MAKERERE UNIVERSITY

DEVELOPMENT OF A BIOACTIVE GAUZE DRESSING FUNCTIONALIZED WITH HERB-LOADED NANOPARTICLES TO FACILITATE WOUND HEALING

BY

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DECLARATION

I, **Namuga Catherine**, declare that this thesis is my original work and has not been submitted to any other university or institution for the award of any degree or academic qualification. Any work of others that has been used in this thesis has been duly acknowledged and appropriately referenced.

Namuga Catherine

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Date: ……………………………………………..

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LIST OF ABBREVIATIONS

AFM: Atomic Force Microscope

AHL: N-Acyl Homoserine Lactone

ANOVA: Analysis Of Variance

ARRIVE: Animal Research Reporting of In vivo Experiments

ASTM: American Society for Testing and Materials

ATR: Attenuated Total Reflectance

ATCC: American Association of Textile Chemists and Colourists

CEDAT: College of Engineering, Art, Design, and Technology

COVAB: College of Veterinary Medicine, Animal Resources and Biosecurity

DLS: Dynamic Light Scattering

DMSO: Dimethyl Sulphoxide

DPPH: 2, 2-diphenyl-1-picrylhydrazyl

DGAL: Directorate of Government Analytical Laboratory

EPS: Extracellular Polymeric Substance

ECM: Extracellular Matrix

FTIR: Fourier Transform Infrared

FRAP: Ferric Reducing Assay

FWHM: Full Width with the Half-Maximum

GC-MS: Gas Chromatography-Mass spectrometry

HSD: Honestly Significant Difference

ICH: International Council for Harmonisation

IRB: Institutional Review Board

LC-MS: Liquid Chromatography-Mass spectrometry

MBEC: Minimum Biofilm Eradication Concentration

MBIC: Minimum Biofilm Inhibition Concentration

MDR: Multi-Drug Resistant

MHB: Mueller Hinton Broth

MIC: Minimum Inhibitory Concentration

MRM: Multiple Reaction Monitoring

MRSA: Methicillin-Resistant Staphylococcus Aureus

MTP: Microtiter Plate

MSCRAMM: Microbial Surface Components Recognising Adhesive Matrix Molecules

NCRI: Natural Chemotherapeutic Research Institute

NDP: National Development Plan

NIST: National Institute of Standards and Technology

OECD: Organisation for Economic Co-operation and Development

ORAC: Oxygen Radical Absorbance Capacity

PBS: Phosphate Buffered Saline

PDI: Polydispersity Index

PII: Primary Irritation Index

RSM: Response Surface Methodology

ROS: Reactive Oxygen Species

SDG: Sustainable Development Goals

SEM: Scanning Electron Microscopy

SVARREC: School of Veterinary and Animal Resources Research Ethics Committee

TEAC: Trolox Equivalent Antioxidant Capacity

TEM: Transmission Electron Microscopy

T-PACC: Textile Protection and Comfort Centre

TRAP: Total Peroxyl Radical-Trapping Antioxidant Parameter

TOSC: Total Oxyradical Scavenging Capacity

UIRI: Uganda Industrial Research Institute.

UNCST: Uganda National Council of Science and Technology

UV: Ultraviolet

UV-Vis: Ultraviolet Visible

VRE: Vancomycin-Resistant Enterococci

WHO: World Health Organisation

XRD: X-ray Diffraction

XPS: X-ray Photonic Spectroscopy

LIST OF PUBLICATIONS

1. Namuga, C., Muwonge, H., Nasifu, K., Sekandi, P., Sekulima, T., & Kirabira, J. B. (2024). Hoslundia opposita vahl; a potential source of bioactive compounds with antioxidant and antibiofilm activity for wound healing. *BMC Complementary Medicine and Therapies*, *24*(1), 236. https://doi.org/10.1186/s12906-024-04540-z.
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3. Namuga, C., Muwonge, H., Lubwama, M., Janet, N., Sekulima, T., & Kirabira, J. B. (2022). Antibacterial activities of Bidens pilosa L, Hoslundia opposita Vahl, and Ageratum conyzoides L against some common wound pathogens. *African Journal of Pharmacy and Pharmacology*, *16*(5), 64-78 https://doi.org/10.5897/AJPP2022.5296.
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ABSTRACT

Wound healing is crucial in healthcare due to its significant physiological and economic impacts on patients. Among wound dressings, gauze is the most widely used; however, it is associated with a high risk of bacterial wound infections that delay healing. Hence, enhancing its effectiveness remains critical. In Uganda, herbal medicines are still utilised in the treatment of wounds and other illnesses; however, they require high dosages and prolonged treatment, leading to poor patient compliance. Nanoencapsulation offers a promising solution by improving the drug's therapeutic effect through sustained release while minimising toxicity and dosage. The main objective of this study was to develop and evaluate a gauze dressing functionalized with herb-loaded chitosan nanoparticles for enhanced wound healing. This study explored the extraction of selected medicinal herbs (*Bidens pilosa* L., *Ageratum conyzoides* L., and *Hoslundia opposita* Vahl) using different methods and solvents with varying polarity. The extraction yield, in vitro antibacterial (on *Staphylococcus aureus/Methicillin-Resistant Staphylococcus Aureus (MRSA), Enterococcus faecalis, Escherichia coli, Klebsiella Pneumoniae, and Pseudomonas aeruginosa*), antibiofilm, and antioxidant activities, as well as phytochemicals present, were determined. The most efficacious extract was subjected to GC-MS and LC-MS analysis. It was nanoencapsulated in chitosan nanoparticles via the ionic gelation method, and optimisation was performed using Response Surface Methodology (RSM). The herb-loaded nanoparticles were evaluated for in vitro antibacterial activity and incorporated into the gauze dressing. Their in vitro antibacterial activity, in vivo wound healing in Wistar rats, water absorption, and retention capacity, as well as in vivo skin irritation in rabbits, were tested. High extraction yields were obtained for samples extracted with highly polar solvents. The methanol (100%) extract of *H. opposita* extracted by maceration, displayed better bioactivity (antibacterial, antibiofilm and antioxidant). The biological activity of the plants was attributed to the presence of various phytochemicals. The herb nanoparticles obtained were spherical with size 212 nm, zeta potential 40 mV, Polydispersity Index (PDI) 0.22, encapsulation efficiency 79.1% and loading capacity 9.82 %. Their in vitro drug-release profile showed sustained release of 50% over 24 hours, compared with 100% for the free extract. They exhibited enhanced antibacterial activity with minimum inhibitory concentrations (1.875 to 3.275 mg/mL) against *S. aureus, MRSA, E. faecalis, E. coli, K. pneumoniae,* and *P. aeruginosa*. Gauze incorporated with nanoparticles exhibited no antibacterial activity but significantly accelerated wound healing, achieving 93% wound closure by day 18 compared with 41% in untreated gauze. The dressing also exhibited improved water-holding capacity; no skin irritation was observed in rabbits, and its water absorption capacity remained unaltered. Extract fractionation identified rutin and 4^{111}-acetylvitexin-2^{11}-O-rhamnoside as the possible major bioactive compounds in the methanol extract of *H. opposita.* In conclusion, integrating the *H. opposita* extract-loaded chitosan nanoparticles into gauze significantly enhanced wound healing, offering a promising advancement in wound care technology.

Keywords: *Wound healing, gauze dressing, herbal medicine, chitosan nanocarriers, nanoencapsulation, antibacterial activity, phytochemical analysis.*