-9.97	3.31E-08	1.06E-10	Poorly soluble	-6.91	3.84E-05	1.23E-07	Poorly soluble

beta-Sitosterol-beta-D-glucoside	-7.7	1.15E-05	2.00E-08	Poorly soluble	-9.67	1.23E-07	2.14E-10	Poorly soluble	-4.4	2.28E-02	3.94E-05	Moderately soluble
Decanoic acid	-2.96	1.90E-01	1.10E-03	Soluble	-4.58	4.54E-03	2.64E-05	Moderately soluble	-2.87	2.30E-01	1.34E-03	Soluble
Lauric acid	-3.07	1.71E-01	8.55E-04	Soluble	-4.69	4.06E-03	2.03E-05	Moderately soluble	-3.69	4.05E-02	2.02E-04	Soluble
Linoleic acid	-5.05	2.49E-03	8.87E-06	Moderately soluble	-7.58	7.42E-06	2.64E-08	Poorly soluble	-4.67	5.93E-03	2.11E-05	Moderately soluble
Linolenic acid	-4.78	4.64E-03	1.67E-05	Moderately soluble	-7.04	2.55E-05	9.16E-08	Poorly soluble	-3.96	3.08E-02	1.11E-04	Soluble
Myristic acid	-4.31	1.11E-02	4.86E-05	Moderately soluble	-6.67	4.83E-05	2.11E-07	Poorly soluble	-4.51	7.12E-03	3.12E-05	Moderately soluble
Oleic acid	-5.41	1.09E-03	3.85E-06	Moderately soluble	-8.26	1.54E-06	5.46E-09	Poorly soluble	-5.39	1.14E-03	4.04E-06	Moderately soluble
Palmitic acid	-5.02	2.43E-03	9.49E-06	Moderately soluble	-7.77	4.31E-06	1.68E-08	Poorly soluble	-5.31	1.25E-03	4.88E-06	Moderately soluble

Palmitoleic acid	-4.7	5.02E-03	1.97E-05	Moderately soluble	-7.16	1.75E-05	6.88E-08	Poorly soluble	-4.59	6.49E-03	2.55E-05	Moderately soluble
Stearic acid	-5.73	5.26E-04	1.85E-06	Moderately soluble	-8.87	3.80E-07	1.33E-09	Poorly soluble	-6.11	2.19E-04	7.71E-07	Poorly soluble
Tetracosanoic acid	-7.89	4.71E-06	1.28E-08	Poorly soluble	-12.23	2.19E-10	5.94E-13	Insoluble	-8.49	1.18E-06	3.21E-09	Poorly soluble
Apigenin 7-glucuronide	-3.63	1.04E-01	2.33E-04	Soluble	-5	4.51E-03	1.01E-05	Moderately soluble	-2.22	2.67E+00	5.98E-03	Soluble
L-Quebrachitol	1.02	2.03E+03	1.05E+01	Highly soluble	1.42	5.11E+03	2.63E+01	Highly soluble	2.58	7.37E+04	3.79E+02	Soluble
Proanthocyanidin	-5.36	2.60E-03	4.39E-06	Moderately soluble	-6.75	1.06E-04	1.79E-07	Poorly soluble	-4.6	1.50E-02	2.52E-05	Moderately soluble
Rutin	-3.3	3.08E-01	5.05E-04	Soluble	-4.87	8.30E-03	1.36E-05	Moderately soluble	-0.29	3.15E+02	5.15E-01	Soluble
Stigmasterol	-7.46	1.43E-05	3.46E-08	Poorly soluble	-8.86	5.71E-07	1.38E-09	Poorly soluble	-5.47	1.40E-03	3.39E-06	Moderately soluble

5.1.2.4 Pharmacokinetic Parameters

Tables 5.9 and 5.10 provide information on the pharmacokinetic parameters of phytochemicals from *Artocarpus hirsutus* and *Cardiospermum halicacabum*. Parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (Pgp) substrate, and inhibition of cytochrome P450 (CYP) enzymes are crucial for understanding the absorption, distribution, metabolism, and excretion (ADME) properties of these compounds. Additionally, the log K_p values represent the predicted skin permeability. These parameters are essential for assessing the pharmacological potential and safety profiles of phytochemicals, aiding in drug discovery and development processes.

5.1.2.5 Drug Likeness Properties

Tables 5.11 and 5.12 provide insights into the drug-likeness properties of the phytochemicals, which are important for a comprehensive assessment of the suitability of the phytochemicals for drug development, considering factors such as molecular weight, lipophilicity, hydrogen bond donors and acceptors, and other physicochemical properties relevant to drug-likeness.

5.1.2.6 Medical Chemistry Properties

Tables 5.13 and 5.14 provide insights into the lead-like characteristics of the phytochemicals, including their potential to serve as drug candidates based on various molecular descriptors and alerts for undesirable features. These properties help assess these phytochemicals' suitability for drug development, providing information on their potential pharmacological activities, synthetic accessibility, and structural alerts for undesirable features.

Table. 5.9 Pharmacokinetic Parameters of *Artocarpus hirsutus*

Phytochemical name	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
Artocarpin	High	No	No	No	Yes	No	No	No	-4.9
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	High	No	No	No	Yes	Yes	No	No	-4.68

Table. 5.10 Pharmacokinetic Parameters of *Cardiospermum halicacabum*

Phytochemical name	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid	Low	No	Yes	No	No	No	No	Yes	-7.43
Apigenin	High	No	No	Yes	No	No	Yes	Yes	-5.8
Apigenin-7-o-glucuronide	Low	No	Yes	No	No	No	No	No	-7.99
Chrysoeriol	High	No	No	Yes	No	Yes	Yes	Yes	-5.93
D-Pinitol	Low	No	Yes	No	No	No	No	No	-9.74

Luteolin	High	No	No	Yes	No	No	Yes	Yes	-6.25
Oxalic acid	High	No	No	No	No	No	No	No	-7.03
beta-Sitosterol	Low	No	No	No	No	No	No	No	-2.2
Arachidic acid	Low	No	No	Yes	No	No	No	No	-1.61
beta-Sitosterol-beta-D-glucoside	Low	No	No	No	No	No	No	No	-4.32
Decanoic acid	High	Yes	No	No	No	No	No	No	-4.45
Lauric acid	High	Yes	No	No	No	No	No	No	-4.54
Linoleic acid	High	Yes	No	Yes	No	Yes	No	No	-3.05
Linolenic acid	High	Yes	No	Yes	No	Yes	No	No	-3.41
Myristic acid	High	Yes	No	Yes	No	No	No	No	-3.35
Oleic acid	High	No	No	Yes	No	Yes	No	No	-2.6
Palmitic acid	High	Yes	No	Yes	No	Yes	No	No	-2.77
Palmitoleic acid	High	Yes	No	Yes	No	Yes	No	No	-3.18
Stearic acid	High	No	No	Yes	No	No	No	No	-2.19
Tetracosanoic acid	Low	No	No	Yes	No	No	No	No	-0.41
Apigenin 7-glucuronide	Low	No	Yes	No	No	No	No	No	-7.99
L-Quebrachitol	Low	No	Yes	No	No	No	No	No	-9.74
Proanthocyanidin	Low	No	No	No	No	No	No	Yes	-8
Rutin	Low	No	Yes	No	No	No	No	No	-10.26
Stigmasterol	Low	No	No	No	No	Yes	No	No	-2.74

Table 5.11 Drug Likeness Properties of *Artocarpus hirsutus*

Phytochemical name	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Artocarpin	Yes	No; 1 violation: WLOGP>5.6	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one	Yes	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55

Table 5.12 Drug Likeness Properties of *Cardiospermum halicacabum*

Phytochemical name	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid	No; 2 violations: MW>500, NorO>10	No; 3 violations: MW>480, MR>130, #atoms>70	No; 1 violation: TPSA>140	No; 1 violation: TPSA>131.6	No; 3 violations: MW>600, TPSA>150, H-acc>10	0.11
Apigenin	Yes	Yes	Yes	Yes	Yes	0.55

Apigenin-7-o-glucuronide	No; 2 violations: NorO>10, NHOrOH>5	Yes	No; 1 violation: TPSA>140	No; 1 violation: TPSA>131.6	No; 3 violations: TPSA>150, H-acc>10, H-don>5	0.11
Chrysoeriol	Yes	Yes	Yes	Yes	Yes	0.55
D-Pinitol	Yes	No; 1 violation: WLOGP<-0.4	Yes	Yes	No; 2 violations: MW<200, XLOGP3<-2	0.55
Luteolin	Yes	Yes	Yes	Yes	Yes	0.55
Oxalic acid	Yes	No; 4 violations: MW<160, WLOGP<-0.4, MR<40, #atoms<20	Yes	Yes	No; 2 violations: MW<200, #C<5	0.85
beta-Sitosterol	Yes; 1 violation: MLOGP>4.15	No; 3 violations: WLOGP>5.6, MR>130, #atoms>70	Yes	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Heteroatoms<2	0.55
Arachidic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Rotors>15	0.85

beta-Sitosterol-beta-D-glucoside	Yes; 1 violation: MW>500	No; 4 violations: MW>480, WLOGP>5.6, MR>130, #atoms>70	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
Decanoic acid	Yes	Yes	Yes	Yes	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	0.85
Lauric acid	Yes	Yes	Yes	Yes	Yes	0.85
Linoleic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 1 violation: XLOGP3>5	0.85
Linolenic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	Yes	No; 1 violation: XLOGP3>5	0.85
Myristic acid	Yes	Yes	No; 1 violation: Rotors>10	Yes	No; 1 violation: XLOGP3>5	0.85
Oleic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 1 violation: XLOGP3>5	0.85
Palmitic acid	Yes; 1 violation: MLOGP>4.15	Yes	No; 1 violation: Rotors>10	Yes	No; 1 violation: XLOGP3>5	0.85
Palmitoleic acid	Yes	Yes	No; 1 violation: Rotors>10	Yes	No; 1 violation: XLOGP3>5	0.85

Stearic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Rotors>15	0.85
Tetracosanoic acid	Yes; 1 violation: MLOGP>4.15	No; 2 violations: WLOGP>5.6, #atoms>70	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Rotors>15	0.85
Apigenin 7-glucuronide	No; 2 violations: NorO>10, NHorOH>5	Yes	No; 1 violation: TPSA>140	No; 1 violation: TPSA>131.6	No; 3 violations: TPSA>150, H-acc>10, H-don>5	0.11
L-Quebrachitol	Yes	No; 1 violation: WLOGP<-0.4	Yes	Yes	No; 2 violations: MW<200, XLOGP3<-2	0.55
Proanthocyanidin	No; 3 violations: MW>500, NorO>10, NHorOH>5	No; 3 violations: MW>480, MR>130, #atoms>70	No; 1 violation: TPSA>140	No; 1 violation: TPSA>131.6	No; 3 violations: TPSA>150, H-acc>10, H-don>5	0.17
Rutin	No; 3 violations: MW>500, NorO>10, NHorOH>5	No; 4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70	No; 1 violation: TPSA>140	No; 1 violation: TPSA>131.6	No; 4 violations: MW>600, TPSA>150, H-acc>10, H-don>5	0.17

Stigmasterol	Yes; 1 violation: MLOGP>4.15	No; 3 violations: WLOGP>5.6, MR>130, #atoms>70	Yes	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Heteroatoms<2	0.55
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Table 5.13 Medical Chemistry Properties of *Artocarpus hirsutus*

Phytochemical name	PAINS (alerts)	Brenk (alerts)	Leadlikeness	Synthetic Accessibility
Artocarpin		1 alert: 0 isolated_alkene	No; 2 violations: MW>350, XLOGP3>3.5	4.43
3,8-dihydroxy-10-methoxy-9-[(E)-3-methylbut-1-enyl]-6-(2-methylprop-1-enyl)-6H-chromeno[4,3-b]chromen-7-one		1 alert: 0 isolated_alkene	No; 2 violations: MW>350, XLOGP3>3.5	4.9

Table 5.14 Medical Chemistry Properties of *Cardiospermum halicacabum*

Phytochemical name	PAINS (alerts)	Brenk (alerts)	Leadlikeness	Synthetic Accessibility
(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid	0	2 alerts: isolated_alkene, oxygen nitrogen_single_bond	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	8.14
Apigenin	0	0	Yes	2.96
Apigenin-7-o-glucuronide	0	0	No; 1 violation: MW>350	5.06
Chrysoeriol	0	0	Yes	3.06
D-Pinitol	0	0	No; 1 violation: MW<250	3.76
Luteolin	1 alert: catechol_A	1 alert: catechol	Yes	3.02
Oxalic acid	0	1 alert: diketo_group	No; 1 violation: MW<250	1
beta-Sitosterol	0	1 alert: isolated_alkene	No; 2 violations: MW>350, XLOGP3>3.5	6.3
Arachidic acid	0	0	No; 2 violations: Rotors>7, XLOGP3>3.5	2.77

beta-Sitosterol-beta-D-glucoside	0	1 alert: isolated_alkene	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	8.02
Decanoic acid	0	0	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	1.67
Lauric acid	0	0	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	1.87
Linoleic acid	0	1 alert: isolated_alkene	No; 2 violations: Rotors>7, XLOGP3>3.5	3.1
Linolenic acid	0	1 alert: isolated_alkene	No; 2 violations: Rotors>7, XLOGP3>3.5	3.03
Myristic acid	0	0	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	2.09
Oleic acid	0	1 alert: isolated_alkene	No; 2 violations: Rotors>7, XLOGP3>3.5	3.07
Palmitic acid	0	0	No; 2 violations: Rotors>7, XLOGP3>3.5	2.31

Palmitoleic acid	0	1 alert: isolated_alkene	No; 2 violations: Rotors>7, XLOGP3>3.5	2.84
Stearic acid	0	0	No; 2 violations: Rotors>7, XLOGP3>3.5	2.54
Tetracosanoic acid	0	0	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.24
Apigenin 7-glucuronide	0	0	No; 1 violation: MW>350	5.06
L-Quebrachitol	0	0	No; 1 violation: MW<250	3.76
Proanthocyanidin	0	0	No; 1 violation: MW>350	5.45
Rutin	1 alert: catechol_A	1 alert: catechol	No; 1 violation: MW>350	6.52
Stigmasterol	0	1 alert: isolated_alkene	No; 2 violations: MW>350, XLOGP3>3.5	6.21

5.1.3 Computational Target Fishing of the Phytochemicals:

There are 20 targets of the *Artocarpus hirsutus* and 319 targets of the *Cardiospermum halicacabum* in total. There are 19 common targets of the *Artocarpus hirsutus* and 235 targets of the *Cardiospermum halicacabum* that are related to the treatment of the wounds. The 327 combined targets of the *Artocarpus hirsutus* and *Cardiospermum halicacabum* has 183 common protein targets that are related to the wound healing which has 2764 interactions.

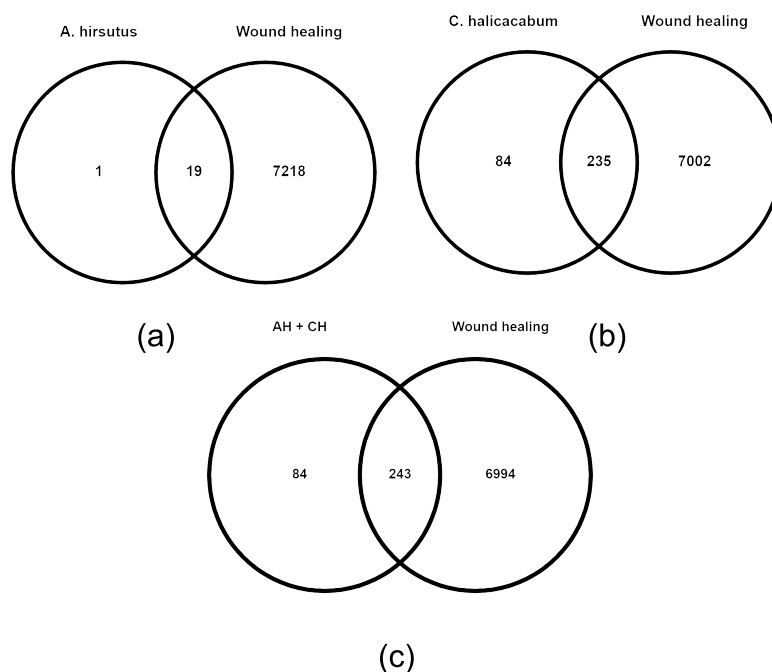


Figure 5.1 Venn Diagram for the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating wounds

There are 10 common targets of the *Artocarpus hirsutus* and 178 targets of the *Cardiospermum halicacabum* that are related to the treatment of the wounds. The 327 combined targets of the *Artocarpus hirsutus* and *Cardiospermum halicacabum* has 183 common protein targets that are related to the bone fracture which has 1979 interactions.

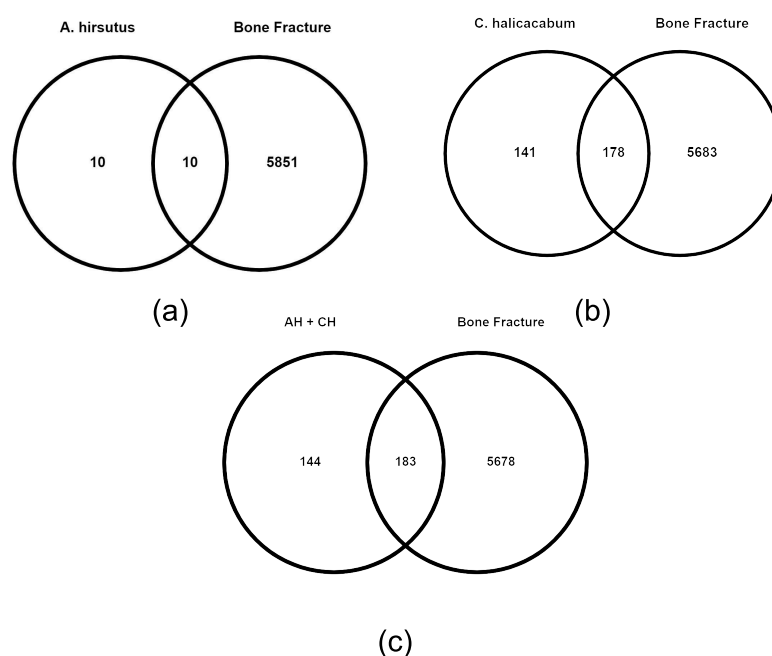


Figure 5.2 Venn Diagram for the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating fractures.

5.1.4 Network Construction and Analysis:

The network analysis of gene interactions in *Artocarpus hirsutus* and *Cardiospermum halicacabum* highlights key genes like UBA2 and THRB as central players, regulating multiple biological processes. UBA2 acts as a crucial hub gene with moderate connectivity but high centrality values, suggesting its pivotal role in the network. Conversely, genes like KLK1 and KDM2A exhibit lower connectivity and centrality, indicating a more limited regulatory influence. NR1H2 emerges as another significant node, potentially orchestrating various biological pathways. While genes like RORA and HAO1 have specific functions, their overall impact on gene interactions is comparatively minor. This analysis provides valuable insights into the complex regulatory networks of these plant species, offering opportunities for further experimental validation and advancing our understanding of molecular mechanisms underlying their biological processes, with implications for crop improvement and pharmacological research.

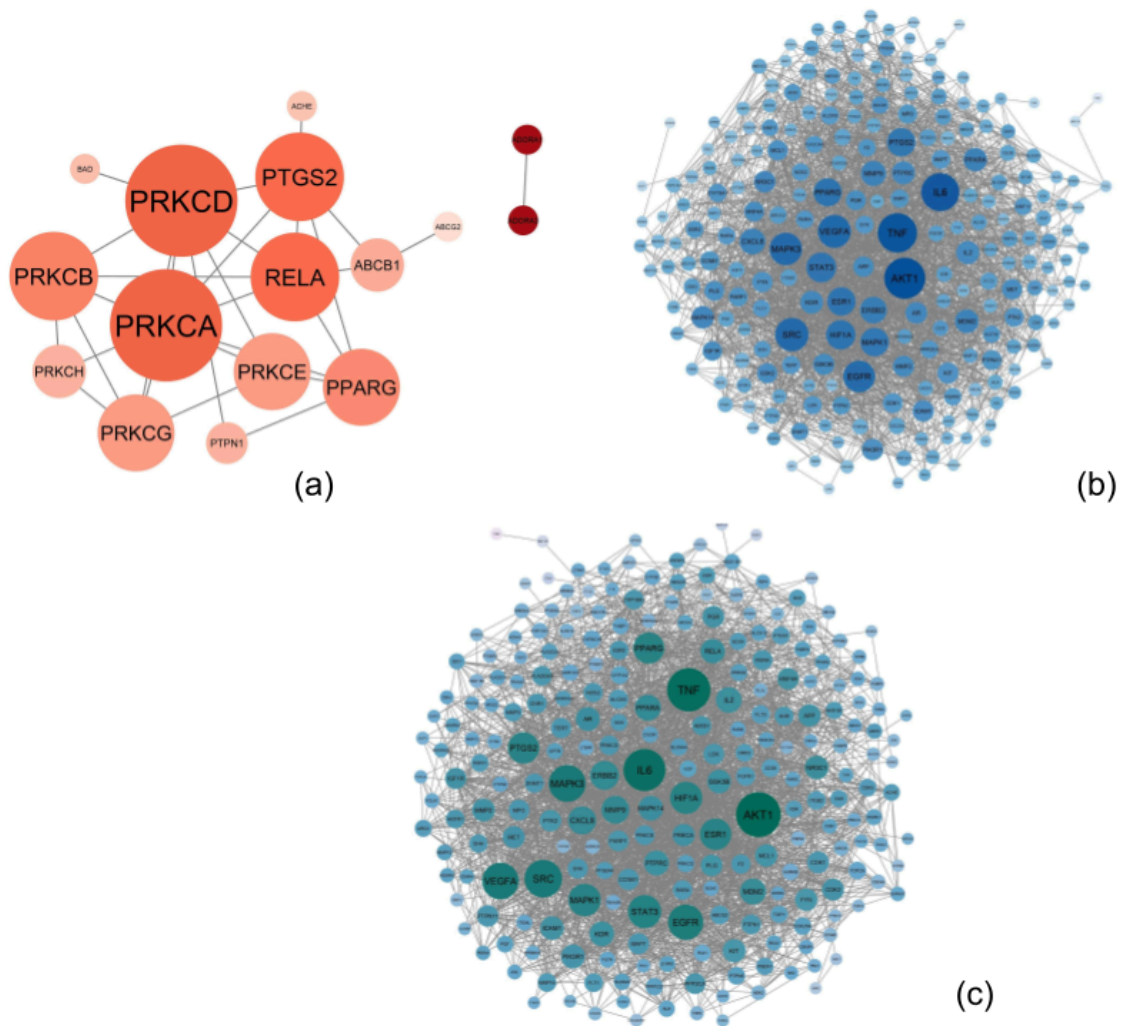


Figure 5.3 Protein-protein interactions (PPIs) of the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating wounds

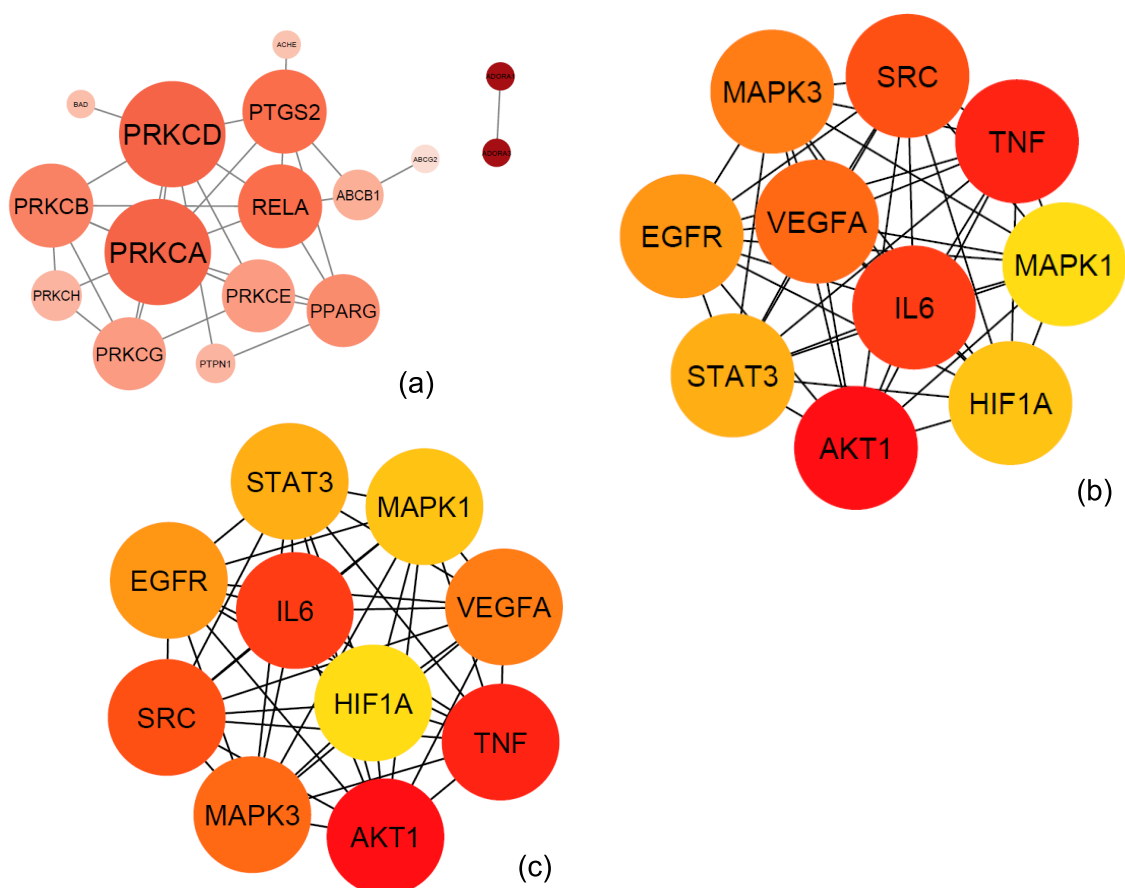


Figure 5.4 Top nodes for PPIs of the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating wounds

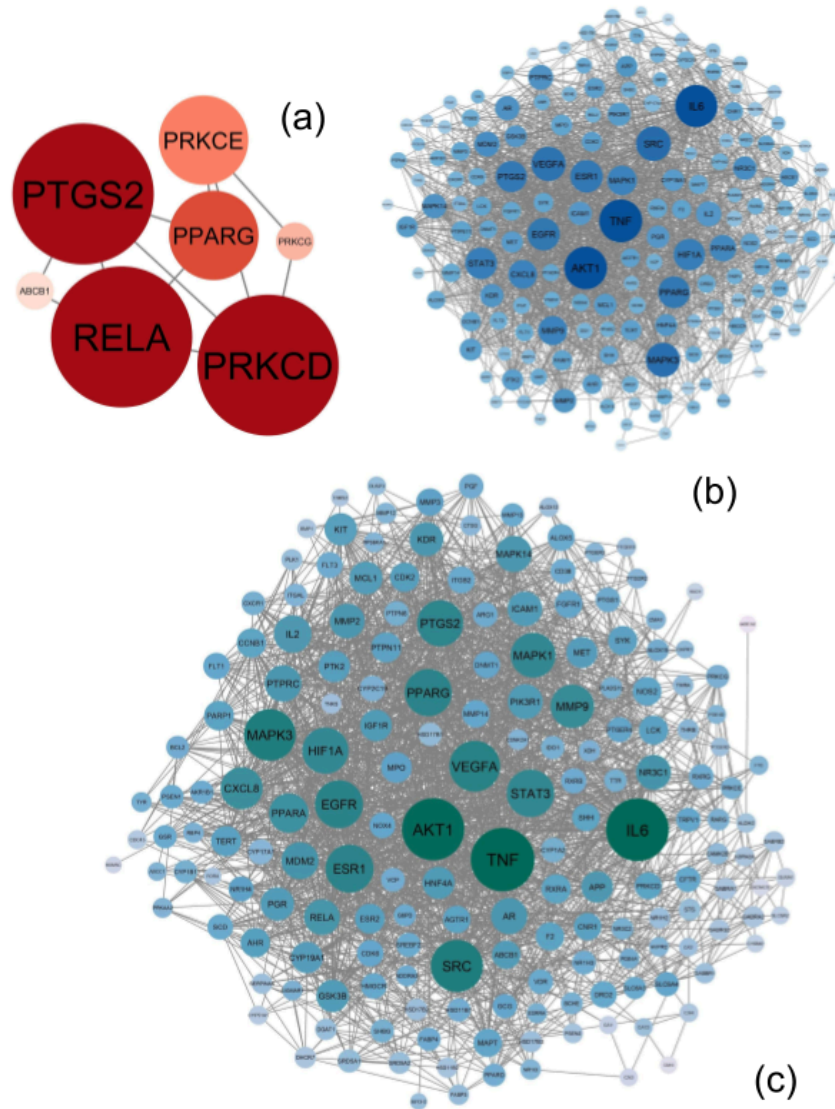


Figure 5.5 Protein-protein interactions (PPIs) of the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating fractures

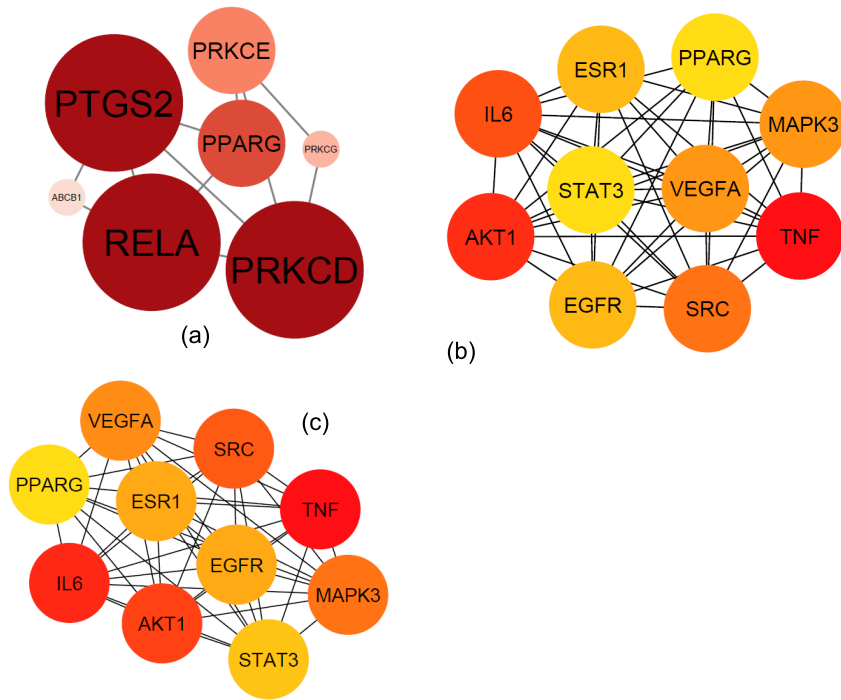


Figure 5.6 Top nodes for PPIs of the common targets of (a) *Artocarpus hirsutus* (b) *Cardiospermum halicacabum* (c) *Artocarpus hirsutus* and *Cardiospermum halicacabum* for treating fractures

5.1.5 Gene Ontology (GO) and Pathway Analysis:

Among the molecular functions identified, binding (GO:0005488) and catalytic activity (GO:0003824) were prominently enriched. These functions play crucial roles in mediating protein-protein interactions and catalyzing biochemical reactions, respectively, which are essential for cellular signaling and metabolic processes during bone repair and tissue regeneration.

Furthermore, the identification of molecular function regulator activity (GO:0098772), molecular transducer activity (GO:0060089), and transcription regulator activity (GO:0140110) highlights the importance of regulatory mechanisms in orchestrating gene expression patterns and cellular responses during the healing process. These regulatory activities govern the activation or repression of target genes involved in bone remodeling, angiogenesis, and inflammation resolution.

In terms of biological processes, several key pathways were significantly enriched, including biological regulation (GO:0065007), cellular process (GO:0009987), and developmental process (GO:0032502). These processes encompass a wide range of activities, such as cellular signaling, proliferation, differentiation, and tissue morphogenesis, which are critical for tissue repair and regeneration following injury.

Moreover, the identification of immune system process (GO:0002376) and response to stimulus (GO:0050896) underscores the involvement of the immune system and the ability of cells to sense and respond to environmental cues during the healing process. These processes play vital roles in coordinating inflammatory responses, fighting off pathogens, and promoting tissue repair and regeneration.

Several key pathways were found to be significantly enriched, including angiogenesis, apoptosis signaling pathway, inflammation mediated by chemokine and cytokine signaling pathway, and TGF-beta signaling pathway. These pathways are known to play crucial roles in orchestrating various aspects of tissue repair, including angiogenesis, cell survival, inflammation resolution, and extracellular matrix remodeling.

Furthermore, the identification of pathways such as Wnt signaling pathway, EGF receptor signaling pathway, and VEGF signaling pathway highlights the importance of growth factors and signaling molecules in regulating cell proliferation, migration, and differentiation during the healing process. These pathways contribute to the recruitment of stem/progenitor cells, promotion of angiogenesis, and enhancement of tissue regeneration at the site of injury.

Moreover, the involvement of pathways such as p38 MAPK pathway, Insulin/IGF pathway, and JAK/STAT signaling pathway underscores the significance of intracellular signaling networks in modulating cellular responses to injury stimuli. These pathways regulate various cellular processes, including inflammation, cell proliferation, and differentiation, thereby influencing the overall outcome of tissue repair and regeneration.

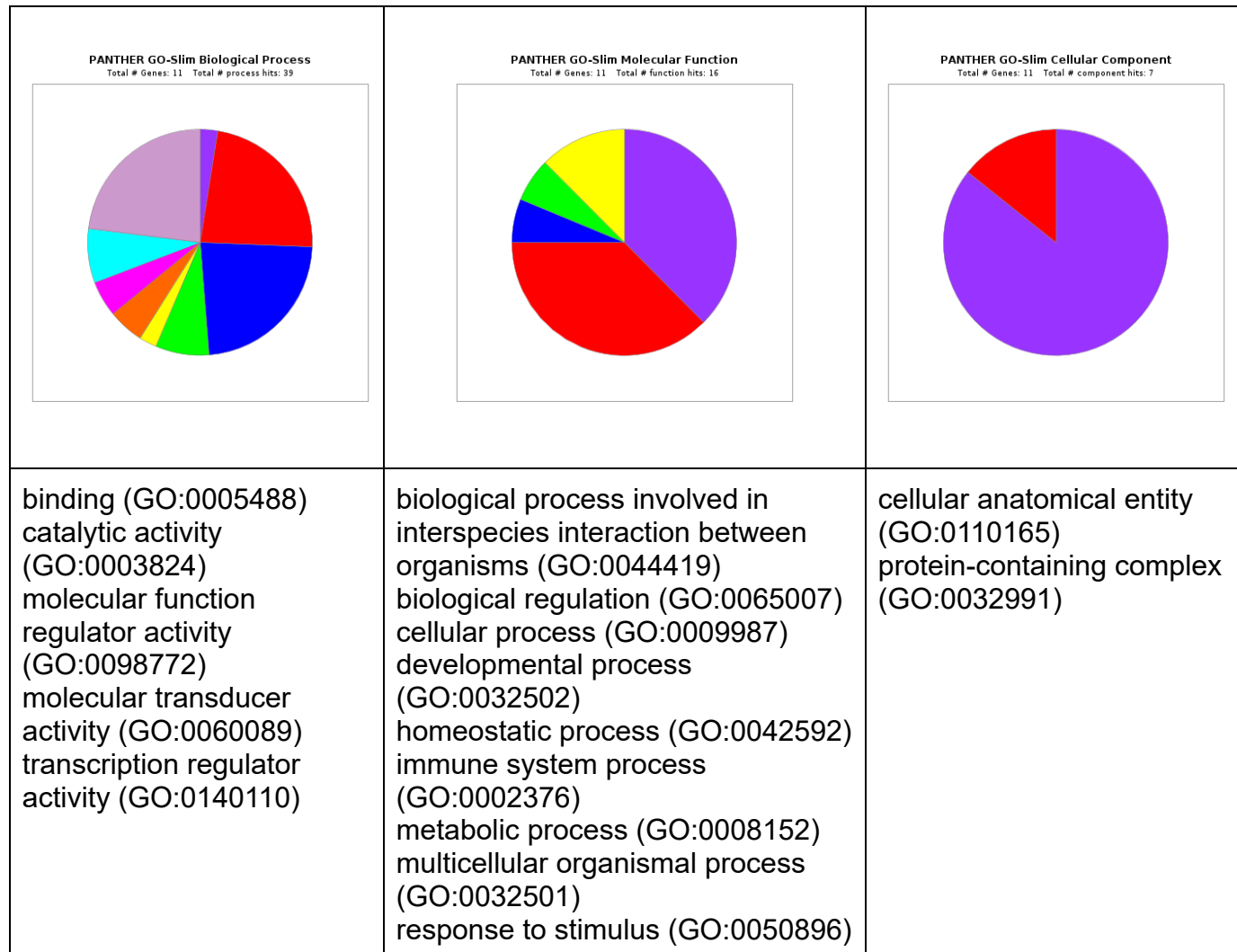


Figure 5.7 Gene Ontology (GO) Analysis for Top 10 hub nodes of Wound Healing

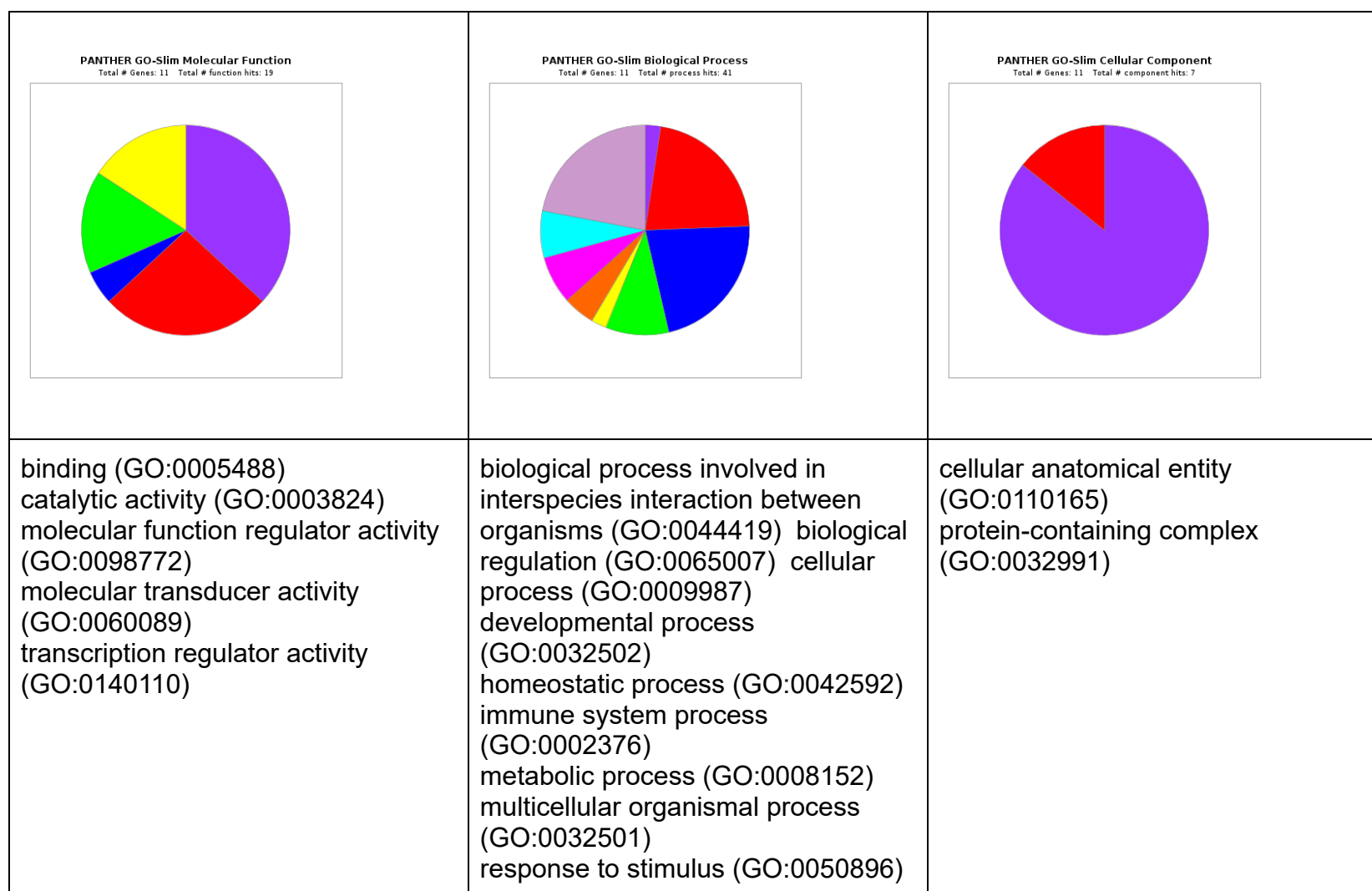
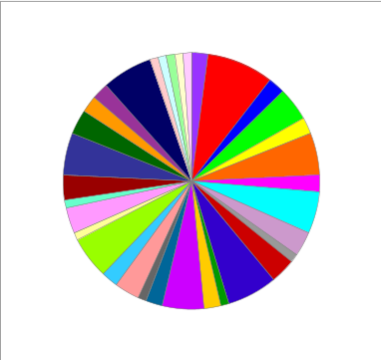
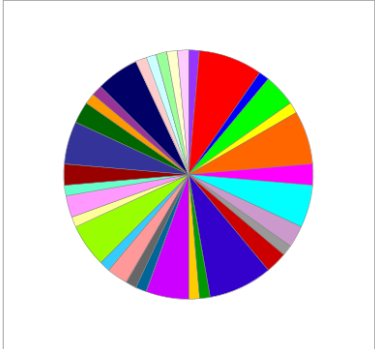


Figure 5.8 Gene Ontology (GO) Analysis for Top 10 hub nodes of Fracture

PANTHER Pathway Total # Genes: 11 Total # pathway hits: 95 	PANTHER Pathway Total # Genes: 11 Total # pathway hits: 72 
• Alzheimer disease-amyloid secretase pathway (P00003) • Angiogenesis (P00005) • Angiotensin II-stimulated signaling through G proteins and beta-arrestin (P05911) • Apoptosis signaling pathway (P00006) • B cell activation (P00010) • CCKR signaling map (P06959) • Cadherin signaling pathway (P00012) • EGF receptor signaling pathway (P00018) • Endothelin signaling pathway (P00019) • FAS signaling pathway (P00020) • FGF signaling pathway (P00021) • Gonadotropin-releasing hormone receptor pathway (P06664)	• Alzheimer disease-amyloid secretase pathway (P00003) • Angiogenesis (P00005) • Angiotensin II-stimulated signaling through G proteins and beta-arrestin (P05911) • Apoptosis signaling pathway (P00006) • B cell activation (P00010) • CCKR signaling map (P06959) • Cadherin signaling pathway (P00012) • EGF receptor signaling pathway (P00018) • Endothelin signaling pathway (P00019) • FAS signaling pathway (P00020) • FGF signaling pathway (P00021) • Gonadotropin-releasing hormone receptor pathway (P06664)

• Huntington disease (P00029) • Hypoxia response via HIF activation (P00030) • Inflammation mediated by chemokine and cytokine signaling pathway (P00031) • Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade (P00032) • Insulin/IGF pathway-protein kinase B signaling cascade (P00033) • Integrin signalling pathway (P00034) • Interferon-gamma signaling pathway (P00035) • Interleukin signaling pathway (P00036) • JAK/STAT signaling pathway (P00038) • PDGF signaling pathway (P00047) • PI3 kinase pathway (P00048) • Parkinson disease (P00049) • Ras Pathway (P04393) • T cell activation (P00053) • TGF-beta signaling pathway (P00052) • Toll receptor signaling pathway (P00054) • VEGF signaling pathway (P00056) • Wnt signaling pathway (P00057) • p38 MAPK pathway (P05918) • p53 pathway by glucose deprivation (P04397) • p53 pathway feedback loops 2 (P04398) • p53 pathway (P00059)	• Huntington disease (P00029) • Hypoxia response via HIF activation (P00030) • Inflammation mediated by chemokine and cytokine signaling pathway (P00031) • Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade (P00032) • Insulin/IGF pathway-protein kinase B signaling cascade (P00033) • Integrin signalling pathway (P00034) • Interferon-gamma signaling pathway (P00035) • Interleukin signaling pathway (P00036) • JAK/STAT signaling pathway (P00038) • PDGF signaling pathway (P00047) • PI3 kinase pathway (P00048) • Parkinson disease (P00049) • Ras Pathway (P04393) • T cell activation (P00053) • TGF-beta signaling pathway (P00052) • Toll receptor signaling pathway (P00054) • VEGF signaling pathway (P00056) • Wnt signaling pathway (P00057) • p38 MAPK pathway (P05918) • p53 pathway by glucose deprivation (P04397) • p53 pathway feedback loops 2 (P04398) • p53 pathway (P00059)
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Figure 5.9 Pathway Analysis for Top 10 hub nodes of Wound Healing and Bone Fracture

5.2 EXTRACTION AND CHARACTERISATION

5.2.1 Plant Extraction - Soxhlation:

The Soxhlet extraction method was utilized to extract rich phytochemicals from the plant materials, including phenols, alkaloids, pigments, carbohydrates, terpenes, and amino acids. After 72 hours of processing, the plant extracts were successfully obtained. Both fine and coarse powder samples of each plant were processed, resulting in cloudy aqueous extracts. The volume of the aqueous extract obtained was 80 mL for *Artocarpus hirsutus* and 50 mL for *Cardiospermum halicacabum*, from an initial volume of 125 mL of water used for extraction.

Freeze-drying is a preferred method for preserving delicate compounds such as phytochemicals, as it prevents thermal degradation and maintains the bioactivity of the extracted substances. The resulting freeze-dried extracts can be further analyzed or utilized for various applications in research, pharmaceuticals, or nutraceuticals, ensuring the retention of the plant's therapeutic properties.

5.2.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

5.2.2.1 *Artocarpus hirsutus*:

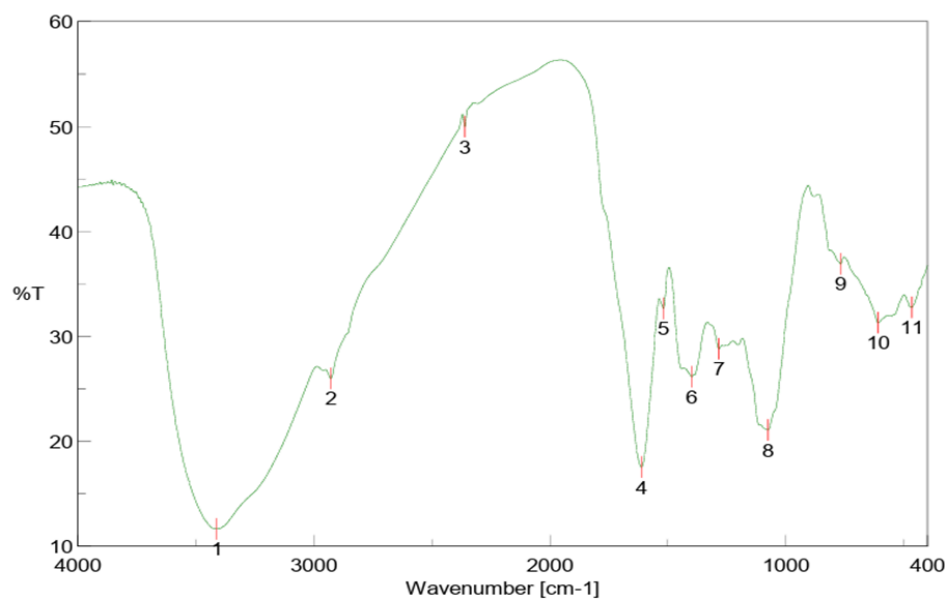


Figure 5.10 FTIR peaks of *Artocarpus hirsutus* extract

Table 5.15 FTIR spectrum of *Artocarpus hirsutus* extract

PEAK NO.	WAVELENGTH	INTENSITY	COMPOUND NAME	BOND VIBRATION
1	3411.46	11.6421	Alcohols, Phenols	O-H stretching
2	2927.41	25.993	Lipids	C-H stretching
3	2360.44	49.9783	Nitriles	(C $\equiv$ N) stretching
4	1612.2	17.5367	Alkenes	C=C stretching
5	1518.67	32.6605	Proteins	C=O stretching
6	1399.1	26.1455	Alkanes	C-H stretching
7	1283.39	28.8127	Ethers	C-O stretching
8	1076.08	21.0752	Ethers	C-O stretching
9	768.494	36.9088	Alkenes	C-H bending
10	609.396	31.3131	-	-
11	466.689	32.7578	Sulphur group	S-S stretching

5.2.2.2 *Cardiospermum halicacabum*:

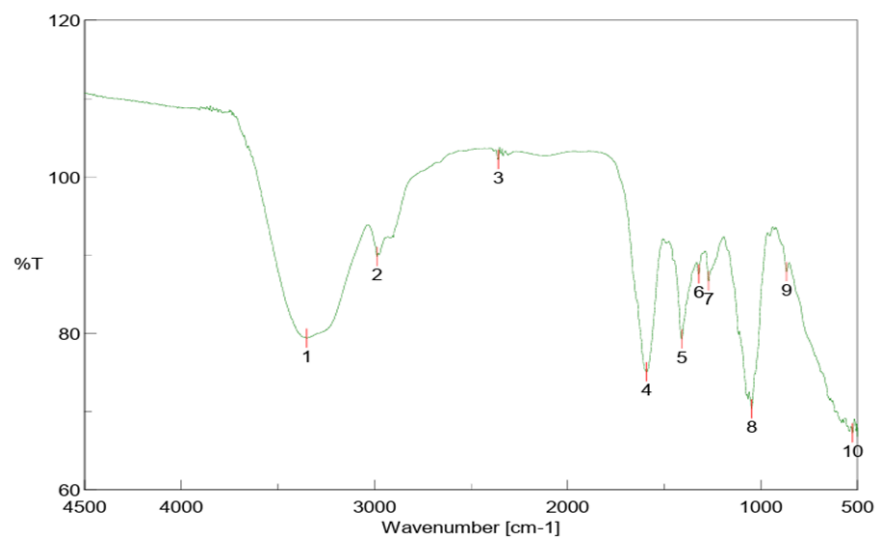


Figure 5.11 FTIR peaks of *Cardiospermum halicacabum* extract

Table 5.16 FTIR spectrum of *Cardiospermum halicacabum* extract

PEAK NO.	WAVELENGTH	INTENSITY	COMPOUND NAME	BOND VIBRATION
1	3350.71	79.3793	Amines	N-H stretching of amines
2	2987.2	89.8226	Lipids	C-H stretching
3	2358.52	102.215	Nitriles	(C≡N) stretching
4	1590.99	75.0865	Proteins	C=O stretching
5	1409.71	79.2787	Sulphur	C=S stretching
6	1321	87.5888	Alkynes	C-H bending
7	1269.9	86.697	Amines	C-N stretching
8	1041.16	70.35	Alcohols, Phenols	C-O stretching
9	866.846	87.8887	CH group	C-H bending
10	525.507	67.2787	Iodo group	C-I stretching of iodo compound

5.3 IN-VITRO STUDIES:

5.3.1 Anti-Microbial Assay

The anti-bacterial activity of *Artocarpus hirsutus* and *Cardiospermum halicacabum* extracts against *Streptococcus* and *Pseudomonas* strains was investigated at varying concentrations. *Artocarpus hirsutus* showed stronger anti-bacterial activity against both strains compared to *Cardiospermum halicacabum*. The inhibition zones increased with higher extract concentrations, indicating dose-dependent efficacy. Both extracts exhibited notable anti-bacterial activity against *Pseudomonas* strains at higher concentrations. These findings suggest the potential of these extracts as natural anti-bacterial agents, warranting further exploration of their bioactive compounds and mechanisms of action.

Table 5.17 Anti-Bacterial Activity on *Streptococcus*

Sample Concentration	<i>Artocarpus hirsutus</i>	<i>Cardiospermum halicacabum</i>
50 µg	3 mm	4 mm
100 µg	9 mm	8 mm
250 µg	10 mm	10 mm
500 µg	12 mm	16 mm

Table 5.18 Anti-Bacterial Activity on *Pseudomonas*

Sample Concentration	<i>Artocarpus hirsutus</i>	<i>Cardiospermum halicacabum</i>
50 µg	0 mm	1 mm
100 µg	4 mm	5 mm
250 µg	9 mm	10 mm
500 µg	10 mm	12 mm

5.3.2 Cytotoxicity Assay:

The cytotoxicity of *Artocarpus hirsutus* and *Cardiospermum halicacabum* extracts was evaluated using the MTT assay against the 3T3 cell line. After incubating cells with the extracts at 100 µg/mL for 24 hours, cell viability was measured. The results showed cell viabilities of 89.5% for *Artocarpus hirsutus* and 85.4% for *Cardiospermum halicacabum*. These findings suggest low cytotoxicity of the extracts against 3T3 cells, indicating their potential for biomedical applications. Further studies are needed to explore safety profiles and mechanisms of cytotoxicity.

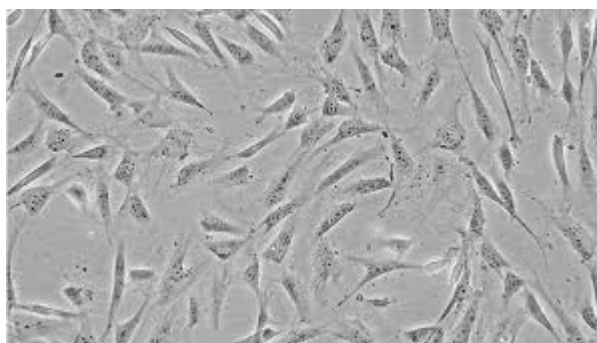
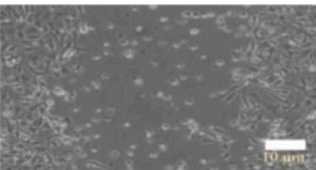
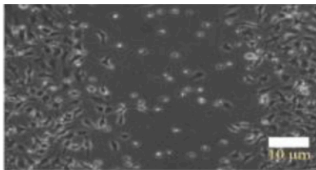
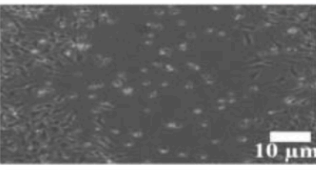
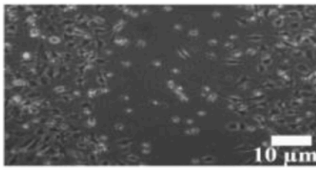
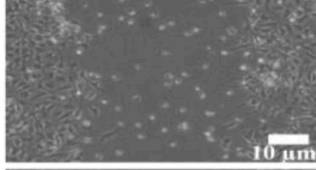
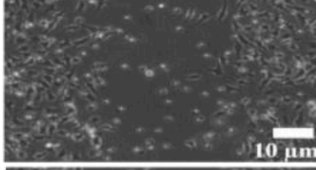
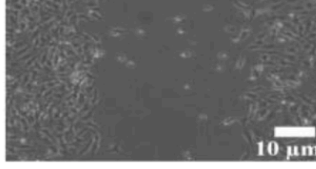
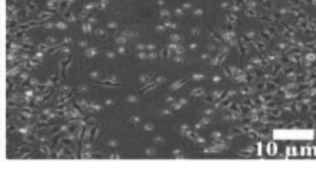


Figure 5.12 3T3 Cell Line

5.3.3 Wound Scratch Assay:

The wound scratch assay performed on the 3T3 fibroblast cell reveals the wound healing capacity of the combined *Artocarpus hirsutus* and *Cardiospermum halicacabum*, which were better when compared to separate plant extracts. In comparison, *Cardiospermum halicacabum* also showed a better wound closure rate when compared to *Artocarpus hirsutus*. Hence, the combination of *Artocarpus hirsutus* and *Cardiospermum halicacabum* plant extracts had the greatest wound healing capacity.

Table 5.19 In-vitro Wound Scratch Assay at 24, 48 and 72 h

Sample	Day 0	Day 1	Day 2
Control			
<i>Artocarpus hirsutus</i>			
<i>Cardiospermum halicacabum</i>			
<i>Artocarpus hirsutus</i> and <i>Cardiospermum halicacabum</i>			

5.4 IN-VIVO STUDIES

5.4.1 Zebrafish Embryo Toxicity Test

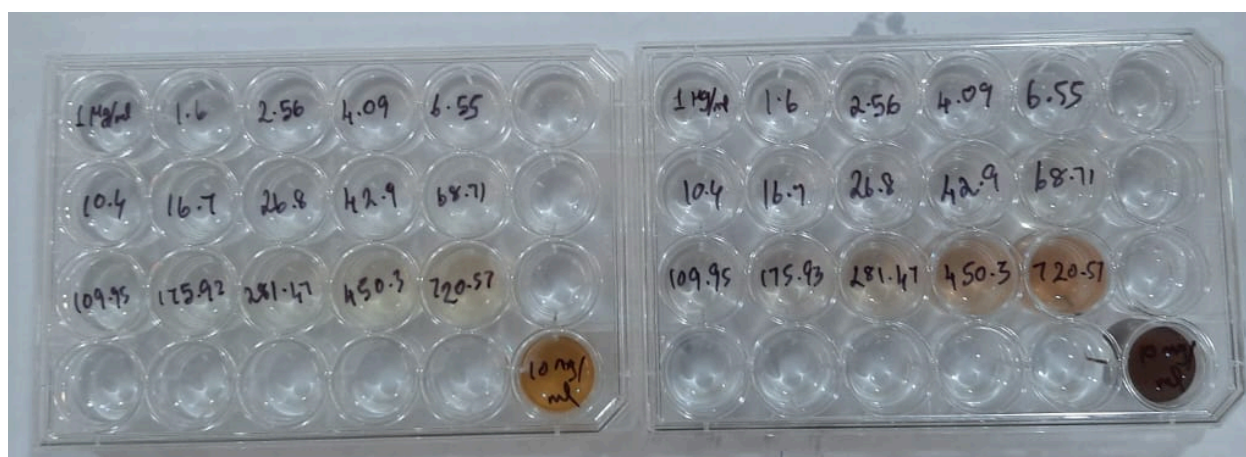
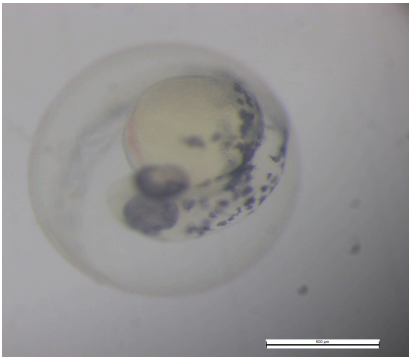
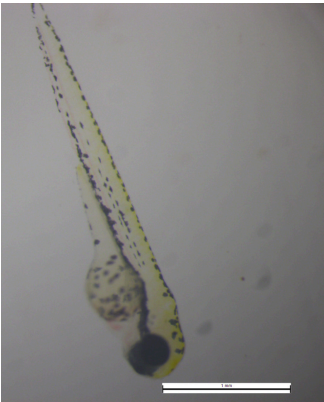
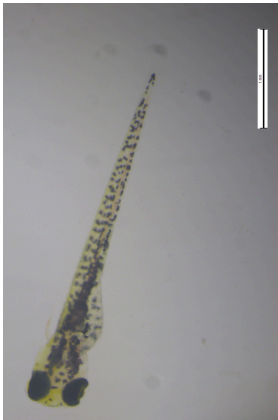
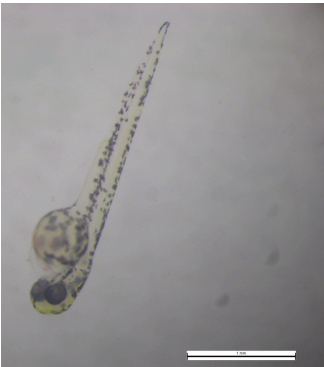
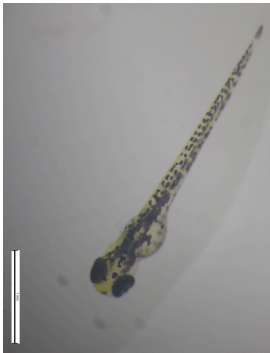


Figure 5.13 Treatment of zebrafish embryos with various concentrations of *Artocarpus hirsutus* and *Cardiospermum halicacabum*

For *Artocarpus hirsutus*, There are no significant changes in the morphology of the untreated embryos (control) and treated ones with concentrations below 200 µg/mL (1, 1.6, 2.56, 4.09, 6.55, 10.4, 16.7, 26.8, 42.9, 68.71, 109.95, 175.92 µg/mL). All embryos treated with concentrations greater than 200 µg/mL (280, 450, 720 µg/mL and 10 mg/mL) resulted in death on the second day of the incubation.

For *Cardiospermum halicacabum*, There are no significant changes in the morphology of the untreated embryos (control) and treated ones with concentrations below 100 µg/mL (1, 1.6, 2.56, 4.09, 6.55, 10.4, 16.7, 26.8, 42.9, 68.71 µg/mL). The growth of all the embryos treated in concentrations between 100 and 200 µg/mL (109.95, 175.92 µg/mL) was slow compared to those treated in concentrations below 100 µg/mL. All embryos treated with concentrations greater than 200 µg/mL (280, 450, 720 µg/mL and 10 mg/mL) resulted in death on the second day of the incubation.

Table 5.20 Embryo morphology at 24, 48 and 72 h at different concentrations extracts of *Artocarpus hirsutus*

Sample concentration	Day 1	Day 2	Day 3
0 (Control - E3 Medium)			
1 µg/mL - 200 µg/mL			

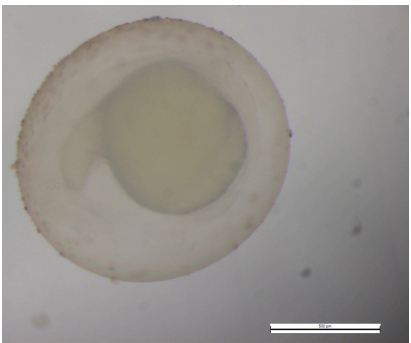
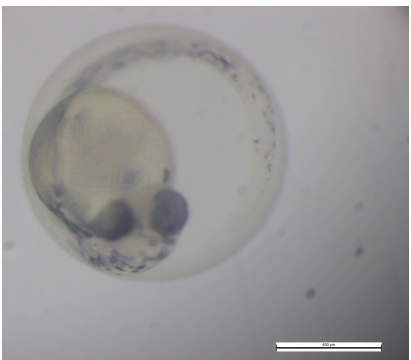
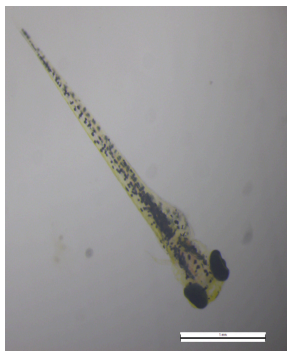
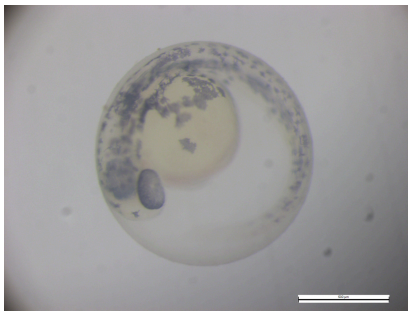
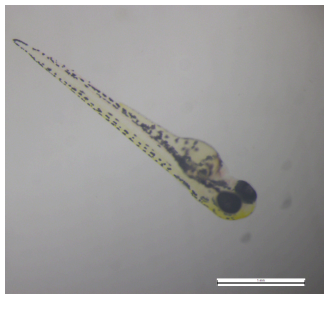
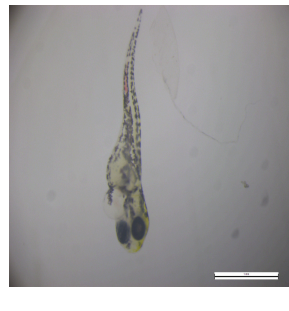
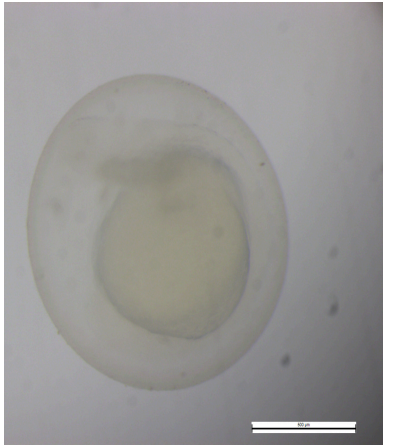
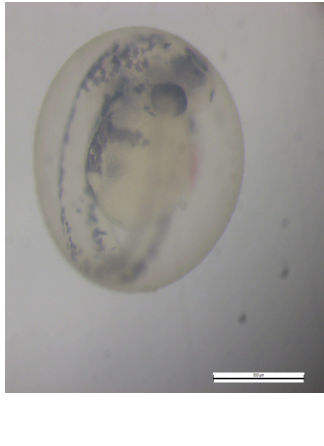
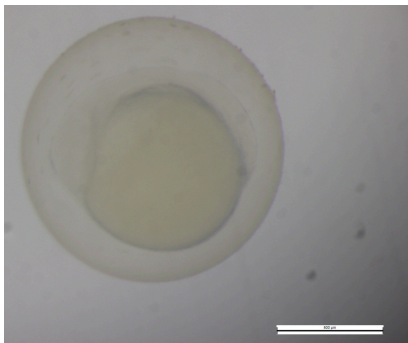
200 µg/mL - 1 mg/mL and 10 mg/mL (Maximum concentration)		-	-
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Table 5.21 Embryo morphology at 24, 48 and 72 h at different concentrations extracts of *Cardiospermum halicacabum*

Sample concentration	Day 1	Day 2	Day 3
0 (Control - E3 Medium)			

1 $\mu\text{g/mL}$ - 100 $\mu\text{g/mL}$			
100 $\mu\text{g/mL}$ - 200 $\mu\text{g/mL}$			
200 $\mu\text{g/mL}$ - 1 mg/mL and 10 mg/mL (Maximum concentration)		-	-

5.4.2 Caudal Fin Regenerative Capacity Test:

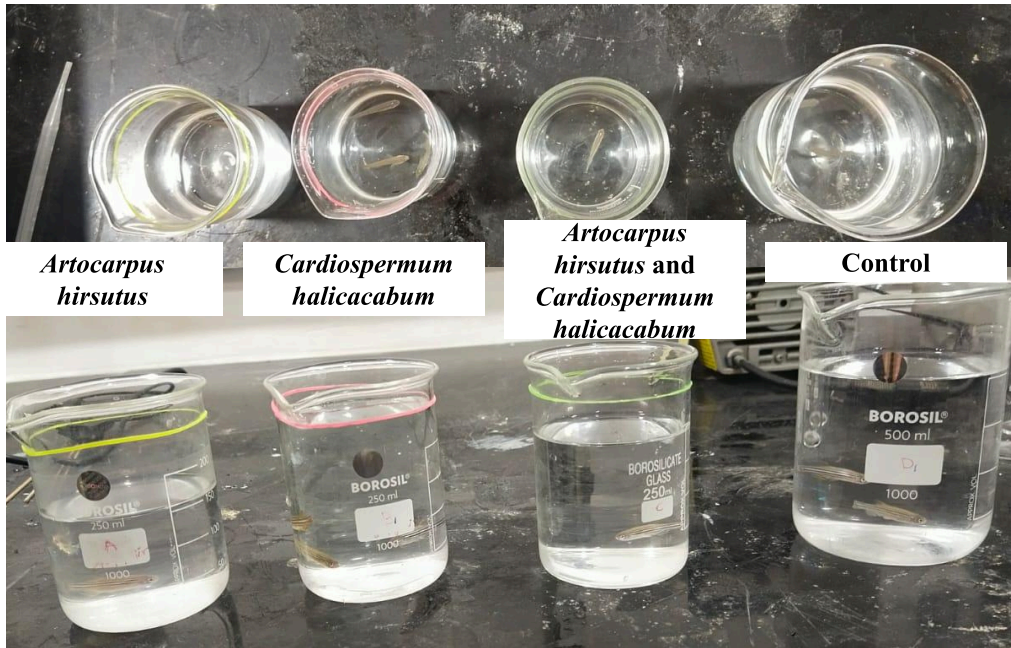


Figure 5.14 Caudal Fin Regenerative Capacity Test



Figure 5.15 Amputation of the Zebrafish

Table 5.22 Regeneration area of Zebrafish caudal fin treated and untreated

Days	<i>Artocarpus hirsutus</i>	<i>Cardiospermum halicacabum</i>	<i>Artocarpus hirsutus</i> and <i>Cardiospermum halicacabum</i>	Control
0 (Before Sectioning)	3.5	3.5	3.7	3.6
0 (After Sectioning)	3	3	3.2	3.1
3	4.4	4.4	4.1	3.7
6	7.7	6.3	6.9	7.2
9	9	6.9	8.3	7.8

The regenerative capacity test assessed the ability of zebrafish caudal fins to regenerate after amputation, with the administration of extracts from *Artocarpus hirsutus* and *Cardiospermum halicacabum* together and separately. Before sectioning, both groups had similar baseline measurements, indicating that the initial size of the caudal fins was comparable.

After sectioning, there was a slight decrease in fin area for both groups, which is expected due to the amputation. For *Artocarpus hirsutus*, The regenerative capacity of treated zebrafish showed a gradual increase in fin area over time. By day 9, the regenerative area reached its peak at 9 mm², indicating significant regeneration potential.

For *Cardiospermum halicacabum*, Treated zebrafish also exhibited an increase in fin area over time although the rate of regeneration appeared to be slightly lower compared to *Artocarpus hirsutus*. By day 9, the regenerative area reached 6.9 mm². The regenerative capacity of zebrafish treated with *Artocarpus hirsutus* extract seemed to be slightly higher compared to those treated with *Cardiospermum halicacabum* extract, as evidenced by the larger regenerative areas observed over the course of the experiment.

CHAPTER 6

SUMMARY AND CONCLUSION

The study investigated the extraction, characterization, and biological activities of phytochemical extracts from two plants, namely *Artocarpus hirsutus* and *Cardiospermum halicacabum*. Soxhlet extraction was employed to obtain the phytochemical-rich extracts, which were subsequently freeze-dried to preserve their bioactivity. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed the presence of various compounds in the extracts, including alcohols, lipids, nitriles, proteins, and others.

In-vitro studies assessed the antimicrobial and cytotoxic properties of the extracts. Both extracts demonstrated notable antimicrobial activity against *Streptococcus* and *Pseudomonas* strains, with *Artocarpus hirsutus* showing stronger efficacy. Moreover, the extracts exhibited low cytotoxicity against 3T3 fibroblast cells, indicating their potential for biomedical applications.

Wound healing potential was evaluated using the wound scratch assay, demonstrating that a combination of both plant extracts had the greatest wound healing capacity compared to individual extracts. In-vivo studies conducted on zebrafish embryos assessed the toxicity and regenerative capacity of the extracts. While both extracts showed concentration-dependent toxicity, they also promoted the regeneration of zebrafish caudal fins, with *Artocarpus hirsutus* exhibiting slightly higher regenerative capacity compared to *Cardiospermum halicacabum*.

The findings of this study highlight the promising therapeutic potential of *Artocarpus hirsutus* and *Cardiospermum halicacabum* extracts. Their demonstrated antimicrobial activity suggests their possible use as natural antimicrobial agents, potentially contributing to the development of new antibiotics. Moreover, their low cytotoxicity and wound healing properties make them suitable candidates for biomedical applications, particularly in the development of wound care products.

The regenerative properties observed in zebrafish suggest that these extracts may also have applications in tissue regeneration and wound healing in mammalian systems. However, further research is needed to elucidate the underlying mechanisms of action and to assess their safety and efficacy in clinical settings. Overall, this study provides valuable insights into the pharmacological potential of *Artocarpus hirsutus* and *Cardiospermum halicacabum* extracts, paving the way for future research and development of novel therapeutics derived from natural sources.

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ANNEXURE

GATE Score Card:



GRADUATE APTITUDE TEST IN ENGINEERING 2024

अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४

ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU

SCORE CARD

Name of the Candidate RIZWAN AHAMED N			
Name of the Parent/Guardian NAJIMUDEEN P			
Registration No.		BT24S57406185	
Test Paper Biotechnology (BT)			
Date of Examination		February 10, 2024	
GATE Score	312	Marks out of 100	36.33
All India Rank (AIR) in the test paper	3618	Qualifying Marks General 38.9 EWS/OBC-NCL 35.0 SC/ST/PwD 25.9	
Number of candidates appeared for the test paper	17078		



Prof. Chandra Sekhar Seelamantula
Organising Chairperson, GATE 2024
On behalf of NCB-GATE
Ministry of Education (MoE)



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A candidate is considered **qualified** if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.

This Score Card is valid up to 31st March 2027.

GATE SCORE COMPUTATION

The GATE 2024 score is calculated using the formula

$$\text{GATE Score} = S_q + (S_i - S_q) \frac{(M - M_q)}{(M_i - M_q)}$$

where

M is the marks obtained by the candidate in the paper mentioned on the GATE 2024 Score Card

M_q is the qualifying marks for general category candidates in the paper

M_i is the mean of marks of top 0.1% or top 10 (whichever is larger) of all the candidates who appeared for the test paper

S_q = 350, is the score assigned to M_q

S_i = 900, is the score assigned to M_i

M_q is 25 marks (out of 100) or μ + σ, whichever is greater. Here, μ is the mean and σ is the standard deviation of marks of all the candidates who appeared for the test paper.

Qualifying in GATE 2024 does not guarantee admission to a postgraduate program or scholarship/financial assistance. Admitting institutes may conduct additional tests or interviews for final selection of candidates.

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Conspectus on nanodiagnostics as an incipient platform for detection of oral potentially malignant disorders and oral squamous cell carcinoma

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One of the most common causes of death around the globe is cancer. One of the most prevalent forms of cancer is the cancer of the oral cavity and oropharynx known as oral squamous cell carcinoma (OSCC). The susceptibility to various viruses, smoking, overindulgence in tobacco, alcohol consumption, occupational exposure, increased sun exposure, and nutritional deficiencies resulting from low socioeconomic status are the preponderant risk factors which lead to genetic and epigenetic transmutation^[1,2]. OSCC potentially spread quickly and the initial stage is referred to as the oral potentially malignant disorder (OPMD). The OPMD symptoms are white or red lesions in the mouth, paresthesia, trismus or jaw hypomobility gradually pronounce into severe vocal changes and oral ulcers which are basically nontreatable^[3]. This illness has the potential to spread quickly, and it can frequently invade nearby tissues^[4]. Since the OPMD indications were usually staved off, the OSCC becomes life-threatening and is thus associated with high morbidity rates. Hence, early detection of the very early OPMD stage is very critical in order to improve the health benefits of the patient and curb the prognosis of the disease. This article provides a brief summary of diagnostic strategies in the detection of oral cancer especially using nanotechnology, and the current trends and future prospects in nanodiagnostics.

There are numerous tests available to diagnose oral and oropharyngeal cancer. Common techniques for diagnosing OSCC are

endoscopy, oral brush biopsy, human papillomavirus testing, positron emission tomography scan, ultrasound, MRI, computed tomography, and radiography. Emerging detection techniques like nanodiagnostics provide advantages over the contrast agents used in modern imaging. Nanoparticles (NPs) will target specific surface molecules, are simpler to synthesize, and are more biocompatible than larger particles. Nanodiagnostics have exceptional characteristics like their size, high mechanical strength, shape, and surface. Nanomaterial-based sensors are significantly more sensitive than normal sensors. They also offer real-time tracking^[5], surface-enhanced Raman spectroscopy, MRI, optical coherence tomography, nano-based ultrasensitive biomarker detection, Quantum Dots (QDs) imaging, photoacoustic imaging, diffusion reflection imaging, and surface plasmon resonance scattering are some of the implementations of nano-based diagnostics^[6]. The NPs currently used in oral cancer diagnosis are listed in Table 1.

Numerous developments have been made in the field of diagnostics as an outcome of the urgent need for sensitive, precise, strong, and quick diagnosis of oral cancer^[15,16]. Recent developments in the field of nanotechnology offer the necessary incentive for nanobiosensor technology development^[17]. Nanobiosensing techniques for OSCC detection are substrate selection, surface cleaning, surface enhancement by incorporation of NPs, surface enhancement by polymers, surface functionalization, biorecognition probe, and its immobilization (Fig. 1)^[18]. In order to diagnose oral cancer, artificial intelligence is applied, such as the detection of precancerous as benign and postcancerous as malignant regions using automated deep learning CNN (Convolutional Neural Network) technique^[19]. CNN is a deep learning algorithm. The major purpose of CNN is that it automatically identifies desired features and provide image recognition, with no human assistance. For the screening of oral cancer, deep CNNs can be a practical way to create low-cost embedded vision systems with constrained memory and processing power^[20]. A significant advancement in recent years is the biosensor based on QDs that help with the diagnosis of some oral cancer biomarkers^[21]. By conjugating QDs with complements of molecular biomarkers, multifunctional NP probes have been developed. QD probes can be often used to determine the molecular biomarker expression in oral cancer cells.

It is now evident that the NPs have demonstrated great promise and success in fundamentally altering the strategy for detecting oral cancer. Also, we sought to discuss key aspects of NP synthesis such as design criteria, cutting-edge experimental techniques, and NP platforms for OSCC diagnosis. The use of unique molecular markers will aid in the future development of active targeting magnetic NPs to significantly enhance tumor localization and diagnosis.

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A commentary on “Potential of nanoemulsions for accelerated wound healing: innovative strategies”

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NETWORK PHARMACOLOGY APPROACH FOR INVESTIGATING THE
PHYTOCOMPOUNDS OF ARTOCARPUS HIRSUTUS & CARDIOSPERMUM
HALICACABUM FOR TREATING WOUND HEALING AND BONE FRACTURE

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ABSTRACT

The exploration of natural compounds for medicinal purposes has long been an integral aspect of pharmacological research. In this study, we investigate the potential therapeutic properties of phytochemicals derived from two traditional plants, *Artocarpus hirsutus* and *Cardiospermum halicacabum*, in the context of wound healing and bone fracture. These plants have a rich history of traditional use and contain diverse phytochemical profiles, making them promising candidates for pharmacological investigation. Wound healing and bone fracture represent critical processes in human health, yet current treatments often face limitations, warranting exploration of alternative therapeutic approaches. Through a network pharmacology approach, we aim to elucidate the mechanisms underlying the efficacy of phytochemicals from *Artocarpus hirsutus* and *Cardiospermum halicacabum* in promoting wound healing and bone fracture. This study employs databases and bioinformatics tools to construct an extensive model aimed at examining the interplay between phytochemicals and key biological targets associated with wound healing and bone fracture. Information on various phytochemicals and their target areas was gathered from diverse sources. We then delved into potential mechanisms by analysing KEGG pathway enrichment and GO biological processes. Molecular docking was employed in order to assess the interaction of key targets to validate network pharmacology findings. Our findings shed light on the multifaceted mechanisms through which phytochemicals from *Artocarpus hirsutus* and *Cardiospermum halicacabum* exert their therapeutic effects on wound healing and bone fracture. This study contributes to the growing body of evidence supporting the use of natural compounds as alternative or complementary therapies for wound healing and bone disorders. Furthermore, Experimental and clinical studies are necessary to validate these in-silico findings, potentially leading to the development of novel pharmacological interventions aimed at enhancing wound healing and bone development processes.

Keywords: Network Pharmacology, Traditional Medicine, Wound Healing, Bone Fracture Therapy, Drug Discovery.



COMPUTATIONAL MODELLING ON PROTEIN ADSORPTION DYNAMICS FOR
ENHANCED BONE TISSUE ENGINEERING

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ABSTRACT

This study investigates and delves into the intricate dynamics of protein adsorption on biomaterial surfaces to advance strategies for enhancing bone tissue engineering. Protein adsorption is a critical process that influences the overall performance and efficacy of biomaterials in various tissue regeneration applications. To gain a comprehensive understanding of the adsorption capacity and intensity under different conditions, the researchers meticulously analysed experimental data collected from a diverse range of protocols. These data were then subjected to rigorous examination to discern the impacts of different conditions on the adsorption process representing experimental data and establish robust isotherm models such as the Langmuir model, representing monolayer adsorption, and the Freundlich model, describing heterogeneous systems. These models were fitted to the experimental data using advanced nonlinear regression techniques implemented in the programming language R. Furthermore, statistical analysis was conducted to detect any significant variations in adsorption behaviour across distinct experimental conditions. Molecular dynamics simulations and computational analysis techniques, we examine the interactions between proteins and biomaterial surfaces to elucidate the underlying mechanisms governing adsorption dynamics. The research focuses on elucidating how factors such as surface chemistry, and topography influence protein adsorption behaviour. By employing computational methods, the research also strives to quantify the rates at which proteins adsorb onto biomaterial surfaces, all the while endeavouring to understand the fundamental mechanisms that govern these intricate interactions. The findings from this computational analysis contribute to a deeper understanding of protein-biomaterial interactions, paving the way for the design and optimisation of innovative biomaterials tailored for enhanced bone tissue regeneration.

Keywords: Protein adsorption, Biomaterial, Bone tissue engineering, Protein – biomaterial interactions, Computational modelling



KINETIC PARAMETER DETERMINATION USING VISUALISATION OF ENZYME
KINETIC GRAPHS IN R AND PYTHON PROGRAMMING

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ABSTRACT

This study employs Python and R programming languages to analyse enzyme kinetics with kinetics plots such as the Michaelis-Menten Plot, Eadie-Hofstee plot, Lineweaver-Burk plot and Hanes-Woolf plot. Kinetic data, including, Vmax, Km, and [S], is collected and processed. Graphical representations of the kinetic plots are obtained for the required enzymes. This study has various applications in the fields of biochemical, pharmaceutical, therapeutic, education and research. The required libraries are installed for both Python and the R programming language. The equations for every plot are applied to calculate reaction velocities and substrate concentration. The accurate inputs for the plots are given based on the programmed x and y axis respectively. The resulting enzyme kinetics data is saved in an Excel file. Python and R scripts generate various plots, and linear regression models extract kinetic parameters. Michaelis-Menten plots, Eadie-Hofstee plots, Hanes-Woolf plots, and Lineweaver-Burk plots are presented, demonstrating the visual representation of enzyme kinetics in both programming languages. The outputs obtained are stored separately for both languages in the Excel file. The results include the y-intercept, x-intercept, and slope which are interpreted to obtain the Vmax and Km values from respective plots. Kinetic parameters obtained from Python and R show close agreement. Python and R are effective tools for enzyme kinetics analysis, providing consistent and reliable results. The study showcases the applicability of programming languages in biochemical research of enzymes, streamlining data processing and analysis while reducing manual errors and labour.

Keywords: Enzyme kinetics, Python programming language, R programming language, Michaelis-Menten plot, Eadie-Hofstee plot, Lineweaver-Burk plot, Hanes-Woolf plot



EXPLORING THE THERAPEUTIC NEXUS IN NETWORK PHARMACOLOGY OF SELECTED PHYTOSTEROLS IN WOUND HEALING AND BONE FRACTURE

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ABSTRACT

This study delves into the multifaceted roles of phytosterols, plant-derived compounds that share structural similarities with cholesterol, in the realms of wound healing and bone fracture repair. Saturated phytosterols demonstrate superior efficacy in reducing cholesterol levels compared to their unsaturated counterparts. Various phytosterols, including Beta-Sitosterol, Stigmasterol, and Campesterol, have garnered attention for their potential cardiovascular benefits, leading to their incorporation into functional foods and dietary supplements. Phytosterols emerge as potential facilitators of bone healing by modulating inflammation and promoting osteogenic activity. Additionally, Phytosterols show promise in enhancing bone mineralization and influencing cellular processes vital for bone formation and repair. This study aims to validate the efficacy of commonly encountered phytosterols in the treatment of wound healing and bone fracture repair utilizing databases and bioinformatics tools to establish a comprehensive model with the intention of scrutinizing the intricate relationship between phytosterols and pivotal biological targets affiliated with the process of wound healing and bone fracture. Information regarding diverse phytosterols and their respective target areas was collected from an array of sources. Subsequently, we thoroughly examined potential mechanisms by scrutinizing KEGG pathway enrichment and GO biological processes. The utilization of molecular docking enabled us to evaluate the interaction of targets, thereby validating the findings of network pharmacology. The findings contribute valuable insights into the therapeutic potential of these plant-derived compounds, shedding light on their applications in promoting wound healing, and bone regeneration. Further research is essential to unravel specific mechanisms and determine optimal concentrations for effective wound and fracture management.

Keywords: Network Pharmacology, Phytosterols, Wound Healing, Bone Fracture Therapy, Drug Discovery.



EFFECT OF Capparis decidua - GHEE COMPOSITE IN FRACTURE BONE HEALING

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Abstract

Scaffolds are the substrate for cells which acts as the models in tissue regeneration. Biopolymer scaffolds have been well - studied and allowed many scientists to develop the fields of biomedicine and nanomedicine and be employed in a variety of tissue engineering applications. Different biomaterial combinations are available to construct biomaterials based scaffolds. Several scaffolds have been developed by combining natural biopolymers with synthetic polymers. Those scaffolds are employed in the tissue regeneration of bones, damaged blood vessels, ligaments, cartilage, urethral and neural. Thus, incorporating ethnomedicinal plant extracts in the existing biopolymer scaffolds hastens damaged tissue repair and regeneration. In the field of bone tissue engineering, the design of effective tissue - compatible plant - based composite scaffolds is essential due to their lesser toxicity effects, biocompatibility, cost - effectiveness, physical stability and biodegradability. From the traditional references and literature, Capparis decidua has many pharmacological features. The plant stems help to enhance the healing of fractured bone. Also, Ghee possesses some healing properties and promotes healing in other formulations. The purpose of the present work was to develop various herbal - based biopolymer scaffolds incorporating the medicinal plant Capparis decidua with Ghee as a carrying agent and establish the effectiveness of this Capparis decidua - Ghee biopolymer composite in fractured bone healing.

Keywords: Traditional medicine, tissue engineering, Bone regeneration, Capparis decidua, Ghee, Scaffolds

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