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Biological properties and safety profile of extracts from locally grown banana leaves in Arusha, Tanzania

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BIOLOGICAL PROPERTIES AND SAFETY PROFILE OF EXTRACTS FROM LOCALLY GROWN BANANA LEAVES IN ARUSHA, TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The current study assessed the biological properties and safety profile of extracts from locally grown banana leaves in Arusha (Tanzania) to affirm their possible use for wound dressing. Phytoconstituents screening from studied banana plant species, *ijihu inkundu (IJ), mlelembo (ML),* and *kimalindi (KIM),* revealed the presence of secondary metabolites: *anthraquinones, alkaloids, flavonoids, tannins, terpenoids, phenols*, *phytosterol, and saponins*. Susceptibility of microorganisms to studied banana varieties were in the order of KIM > ML > IJ. One-way analysis of variance (ANOVA) revealed a statistical difference of mean among all extracts ($p < 0.05$). Moreover, results also revealed that all tested organisms were susceptible to the studied banana extracts, and their susceptibility was in the order of *C. albicans* > *C. neoformans* > *S. aureus* > *S. typhi* >*E. coli* > *P. aeruginosa.* These results suggest that the studied leaves, especially *kimalindi*, may be used to dress wounds involving fungal infections (*C. albicans* and *C. neoformans*) and bacterial infections (*S. aureus*), all of which are common wound infections. Antioxidant activity was evaluated by measuring the ability of extracts to scavenge the 2, 2-diphenylpicrylhydrazyl (DPPH) free radical. Results revealed that the scavenging of DPPH free radical was in the order of *kimalindi*> *ijihu inkundu*> *mlelembo,* these results also affirming that *kimalindi* extract had better scavenging of DPPH radical, and hence presents better antioxidant activity. Brine shrimp results for toxicity showed that almost all banana leaves extracts were practically non-toxic to the shrimps, exhibiting mild toxicity by giving the LC_{50} values higher than 100 µg/mL.

DECLARATION

I, Aidani Telesphory, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concomitantly submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read the dissertation titled, "Biological properties and safety profile of extracts from locally grown banana leaves in Arusha, Tanzania" and recommend for examination in fulfillment for the requirements for the award of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

To God, the almighty Father of Heaven and Earth I dedicate this dissertation.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collections
BLD	Banana Leaf Dressing
BST	Brine Shrimps Toxicity Assay
DMSO	Dimethyl sulfoxide
DPPH	2, 2-diphenylpicrylhydrazyl
EA	Ethyl acetate
EAHB	East African Highland Bananas
EGF	epidermal growth factor
FGF	fibroblast growth factor
Hex	*n*-Hexane
IITA	International Institute of Tropical Agriculture
INT	para- Iodonitrotetrazolium
ITM	Institute of Traditional Medicine
MeOH	Methanol
MUHAS	The Muhimbili University of Health and Allied Sciences
N	Number of Replications
NB	Nutrient broth
NM-AIST	The Nelson Mandela African Institution of Science and Technology
PDGF	platelets derived growth factor
PVA	polyvinyl alcohol
ROS	Reactive Oxygen Species
SDB	Sabouraud's dextrose broth and
SE	Standard Error
TGF-β	Transforming growth factor-beta

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Wounds refer to the distraction of anatomical and functional integrity of living tissues. Such distraction may be due to physical, chemical, or thermal injuries (Agyare *et al.*, 2016). According to the wound healing society, wounds often distort the epithelium with or without losing the underlying connective tissues (Strodtbeck, 2001). Wound healing is the restoration of the bruised tissues (Boateng *et al.*, 2008); the process that is brought by an orchestrated wound management that involves: administrations of topical antimicrobial agents, pain killers, and other healing promoting agents, such as antioxidants and antiinflammatory agents (Thakur *et al.*, 2011). Some wounds heal faster and quickly under standard physiological mechanisms for wound healing; such wounds are called acute wounds. However, some do not follow the normal healing process and eventually develop into chronic situations (Beldon, 2010; Enoch & Leaper, 2008). Wounds that exhibit an impaired healing process are referred to as delayed acute or chronic wounds; as a consequence of their postponed or delayed healing, they pass into a state of pathologic inflammation (Agyare *et al.*, 2016; Janis & Harrison, 2014).

Wound dressings play a vital role in wound management, and the ideal wound dressing material should be able to act as a protective barrier to microorganisms, non-adherence, absorbs excess exudes from the wound, non-allergenic, easily removed without further tissue injury, fasten wound healing process and cost-effective (Boateng *et al.*, 2008; Jin *et al.*, 2016; Ruszczak, 2003; Ruszczak & Schwartz, 2000). Most of the modern dressing materials satisfy the above requirements; however, their use in an impoverished setting economic societies (for example in Sub Saharan Africa and Tanzania particularly) is hampered due to costs, specifically when applied over a long period (Boateng & Catanzano, 2015; Boateng *et al.*, 2008; Mayet *et al.*, 2014; Rezvani Ghomi *et al.*, 2019).

Traditional products from medicinal plants are an alternative way of addressing these challenges (Thakur *et al.*, 2011). In addition to that, Agyare *et al*. (2016) estimated that more than 5000 plant species in Africa are used as medicinal plants to manage various diseases, including wounds. Furthermore, approximately 80% of people in developing countries, particularly in Africa, depend on traditional medicines from herbal species to support their

health needs, such as wounds management, infectious, and other metabolic diseases (Agarwal *et al.*, 2009).

Banana leaves from banana trees (*Musa accumunata or Musa balbisiana*) have been thought to possess wound healing properties; possibly due to their phytochemical constituents (Chongchet, 1980; Hoetzenecker *et al.*, 2013). The practice of using banana leaves in wound management is dated years back in India, where patients with smallpox were treated lying on banana leaves due to their coolness and non-adherent properties (Gore & Akolekar, 2003b). As wound dressing materials, banana leaves have been used to manage various wounds, particularly burns. Chongchet (1980) was the first to report sterile banana leaves as the dressing biomaterials on burn wounds in about 100 burn patients, where banana leaves showed atraumatic properties.

Core *et al*. (2003) extended the work of Chongchet by comparison to petroleum jelly gauze dressing. They found that banana leaf dressing (BLD) resulted in a rapid epithelization of wounds, with less pain during dressing changes and atraumatic properties than that of petroleum jelly gauze dressing (Gore & Akolekar, 2003a). Sterilization aspects for banana leaves have demonstrated steam sterilization to be the optimal sterilization technique without altering the wound healing property of the banana leaves (Guenova *et al.*, 2013).

To the best of our knowledge, phytochemical and biological properties of banana leave grown locally in Arusha (Tanzania) have never been studied despite their proposed wound healing properties as observed at Nkoaranga hospital in Arusha, where the incident of burns and cutting wounds is high among the community that cannot afford daily wound management medications. This research has therefore addressed the above speculations by analyzing phytochemicals and biological properties of banana leaf extracts from locally grown banana in Arusha, to affirm their wound healing property.

1.2 Statement of the problem

Wound management is a public health issue that necessitates costly medications and a lengthy healing period. As a result, wound care raises medical expenditures in areas where the majority of middle- and low-income people cannot afford it (Hoetzenecker *et al.*, 2013). As a result, any endeavor aimed at lowering the cost of wound management is critical. One way to address the limits of current wound dressing materials, particularly the costs incurred when used for a long time, is to use traditional therapeutic remedies derived from plants (Hoetzenecker *et al.*, 2013).

The phytoconstituents and other related biological properties from banana leaves may modulate cascades of processes during the healing by acting as antimicrobial, antioxidant, and antiinflammatory agents (Tsala *et al.*, 2013). To date, several plant natural products are reported to have wound healing properties as reviewed by recent publications (Budovsky *et al.*, 2015; Ibrahim *et al.*, 2018; Pereira & Bartolo, 2016; Shedoeva *et al.*, 2019). Banana leaves from locally grown *Mchare* bananas have been used to dress burns in children at Nkoaranga hospital, and the results are promising. However, unlike other herbal products with medicinal properties, the application of banana leaves from the locally grown *Mchare* banana in wound dressing has never been justified scientifically. Evidently, studies aiming at identifying the phytochemical and biological parameters underlying their wound healing properties cannot be understated. Therefore, this study aimed at determining the phytochemical and biological wound healing properties of banana leaves among the selected two Mchare banana species (*Ijihu inkundu* and *Mlelembo*) and a *Musa cavendish* (Dwarf Cavendish banana) locally known as *Kimalindi* from Arusha, Tanzania.

1.3 The rationale of the study

Information regarding the biological properties of bananas leaves from locally grown Mchare bananas (*Mlelembo* and *Ijihu inkundu*), and *Musa Cavendish* at Nkoroanga hospital is unavailable, despite their application in wound dressing. Therefore, the study aimed to evaluate the wound healing properties from the extracts of *kimalindi, ijihu inkundu,* and *mlelembo*. The study also aimed to provide baseline information regarding available phytochemical compounds and toxicity in banana leaves from locally grown bananas in Arusha, Tanzania.

1.4 Research objectives

1.4.1 General objective

To determine phytochemical and biological properties of banana leaves' extracts from locally grown bananas species (*kimalindi, ijihu inkundu,* and *mlelembo)* underlying their wound dressing application.

1.4.2 Specific objectives

(i) To screen the phytoconstituents within the banana leaves extracts from *kimalindi, ijihu inkundu*, and *mlelembo* responsible for their wound healing properties.

(ii) To determine the antimicrobial properties of banana leaves' extracts from *kimalindi*, *ijihu inkundu,* and *mlelembo* against pathogenic microorganisms associated with wound infections.

(iii) To determine the antioxidant properties of banana leaves' extracts from *kimalindi*, *ijihu inkundu,* and *mlelembo* species.

(iv) To determine toxicity profiles of bioactive banana leaves' extracts which might hinder their applicability as wound dressing materials.

1.4 Research questions

(i) What are the phytoconstituents present within the leaves of the kimalindi, mlelembo, and ijihu inkundu?

(ii) What are the antimicrobial properties of *kimalindi*, *mlelembo,* and *ijihu inkundu* against pathogenic microorganisms associated with wound infections?

(iii) What are the antioxidant properties of *kimalindi*, *mlelembo,* and *ijihu inkundu* leaves?

(iv) What are the safety profiles of *kimalindi*, *mlelembo,* and *ijihu inkundu* leaves favoring their applicability as wound dressing materials?

1.6 Significance of the study

(i) Information presented in this study provides baseline information on the phytoconstituents, antimicrobial properties, antioxidant properties, and safety profiles of two locally *Mchare* banana leaves and the dwarf *Musa cavendish* of the northern part of Tanzania.

(ii) The study findings carry the potential for applying banana leaves from the studied locally grown Mchare banana and the dwarf *M. cavendish* as an alternative to the currently available wounds dressing materials.

(iii) The study provides information on the best solvent that gives the best results when considering the preparation of topical extracts that might serve in parallel with the petroleum jelly in wound management.

1.7 Delineation of the study

The study focused on evaluating the potential biological properties underlying the banana leaves' wound healing potential from the two *Mchare* bananas and the dwarf *M. cavendish* spp, locally known as the mlelembo *ijihu inkundu and the kimalindi*, respectively. The samples for research materials were obtained from the International Institute of Tropical Agriculture (IITA) in 2019. The study did not cover other Mchare members apart from the mentioned one. Thus, the presented information presented in this document should specifically be referred to the two Mchare bananas (*mlelembo* and *ijihu inkundu*) and dwarf *M. cavendish* (*kimalindi*) and not otherwise.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wound dressing

According to Selvaraj *et al.* (2015), appropriate dressing materials should be chosen based on their ability to: Maintain a moist environment, promote the creation of new blood vessels and connective tissue synthesis, enable gas exchange between wounded tissue and the surroundings, and be non-adherent to the wound upon healing without triggering tissue trauma, promoting leucocyte and epidermal growth factor migration; and, last, it ought to be antiseptic, non-allergic, non-toxic, and sterile (Selvaraj *et al.*, 2015). Dressings are designed to be in contact with the wound, historically the application of wound dressings is dated since 1600 BC where the linen strips covered with oil and grease were used in dressings of wounds. Wools and animal skins boiled in honey, wine, and vinegar were used by Greece during 460-370 BC (Daunton *et al.*, 2012). A breakthrough in the field of microbiology revolutionized the field of wound dressings by allowing the incorporation of antibiotics in wound dressing materials aiming at controlling infections within wounds (Baranoski & Ayello, 2012).

Wound dressings operate by facilitating quick re-epithelization, increasing collagen and blood vessel development, and changing the pH of the wound bed, which together significantly reduce rates of infection in wounds (Dabiri *et al.*, 2016). Several modern dressings, such as polyurethane foams, hydrocolloids, and iodine-containing gels, were launched in the mid-1980s, were designed to maintain moisture and absorb wound fluids (Daunton *et al.*, 2012). Since then, wound dressings have progressed to include synthetic foam dressings, silver/collagen-containing dressing hydrogels, hydrocolloids, alginates, silicone meshes, tissue adhesives, and vapor-permeable adhesive films, among others.

Despite the availability of the above-mentioned wound dressings, economical dressings are still required, especially because wound treatment is costly to the point where it may be out of reach for societies with limited clinical setting resources. In light of this rationale, the biological and safety characteristics of extracts from locally grown banana leaves that may have wound dressing properties were investigated in this work.

2.2 Overview of East African banana cultivars

East African cultivars of bananas are too many to categorize, mostly referred to by their local/native names based on their vernacular tongue (Karamura, 1999). They can be assembled into three subclasses: dessert banana, plantain banana, and the East African Highland Bananas (EAHB); the latter is meant for cooking and local beer productions (Pillay *et al.*, 2001; Sebasigari *et al.*, 1987). *Mchare* species located in the northern part of Tanzania: Moshi, Arusha, and Tanga fall under the group of EAHB (Kilimo Trust, 2012). They are the triploid of the *M. acuminata* (AAA-EA) (Karamura *et al.*, 2012), and they serve for both staple and commercial purposes among the communities. The nutritional value of Mchare bananas has been recently evaluated (Dotto *et al.*, 2019): however, their medicinal benefits, such as their wound dressing property, are yet to be documented. Other vernacular names and uses for both EAHB and plantain cultivars common to Tanzania are stipulated in the table below:

Table 1: Vernacular names and uses for both EAHB and plantain cultivars common to Tanzania adapted with modification from (Karamura *et al.*, 2012)

Name	Place where name is referred	Application
Ndizi	Throughout the country	Cooking, roasting, dessert, and brewing
Embire/Enkundi	Kagera	Brewing
Ng'ombe	Kilimanjaro and Arusha	Cooking and brewing
Omutsir	Kigoma	Brewing
Matoke, Ndizi za kupika, Ekitoke kisamunya,	Throughout the country	Cooking
Enkonjwa, Gonja, Ndizi ya kuchoma	Throughout the country	Roasting
Ndizi mbivu, Kiise, Ntotomya	Throughout the country	Dessert

Other common cultivars of bananas in Tanzania are the *Cavendish* cultivars, which fall within the dessert triploid (AAA subtype), which is the utmost grown cluster of edible bananas in the region. It is referred to as either *kimalindi* or *Malindi* among society. Unlike the EAHB and the plantain, the Cavendish is sweet and consumed as a raw flesh fruit when ripen. Unlike for the *Mchare* cultivars, the nutritional and health benefits of Cavendish are evidenced by literature: however, to our best knowledge, this is the first kind of study to document medicinal values of Cavendish found in the northern part of Tanzania.

2.3 Medicinal values of Banana

Bioactive compounds from the leaves, flowers, pseudostem, fruits, pulps, and peels are the reasons for bananas' medicinal values. Their potential includes a range of activities: antioxidant, antiinflammatory, antimicrobial, antidiabetic, antiulcerative, anticancer, as reviewed by Kumar *et al.* (2012) and Singh *et al.* (2016). The presence of minerals such as Potassium and Iron within the fruits has proven the sustainable growth of muscles and controlling blood pressure: furthermore, prevention of depression correlates with the serotonin from bananas (Singh *et al.*, 2016). Fruits from plantain bananas have a resistant starch with low digestibility, unlike the high digestibility sugars from cereals: Resistant starch from plantain bananas is suitable for cardiovascular and diabetic people outstanding to its hypocholesterolemic activity (Cressey *et al.*, 2014; Volp *et al.*, 2008).

Carotenoids-rich bananas serve people from vitamin A deficiency and other chronic degenerative diseases: A severe problem throughout the World (Amah *et al.*, 2019; Ekesa *et al.*, 2015). Ascorbic acids, flavonoids, and phenolic compounds within bananas present the fruits with antioxidative stress by preventing low-density lipoprotein oxidation (Kumar *et al.*, 2012). Several pharmacological studies suggested the antimicrobial potential of different parts of banana that are linked by its several bioactive compounds: Traditional health practitioners exploit the antimicrobial potential of banana in the treatment of infectious disease, and the results are significant as reported by Behiry *et al.* (2019) and Kapadia *et al.* (2015). Researchers have explored the Antiulcerogenic and ulcer healing property of bananas (Goel & Sairam, 2002; Sumbul *et al.*, 2011); it has been reported by Lewis *et al.* (1999) that a natural flavonoid leucocynidin brings ulcer healing from banana. Daily consumption of plantain banana correlates with the reduction of Low-density Lipoprotein/High-density lipoprotein (LDL/HDL) ratio and improvement of insulin sensitivity in patients with hypocholesterolemic conditions (Cressey *et al.*, 2014).

Concerning the above outlined medicinal values of bananas, most of the bioactive compounds of health benefits have been explored from the banana fruits, pulps, and peels; thus, much more of the same information is vital for the case banana leaves. Furthermore, medicinal values of the proposed banana species in this study have not yet exploited to their maximal; thus, the information from this study will enlighten the possible medicinal values of locally grown banana leaves, such as their potential as wound dressing materials.

2.4 The science of wound healing

Wounds healing is a complex process including various biological and molecular phases for achieving tissues remodeling; the process can be grouped into four phases (Fig. 1): Homeostasis (coagulation) phase, inflammatory phase, proliferative phase (formation of granulation tissue and collagen), and tissue remodeling phase (Agyare *et al.*, 2016; Beldon, 2010). Homeostasis involves the actions of platelets where they release an adhesive glycoprotein, leading to platelet aggregations. On top of that, platelets activate several growth factors including, transforming growth factor-beta (TGF-β), platelets derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) (Beldon, 2010; George *et al.*, 2006). The activated growth factors stimulate monocytes and neutrophils' movement to the wound site, which marks the onset of the inflammatory.

In the inflammatory phase, the neutrophils and macrophages provide the first defense line by phagocytosis of cell debris and some microbes (Tsala *et al.*, 2013). Suppose not well-controlled excess neutrophils may release intracellular enzymes into the surrounding matrix, which may further digest the damaged surrounding tissue (Li *et al.*, 2007). Apart from providing the first line of defense, macrophages also facilitate the growth of endothelial and smooth muscle cells, which play a pivotal role during the proliferation phase by activating angiogenesis and collagen deposition (Li *et al.*, 2007).

The proliferation phase starts four days after injury and may last up to 21 days depending on the nature and the size of wounds; it is driven by the activity of endothelial and fibroblast cells, which facilitates angiogenesis, collagen deposition, wound contraction, granulation, and re-epithelization (Süntar *et al.*, 2010). Rearrangements of collagen tissue mark remodeling into a more tensile compared to the normal skin; epithelial tissues formed by fibroblast cells are transformed into the barrier-forming system; depending on the nature of wounds, remodeling might take up two years after wounding (George *et al.*, 2006). Most of the wound dressing materials act by stimulating the wound healing phases.

2.5 Wound dressing materials

Wound dressings are used to protect the wound from contamination, speed up the healing process, and deliver bioactive ingredients to the wound's sites (Lin *et al.*, 2016). The wound dressing is categorized as either traditional or advanced dressing; in traditional dressing like

cotton, wool, and gauze, the dressing material is not actively involved in the wound healing process. In contrast, in advanced dressing, the dressing materials do either involve themselves in the process of wound healing or by the release of bioactive ingredients incorporated within dressings (Hurd *et al.*, 2017). The incorporated drugs act either as a debriding agent or an antimicrobial agent, removing necrotic tissues or preventing and treating the infection to enable tissue regeneration, respectively (Dhivya *et al*., 2015).

Advanced dressing materials are grouped into: Natural inert polymers, natural bioactive polymers, and synthetic polymers made up of molecules that stimulate wound healing processes such as collagen, cellulose, pectin, hyaluronic acid, chitosan, sodium alginate, polyvinyl alcohol (PVA), polyethylene oxide and polyurethane (Dhivya *et al*., 2015).

Traditional wound dressing materials involve a passive wound healing process. They include cotton, wool, gauze, natural or synthetic bandages; however, they are limited by their low healing properties due to their passive participation in wound healing compared to their counterpart advanced dressing materials (Borda *et al.*, 2016). Despite their low healing efficiency, traditional dressing materials are easily reachable to poor clinic settings due to their low expenses and better patient acceptance (Guenova *et al.*, 2013; Hoetzenecker *et al.*, 2013). Enhancement of these dressing materials' properties can be obtained by coating them with other materials or compounds to form a functional dressing. For example, gauze and bandage can be functionalized with topical antimicrobial like povidone-iodine, sodium hypochlorite solutions, hydrogen peroxide, acetic acid, and silver releasing agents to prevent reinfection during dressing.

Paraffin may be impregnated on the surface of gauze to prevent the sticking of dressing on the surface of a wound and, therefore, prevent tissue injury and bleeding during dressing. Traditional medicinal products from plants have been utilized since ancient times as a medicine source, including wound healings (Alam *et al.*, 2011; Bahramsoltani *et al.*, 2014). Some of those products have been justified scientifically; however, others like banana leaves, their wound healing properties have never been scientifically justified (Guenova *et al.*, 2013). Thus, this work aimed at exploring the scientific basis underlying the wound healing properties of banana leaves.

2.6 Application of banana leaves in wound dressings

Banana leaves have been used for more than 40 years as wound dressing materials in burns wound (Chongchet, 1980; Gore & Akolekar, 2003a, 2003b). However, their scientific basis underlying their wound healing properties is unrevealed (Guenova *et al.*, 2013). Several successful trials have reported using banana leaves as dressing materials since the 1980s; however, none of them have demonstrated scientific mechanisms underlying their wound healing properties. Chongchet was the first to report on the application of banana leaves as the dressing material in burns wounds, most of the patients were comfortable with the sterile banana leave. Furthermore, no pain was reported during dressing changes; however, the main challenges were longevity and contamination of sterile banana leaves where they could not last up to a week.

Gore and Akolekar have explored the efficacy of BLD in skin graft donor patients to be superior to Vaseline gauze dressing. They further demonstrated that BLD represented the ideal property of dressing materials: non-adherent, atraumatic, pain-free, readily available, and cheap compared to VG (Gore & Akolekar, 2003a). The cost incurred by BLD is cheaper than other traditional dressing materials. When BLD was compared with boiled potato peel bandage (PPPB), the efficacy of both BLD and BPPB was equivalent in all features, yet BLD was 11 times cheaper compared to the BPPB (Gore & Akolekar, 2003b). Thus, BLD continues to be superior to other dressing materials since it is grown in most parts of the World, especially in low clinic settings, and can be easily prepared used; therefore, strong scientific evidence underlying the applicability of BLD is strongly recommended.

Other parts of banana plants like fruits and peels have been well exploited to the point that their phytochemical and biological properties underlying their wound healing properties are known. Antimicrobial, antioxidant, and antiinflammatory parameters that enhance banana fruits and peels' wound healing process have been documented (Agarwal *et al.*, 2009; Agyare *et al.*, 2016; Imam & Akter, 2011; Lino *et al.*, 2011). Different phytochemical compounds such as tannins and flavonoids, alkaloids, glycosides, and terpenoids extracted from banana fruits have antimicrobial and healing properties (Pereira & Maraschin, 2015; Vu *et al.*, 2018). Furthermore, natural polymers such as cellulose, collagen, and pectin found within the extracts of banana fruits and peels are involved in the whole healing process. Thus, being motivated by those facts, the present study aimed at identifying the phytochemical and biological properties

of banana leaves, which place them as the wound dressing material. Furthermore, the study determined the toxicity profiles of banana leaves as the wound dressing materials.

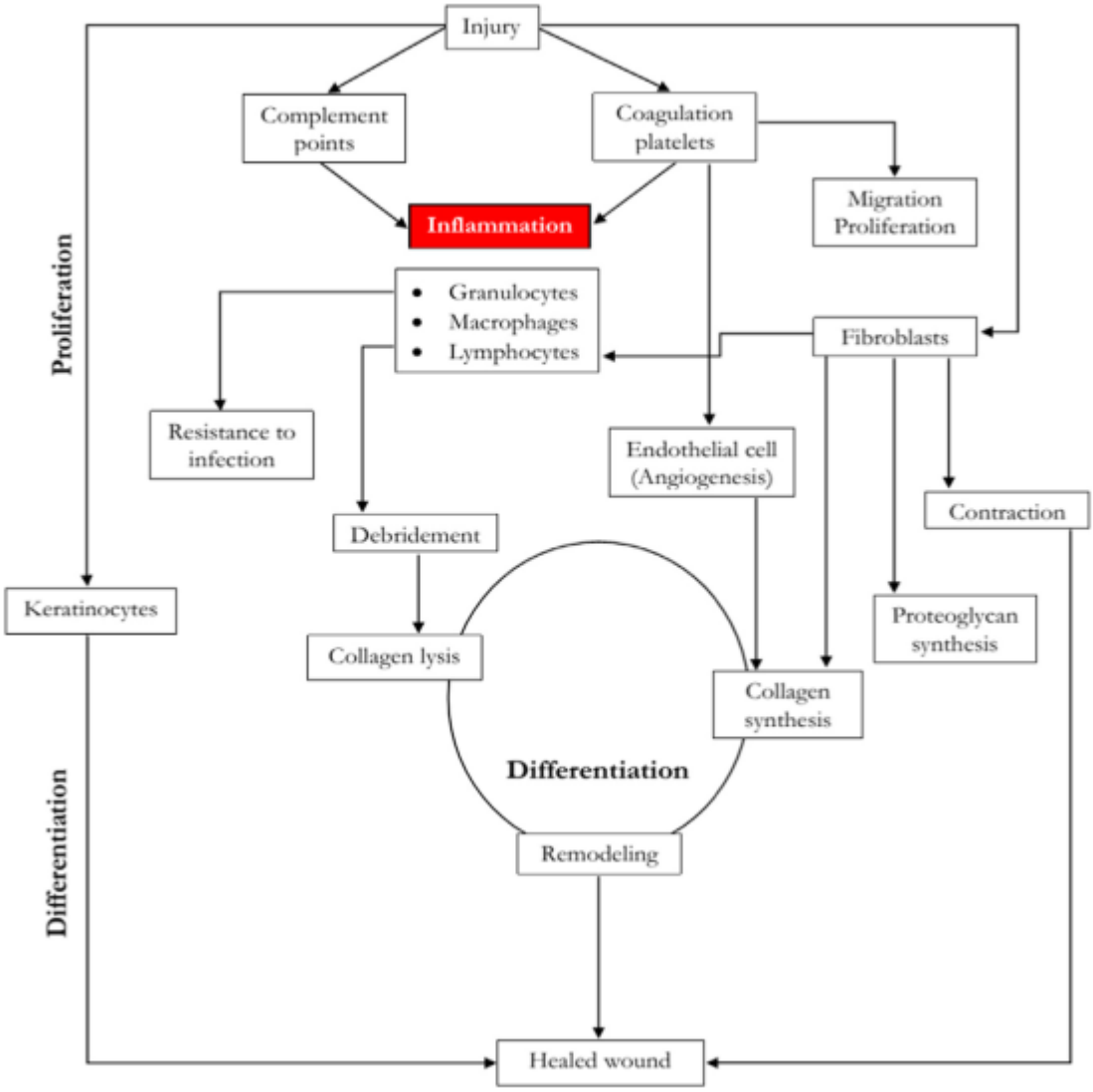

Figure 1: Schematic presentation of wound healing physiology adapted (Agyare *et al.*, 2016)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals and tested microorganisms

Ethyl acetate (EA), *n*-Hexane (Hex), and methanol (MeOH) were procured from Fisher Scientific (UK). At the same time, Dimethyl sulfoxide (DMSO), para- Iodonitrotetrazolium (INT), 2, 2-diphenylpicrylhydrazyl (DPPH), and Phosphate buffered saline pH 7.4 (PBS) were procured from Sigma (Poole, Dorset, UK). Ketoconazole and Ciprofloxacin tablets were bought from S Kant Healthcare LTD and Micro Lab LTD, India respectively, Sabouraud's dextrose broth (SDB) and Nutrient broth (NB) were procured from HIMEDIA (Himedia laboratories Pvt Ltd, India) and Tulip Diagnostic (P) Ltd (Microxpress™, Goa, INDIA). *Escherichia coli (*ATCC 25922*), Pseudomonas aeruginosa (*ATCC 29953*), Salmonella typhi* (ATCC 6539), *Staphylococcus aureus* (ATCC 25925), *Candida ablicans* (ATCC 90028), and *Cryptococcus neoformans* (clinical isolates) were o from the Department of Microbiology (MUHAS). Brine shrimps' eggs were from Aquacultures innovations (Grahamstown 6140), and sea salt was prepared by evaporating ocean water, collected along the Koko coast of the Indian Ocean, Dar es salaam.

3.2 Plant material

The studied banana leaves were from three banana species; two species were indigenous *Mchare* bananas viz; *ijihu inkundu* and *mlelembo,* other species were a *Musa Cavendish,* known as *Kimalindi in the northern part of the county*. They were all collected from the International Institute of Tropical Agriculture (IITA), located in Arusha, Tanzania. The species were selected based on their availability at the time of the study, the ongoing need for developing potential wound dressing materials from banana leaves, and their popularity among the indigenous in the northern zone of Tanzania, particularly Arusha, Moshi, and Tanga (Dotto *et al.,* 2019). The collected banana leaves were dried under shade and finally pulverized into a fine powder for extractions

3.3 Extraction of banana leaves extract

The dried powder from each banana leaves, viz. *Ijihu inkundu*, *Kimalindi*, and *Mlelembo* were sequentially extracted by a cold maceration method by using *n*-hexane (99.9% AR), ethyl

acetate (99.9% AR), and methanol (99.9% AR) solvents. Briefly, 200 g of the leaves powder was first soaked into *n*-hexane solvent for 48 hours. The solvent-containing extracts were filtered by a cotton wool plug, followed by a filter paper (Whatman No 1). A rotary evaporator concentrated the filtrate at 60 °C and reduced pressure to deplete the extraction of bioactive compounds. The remaining residues were further re-soaked into *n*-hexane for 24 hours, followed by filtration and concertation as prior. The remaining *n*-hexane residues were re-soaked into ethyl acetate solvent for 48 hours, followed by filtration and concentration by a rotary evaporator at 60 °C and reduced pressure. The obtained residues were soaked again into ethyl acetate solvent for an additional 24 hours, followed by filtration and concentration as prior. The remaining residues of ethyl acetate were re-soaked into methanol solvents for 48 hours, followed by concentration using rotavapor at 60 °C and reduced pressure. The obtained residues were further subjected to methanol for additional 24 hours, followed by concentration as prior done. Methanol residues were re-soaked into a mixture of ethyl acetate and methanol (1:1) solvents for 48 hours, followed by concentration and filtration; after that, the obtained residues were then soaked again into ethyl acetate and methanol (1:1) solvents for 24 hours, followed by filtration and concentration as prior. The obtained crude extracts were air-dried and stored at -21 °C till the time for conducting bioassay, while the plant residues were reserved for future use.

3.5 Screening for secondary metabolites

The phytoconstituents from the methanolic banana leaves extracts were screened by standard procedures for qualitative phytochemical screening reported from the literature with some modifications. Seven metabolites were screened, viz. anthraquinones, alkaloids, flavonoids, saponins, phenols, tannins, and terpenoids.

3.5.1 Qualitative test for saponins.

The presence of saponins was detected by foam procedure(Yadav & Agarwala, 2011). Briefly, a small number of methanolic extracts was mixed with distilled water to half of the test tube, followed by vigorous shaking. The formation of foam on the top surface of the reaction mixture indicated the presence of saponins.

3.5.2 Qualitative test for tannins

Braemer's test, with some modifications, detected the presence of tannins. Briefly, 250 mg of methanolic extract was mixed with 5 mL of 45% ethanol; the reaction mixture was vigorously mixed, boiled, and filtered. Few drops of 1% Ferric chloride ($FeCl_3$) were added to the extract. Greenish to black coloration signified tannins' presence.

3.5.3 Qualitative test for terpenoids

Terpenoids were detected accordingly to the procedures described with some modifications. 250 mg of methanolic extracts were mixed with 4 mL chloroform and gently mixed, followed by the addition of concentrated sulphuric acid (H_2SO_4) to form a layer. Red-brown coloration at the interface indicated the presence of terpenoids.

3.5.4 Qualitative test for phenols

Phenols were detected according to the procedures described by Yadav and Agarwala (2011). To the test tube, 250 mg of methanolic extracts were taken with 2 mL of water, followed by the addition of two drops of $FeCl_3$. Bluish-black coloration indicated the presence of phenols.

3.5.5 Qualitative test for anthraquinones

Anthraquinones were detected according to the method described by Gul *et al.* (2017). With some modifications, 2 mL of a sample extract solution was taken with 4 mL of chloroform in a test tube incubated for 5 minutes. A reaction mixture was mixed with 5 mL of 10% ammonia solution. A formation of a pink to red coloration at the lower layer of the mixture (ammonia) signified anthraquinones' presence.

3.5.6 Qualitative test for alkaloids

To the small amount of crude extract, 2 mL of 2% H_2SO_4 was added and strongly mixed. Afterward, Meyer's, Wegner's, and Dragendorff's reagents were independently added. Results from this experiment were read as follows:

(i) Meyer's reagent

A creamy-white colored precipitate was considered for the presence of alkaloids.

(ii) Wegner's reagent

A reddish-brown precipitate was taken as an indicator for the presence of alkaloids.

(iii) Dragendorff's reagent

A formation of red precipitate was evidence for the presence of alkaloids.

3.5.7 Qualitative test for flavonoids

The presence of flavonoids was detected by methods reported by Roghini and Vijayalakshmi (2018) with some modifications small amount of extracts was mixed with 2 mL of 2% NaOH. The formation of an intense yellow color indicated the presence of flavonoids.

3.6 Antimicrobial activity

Minimum inhibitory concentration (MIC) Eloff (1998) and Nondo *et al.* (2011) was employed in determining the susceptibility of the tested microorganisms to the banana leaves extracts. The method is a semi-quantitative procedure using 96-microtiter well plates to assess the in vitro antimicrobial activity of an antimicrobial agent (Garcia, 2010). Antimicrobial activity was thus carried out as follows:

3.6.1 Experimental procedures

Microtiter plates were pre-loaded with 50 µL of broth media in each well, followed by 50 µL of the plant extracts (100 mg/mL) to make 100 µL in the first well of the tested rows. The mixture was subsequently mixed by pipetting up and down within a well. Following an in-depth mixing, 50 µL were drawn from each of the first rows of the well and pipetted into the next row wells. The process was subsequently repeated down the columns to the last row wells of which the remaining 50 µL were discarded. Following a successive loading of broth and the plant extracts, 0.5 Mac Farhland standard turbidity (1.5×10^8 CFU) was then loaded into each row of wells to make a final total volume of 100 µL per well. The wells with ciprofloxacin served as a positive control, and that with DMSO was used as a negative control, wells with bacteria/fungi and broth were left to validate the experiment by monitoring the growth of the tested microorganisms. After that, the plates were incubated at 37 °C for 24 hours. Following incubation time, MIC was determined by the addition of 20 µl of 0.002% INT dye in each well, followed by incubation at 37 °C for 1 hour. Bacteria/ fungi growth was indicated by the change

of INT-dye color into a purple colour, while the persistence of INT color indicated the presence of live bacteria/fungi.

3.6.2 Antimicrobial susceptibility data analysis

MIC values were expressed as a mean ± Standard error of the mean (SEM). Analysis of variance (One way-ANOVA) was used for the mean interrelation among groups, and data were considered statistically significant at a $P<0.05$. Tukey and Bonferroni post hoc tests were used to confirm differences among groups.

3.6.3 Antioxidant activity of banana leaves

The antioxidant activity of banana leaves extracts was determined by the scavenging ability of methanolic extracts of banana leaves on 1, 1-diphenyl-2-picryl-hydrazy (DPPH) free radical. Scavenging of DDPH by oxidants involves reducing nitrogen-free radicals by hydrogen atom from the phenolic group (s) of plant extracts (Nakanishi *et al.*, 2005). The reduction of DPPH by phenolic compounds may either be brought by a transfer of H atom to the DPPH free radical or by the electron transfer mechanisms, whereby the antioxidant substance might give an electron to the DPPH free radical (Leopoldini *et al.*, 2004; Wright *et al.*, 2001). Following the reduction of oxidants (free radicals), antioxidants transform from being a very reactive radical into a less reactive radical with a less damaging effect (Fitzmaurice *et al.*, 2011; Maxwell, 1997).

3.6.4 Experimental materials and procedures

Scavenging activity of banana extracts was carried according to the methods reported by Gul *et al.* (2017), Gyamfi *et al.* (1999) and Sahaa *et al.* (2013) with some modifications. A stock solution of 1g/mL from each sample was diluted into a final working concentration of 200, 400, 600, 800, and 1000 mg/mL in methanol, 1 mL of 0.3 mmol /L of DPPH in methanol was mixed with 1 mL of a sample or standard, followed by incubation of the reaction mixture at 37 °C for 30 min in a dark condition. The reaction mixture's absorbance was recorded at 517 nm and transformed into percentage scavenging activity per equation 1 below. A_o stands for OD of control (DPPH), and A_1 stands for OD of extracts or standard ascorbic acid. The DPPH solution without plant extracts was a control, and 80% methanol was a blank. Decoration of DPPH color indicated scavenging of DPPH free radical, marked by decreased optical density (OD). All experiments were in triplicate, and results were expressed as a mean ± SEM.

$$DPPH\ Scavenging\ activity\ (\%) = [(A0 - A1) \div A0] \times 100 \ldots\ldots\ldots\ldots\ldots (1)$$

3.6.5 2, 2-diphenyl-1-picrylhydrazyl scavenging data analysis

Percentage inhibition by banana leaves extracts and ascorbic acid expressed as a mean ±SEM were recorded. One-way ANOVA was used for the mean interrelation among groups. Data were considered statistically significant at a $P<0.05$. Tukey and Bonferroni post hoc tests were used to confirm the existence of differences among groups. Standard curve against % scavenging versus concentration gives the half scavenging concentration (IC_{50}).

3.7 Brine shrimp lethality test (BST)

The brine shrimp's lethality assay serves as a hint for toxicity and cytotoxicity of plant extracts (Hamidi *et al.*, 2014; Nondo *et al.*, 2011; Solis *et al.*, 1993). Brine shrimp's tests is further used to establish critical bioactive compounds sourced from plant extracts, with their potential pharmacological importance and median inhibitory/effective concentration (Nondo *et al.*, 2011). The method is rapid and straightforward as it requires few requirements and conditions compared to the other cytotoxicity screening methods (Hamidi *et al.*, 2014). Linear regression line plotted involving the percentage of the dead shrimps against a sample concentration's logarithmic gives the LC_{50} of the extracts. In the present study, toxicity, cytotoxicity of native banana leaf extracts and their median lethal concentration (LC_{50}) was evaluated by BST. Briefly, the assay involved the use of Brine shrimp larva (Artemia Salina) in the prediction of active bioactive compounds capable of producing a lethal pharmacological response to the shrimps (Solis *et al.*, 1993).

3.7.1 Preparation of Brine shrimp larva

The brine shrimps were prepared according to Meyer *et al.* (1982) and Solis *et al.* (1993), with minor modifications. Briefly, 3.8 g/L of artificial sea salt was prepared by dissolving 3.8 g of sea salt into 1 L of distilled water. The brine solution was dispensed in a sterile hatching rectangular container separated into two parts. The black sheet covered one part that served for brine shrimps' eggs for a conducive dark environment, whilst the second part was illuminated by light (Fig. 2). After that, 500 mg of brine shrimps' eggs were disseminated to the dark side of the hatching container, and the other part was illuminated, purposely for the attraction of the hatched shrimps. Following their hatching, the brine shrimps' larvae (nauplii) were collected after 24 up to 48 h.

3.7.2 Cytotoxicity assays

A stock solution (40 mg/mL) from each plant extracts was prepared by dissolving 40 mg of crude extract into 1 mL of Dimethyl sulfoxide (DMSO). Different working solutions viz. 240, 120, 80, 40, 24, and 8 µg/mL were prepared by pipetting out different volumes from the stock solutions into the vials containing ten brine shrimps larvae; volumes were adjusted into 5 mL by addition of 3.8 g/L of brine solutions. All experiments were duplicated, where DMSO served as negative control while cyclophosphamide served as standard anticancer control drug. After 24 h, toxicity and cytotoxicity were evaluated by counting out the dead larvae. The toxicity profile from two toxicity indexes: Meyer's and Clarkson's toxicity index (Hamidi *et al.*, 2014) is the reference. According to Meyer's index, the LC_{50} > 1000 µg/mL are non-toxic (Meyer *et al.*, 1982) while for the case of Clarkson criterion for toxicity, the LC_{50} > 1000 µg/mL is non-toxic, LC_{50} of between 500 up to 1000 µg/mL exhibits low toxicity, LC_{50} of 100 up to 500 µg/mL are medium toxic the LC_{50} of less than 100 µg/mL is said to be a highly toxic (Clarkson *et al.*, 2004).

3.7.3 Data analysis for Brine Shrimp Lethality Test

Percentage mortality was plotted against their logarithmic concentrations; the linear regression model was used to calculate the concentration that resulted in LC_{50}, LC_{16}, $LC_{84,}$ and 95 confidence interval (95% CI) accordingly to Litchfield and Wilcoxon (1949).

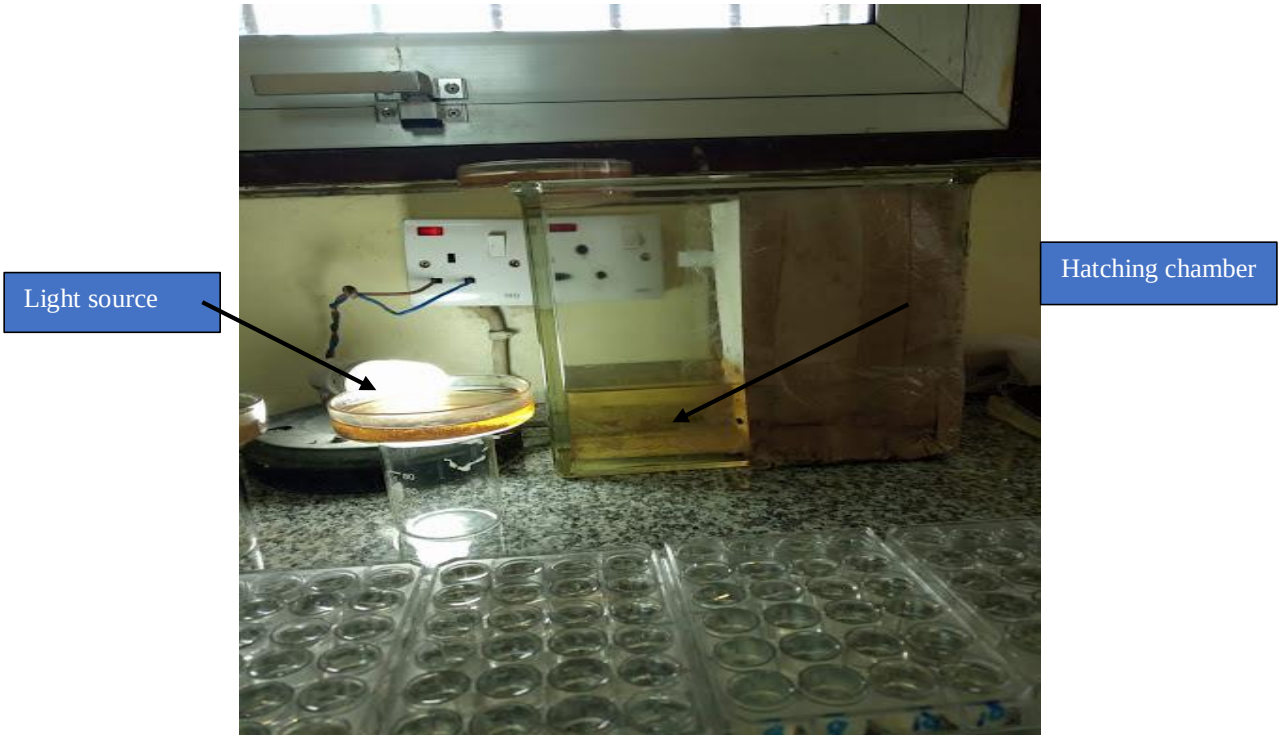

Figure 2: Hatching chamber for shrimps

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Qualitative phytochemical screening

A total of seven secondary metabolites viz. anthraquinones, alkaloids, flavonoids, saponins, tannins, terpenoids, and phenols. Table 2 above shows the screened phytoconstituents present in the methanolic extracts from ijihu *inkundu*, *kimalindi*, and *mlelembo* banana leaves.

Table 2: Phytoconstituents for the methanolic extracts of the studied banana leaves

Secondary Metabolites	*Ijihu Inkundu* MeOH extracts	*Mlelembo* MeOH extracts	*Kimalindi* MeOH extracts
Saponins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Anthraquinones	+	+	+

+, indicates the presence of the screened secondary metabolite

These findings agree with other studies that have highlighted the presence of phytoconstituents in various parts of banana, including leaves, flowers, pulp, and banana fruit, as the underlying factors beyond the medicinal values of banana (Ahmad *et al.*, 2015; Asuquo & Udobi, 2016; Mahmood *et al.*, 2011; Sahaa *et al.*, 2013; Sumathy *et al.*, 2011; Tepal, 2016). The screened phytoconstituents have been reported to have medicinal values, with each bioactive compound exhibiting particular wound healing parameters. For instance, the collagen synthesis is attributed to saponins (Dinda *et al.*, 2010; Sevimli-Gür *et al.*, 2011), while the antiseptic and antioxidant potential is said to be influenced by either tannins (Benzidia *et al.*, 2019; Javed *et al.*, 2020; Shohayeb *et al.*, 2013) and flavonoids (Cheng *et al.*, 2020; Cushnie & Lamb, 2005; Kumar & Pandey, 2013; Panche *et al.*, 2016).

The phytoconstituents mentioned above are believed to modulate the process of wound healing by either interfering with one or more phases of wound healing, most notably by fastening the inflammatory phase, which is a fascinating phase of wound healing (Das *et al.*, 2017; Tsala *et*

al., 2013). Furthermore, the phytoconstituents have been proved to be easily absorbed by the skin's superficial tissues, which further affirms their applicability in the traditional wound dressing (Biswas *et al.*, 2004; Kumar *et al.*, 2007).

Most plant natural products, including those from bananas, have been used since antiquity in wound management. To date, several *in vivo* and *in vitro* studies have shown their effectiveness in the management of wounds, however the lack of several clinical studies that warranty their safety on humans limits their applicability (Ibrahim *et al.*, 2018). The ongoing challenge is on the screening and identification of bioactive compounds from plant materials that are responsible for their wound healing properties and their underlying mechanisms, which is the future focus to further this study.

Several studies regarding the medicinal and nutritional values of bananas have reported the presence of several phytoconstituents as reviewed by Mathew and Negi (2017); Pereira and Maraschin (2015); Singh *et al.* (2016) and Vu *et al.* (2018). Those studies have focused their attention mostly on the extracts from the flower, fruits, peels, and pulps, leaving little tension on the banana leaves' potential medicinal value. Thus, the present study has highlighted seven plant phytoconstituents from the leaves of the three locally grown bananas in Arusha, Tanzania.

4.2 Antimicrobial activity of banana leaves extracts

A total of 12 extracts from banana leaves were evaluated for their antimicrobial activity; most extracts had a promising activity for at least one of the tested organisms (Table 3). The KILEA: MeOH, and IJLAE: MeOH extracts exhibited the highest activity against *P. aeruginosa, C. neoformans, and C. albicans* (MIC = 0.1953 mg/mL).

Among the Ijihu inkundu extracts, ethyl acetate extracts had a MIC value of 0.3906 mg/mL against *E. coli, C. albicans,* and *C. neoformans*. Both E. *coli and C. neoformans* were inhibited to the concentration of 1.5625 mg/mL, *n*-hexane extracts were less sensitive than the rest of the extracts against tested microorganisms except to *C. albicans* and *C. neoformans* (1.5625 mg/mL) while *E. coli and S. typhi* were inhibited to 6.25 mg/mL and *P. aeruginosa* was less sensitive (MIC = 12.5 mg/mL).

Among the extracts from *mlelembo*, Ethyl acetate: Methanol (1:1) extracts had the most potent antimicrobial activity against *C. albicans* (MIC = 0.3906 mg/mL*)*, followed by *E. coli, S. aureus, and C. neoformans* (MIC = 0.78125 mg/mL*)*. For methanolic extracts, the best activity

was against *C. albicans* (MIC = 1.5625 mg/mL) followed by *C. neoformans* (MIC = 3.125 mg/mL). The rest of the tested microorganisms had a weaker activity with the MIC value of 12.5 mg/mL. For ethyl acetate extracts, *C. albicans* was most susceptible, with a MIC value of 0.7812 mg/mL, followed by *E. coli, S. aureus, and C. neoformans* (MIC= 1.5625 mg/mL), *P. aeruginosa*, and *S. typhi* were inhibited to the concentration of 6.25 mg/mL and 3.125 mg/mL respectively. For the case of *n*-hexane extracts, both tested microorganisms were less susceptible to the extracts with MIC values of *12.5* mg/mL against the *S. typhi, S. aureus, and P. aeruginosa* while *C. albicans* and *C. neoformans* were inhibited to the concentration of 6.25 mg/mL.

Among the extracts from *kimalindi*, ethyl acetate: methanol extracts had MIC value of 0.7813 mg/mL against *E. coli, C. ablicans* and *C. neoformans, S. typhi and S. aureus* were inhibited to the concentration of 1.5625 mg/mL. For the case of methanolic extracts, P. aeruginosa was more susceptible (MIC = 0.3906 mg/mL) followed by *S. aureus*, *C. ablicans* and *C. neoformans* (MIC = 1.5625 mg/mL), *E. coli* and *S. typhi* were inhibited to the concentration of 3.125 mg/mL and 6.25 mg/mL respectively. Ethyl acetate extracts were more sensitive against *P. aeruginosa* and *C. neoformans* (MIC = 0.3906 mg/mL), followed by *C. ablicans and* S. *typhi* (MIC = 0.7813 mg/mL), S. aureus (MIC = 1.5625 mg/mL), *E. coli* and (MIC = 3.125 mg/mL), *n*-hexane extracts had a promising activity against *P. aeruginosa* and *C. ablicans* (MIC = 0.3906 mg/mL and 0.7813 mg/mL respectively), followed by *C. neoformans and* S. aureus (MIC = 1.5625 mg/mL), whilst both *E. coli* and *S. typhi* were least inhibited (MIC = 3.125 mg/mL).

Table 3: The minimum inhibitory concentration exhibited by 12 plant extracts

Plant extracts	Minimum Inhibitory Concentration (mg/mL)					
	E. coli	*S. typhi*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*C.neoformans*
IJLHex	6.25	6.25	3.125	12.5	1.5625	1.5625
IJLEA	0.3906	1.5625	6.25	12.5	0.3906	0.3906
IJLMeOH	1.5625	6.25	12.5	25	3.125	1.5625
IJLAE: MeOH (1:1)	0.3906	0.3906	1.5625	6.25	0.1953	0.1953
MLLHex	25	12.5	12.5	12.5	6.25	6.25
MLLEA	1.5625	3.125	1.5625	6.25	0.7813	1.5625
MLLMeOH	12.5	12.5	12.5	12.5	1.5625	3.125
MLLEA: MeOH (1:1)	0.7813	1.5625	0.7813	1.5625	0.3906	0.7813
KILHex	3.125	3.125	1.5625	0.3906	0.78125	1.5625
KILEA	3.125	3.125	1.5625	0.3906	0.78125	0.3906
KILMeOH	3.125	6.25	1.5625	0.390625	1.5625	1.5625
KILEA: MeOH (1:1)	0.7813	1.5625	1.5625	0.1953	0.7813	0.7813
Ketoconazole	*NA*	*NA*	*NA*	*NA*	0.78125	0.39
Ciprofloxacin	0.78125	0.390625	0.78125	0.390625	*NA*	*NA*

Keynote**:** **IJLHex** = *Ijihu inkundu* leaves *n*-hexane extract, **IJLEA** = *Ijihu inkundu* leaves ethyl acetate extracts, **IJLMeOH** = *Ijihu inkundu* leaves methanolic extract, and **IJLAE: MeOH** = *Ijihu inkundu* leaves ethyl acetate: methanol extract (1:1). **MLLHex** = *mlelembo* leaves *n*-hexane extracts, **MLLEA** = *mlelembo* leaves ethyl acetate extracts, **MLLMeOH**= *mlelembo* leaves methanolic extract, and **MLLEA: MeOH** (1:1) = *mlelembo* leaves ethyl acetate: methanol extracts. KILHex = *kimalindi* leaves *n*-hexane-extracts, **KILAE** = *kimalindi* leaves ethyl acetate extract, **KILMeOH** = *kimalindi* leaves methanolic extracts, and **KILEA: MeOH (1:1) =** *kimalind*i leaves ethyl acetate: methanol extracts, NA- not applicable

4.2.1 Effectiveness of banana leaves extracts to the tested organisms

Effectiveness of extracts against microorganisms were in the order of KILEA: MeOH (1:1) > MLLEA: MeOH (1:1) > IJLAE: MeOH (1:1) > KILMeOH > KILEA = KIMHex > MLLEA > IJLEA > IJLHex > IJLMeOH > MLLMeOH > MLLHex (Table 4). One-way analysis of variance (1-way ANOVA) revealed a statistical difference of mean among all 12 extracts ($p < 0.05$). From these results, we can deduce that all *kimalindi* extracts had a better effect against

the tested microorganisms, suggesting that *kimalindi* leaves may present the best option when used in wound dressing, as depicted in (Fig. 3).

Table 4: Mean comparisons exhibited by 12 plant extracts against the tested microorganisms

Plant extracts	Average MIC (mg/mL)
KILEA: MeOH	0.94 ± 0.22
MLLEA: MeOH	0.98 ± 0.22
IJLAE: MeOH	1.50 ± 0.97
KILMeOH	1.56 ± 0.52
KILHex	1.76 ± 0.47
KILEA	1.76 ± 0.47
MLLEA	2.47 ± 0.82
IJLEA	3.58 ± 2.01
IJLHex	5.21 ± 1.70
IJLMeOH	8.33 ± 3.73
MLLMeOH	9.11 ± 2.15
MLLHex	12.5 ± 2.80

Results represent the minimum inhibitory concentration exhibited by each extract. Values are presented as mean (n=72) ± SEM, $p<0.05$.

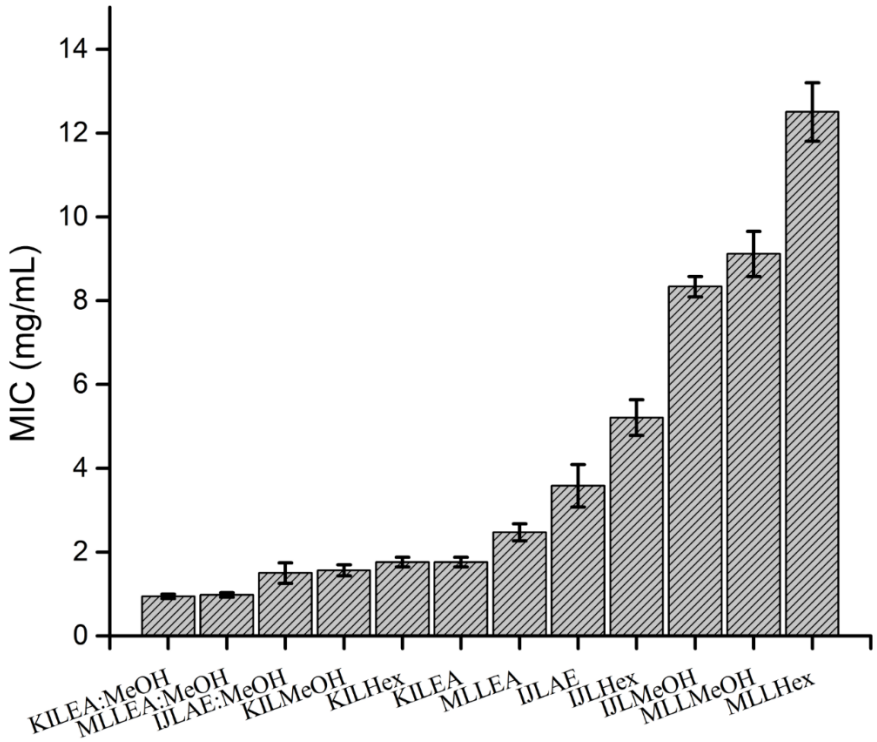

Figure 3: Bar plot indicating the means effectiveness of plant extracts on the tested microorganisms

4.2.2 Susceptibility of tested microorganisms to plant extracts

All tested organisms were susceptible to the extracts; however, their susceptibility was in the order of *C. albicans* > *C. neoformans* > *S. aureus* > *S. typhi* >*E. coli* > *P. aeruginosa,* with the average MIC values viz: 1.45 ± 0.49 mg/mL, 1.64 ± 0.48 mg/mL, 4.59 ± 1.41 mg/mL, 4.75 ± 1.18 mg/mL, 4.88 ± 2.08 mg/mL and 7.54 ± 2.22 mg/mL respectively. Those results show that the studied banana leaves, especially the *kimalindi* leaves, could be used for the dressing of wounds prone to fungi infections (*C. ablicans* and *C. neoformans*) as well as the S. aureus, which commonly associated with wound infections, notably the burns, surgical and pressure/bed sores. However, the obtained finding needs to be tested and confirmed in a trial involving infected wounds. There was no statistical difference in the susceptibility of all six tested microorganisms ($p > 0.05$), implying that, despite being sensitive to the plant extracts further, more research is required to prove their sensitivity towards both *ijihu inkundu mlelembo* and *kimalindi* extract (Fig. 4).

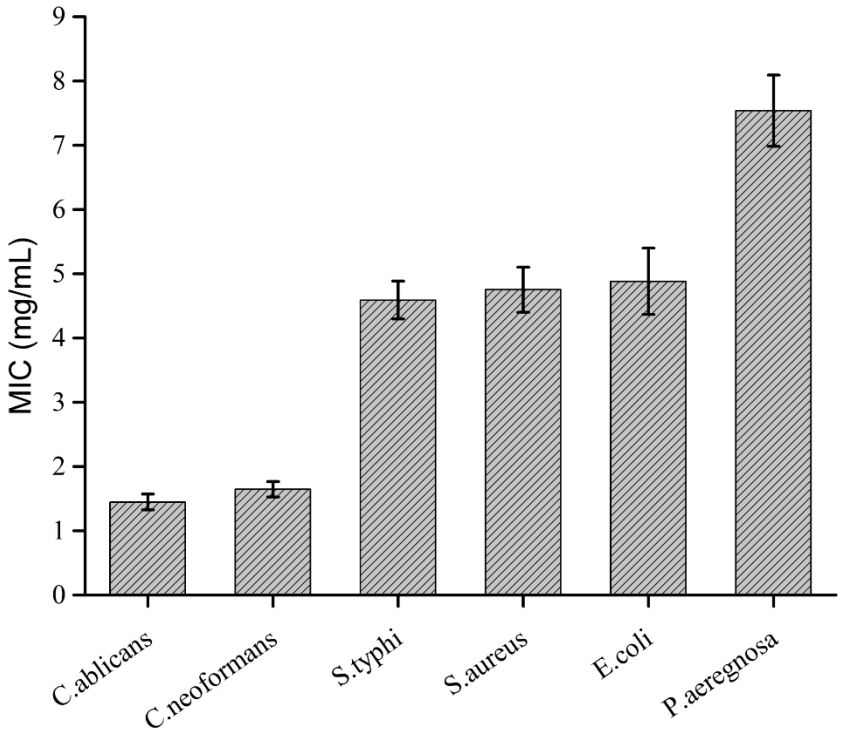

Figure 4: Microbial susceptibility to the extracts (n=72), P > 0.05

4.2.3 Susceptibility of the tested organisms as influenced by the combined effect of both plant varieties and solvents

Interaction between solvents and the banana leaves' varieties in prompting the antimicrobial susceptibility of the studied microorganisms was statistically significant ($p < 0.05$), with *kimalindi* extract showing best effect when extracted in combined solvents (EA: MeOH) and also when used in individual solvents (Fig. 5). This result further confirms the observed individual effect of studied banana leaves extracts (susceptibility of tested microorganisms to tested banana leaves extracts), further affirming the potential of using kimalindi leaves in wound dressing.

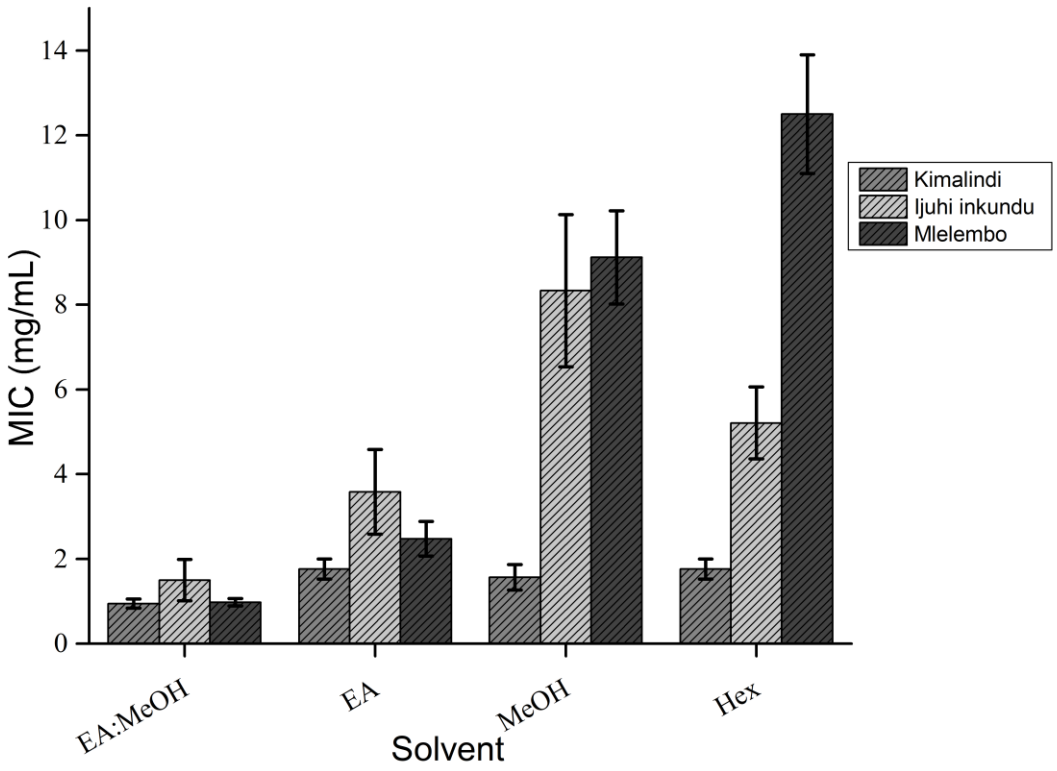

Figure 5: Interaction between solvent and banana leaves varieties influencing the tested microorganisms' susceptibility, P < 0.05

4.2.4 General discussion on antimicrobial activity of banana leaves extracts

Based on the arguments by Eloff (1998), the obtained MIC values are groups as follows: 0.05-0.5 mg/mL are potent antimicrobial agents, 0.6-1.5 mg/mL are considered to be moderate, and the MIC values ranged above 1.5 mg/mL are considered as weak antimicrobial agent. From the obtained results (Table 3), MIC values ranged from strong to weak; ethyl acetate exhibited the most strong antimicrobial activity while ethyl acetate: methanol (1:1) extracts of *kimalindi* and *ijihu inkundu* (MIC = 0.1953 mg/mL) against the *C. ablicans, C. neoformans* and *P. aeruginosa* respectively.

The ethyl acetate extracts from *ijihu inkundu* had strong antimicrobial activity (MIC = 0.3906 mg/mL) against *E. coli, C. ablicans,* and C. *neoformans.* Other tested organisms against the ethyl acetate and n-hexane extracts of ijihu inkundu had a weak antimicrobial activity with the MIC values of > 1.5 mg/mL. The ethyl acetate: methanol extracts of *mlelembo* had potent activity against *C. ablicans (*MIC= 0.3906 mg/mL) and moderate antimicrobial activity against

C. neoformans and *E. coli (*MIC= 0.7813 mg/mL). The rest of the tested organisms were less susceptible to the ethyl acetate extracts of *mlelembo* (MIC > 1.5 mg/mL).

The ethyl acetate extracts from *mlelembo* showed moderate antimicrobial activity against *C. ablicans* (MIC= 0.7813 mg/mL), while other tested organisms tested against the ethyl acetate extracts of mlelembo were weakly inhibited (MIC > 1.5 mg/mL). Both methanolic and n-hexane extracts of mlelembo had weak antimicrobial activity by inhibiting the tested organisms to a concentration above 1.5 mg/mL.

The n-hexane, ethyl acetate, and methanol extracts of *kimalindi* had strong antimicrobial activity on *P. aeruginosa* and C. neoformans (MIC = 0.3906 mg/mL), n-hexane and ethyl acetate extract of kimalindi exhibited further moderate antimicrobial activity on *C. ablicans* (MIC= 0.7813 mg/mL), other tested organisms against the extracts of *kimalindi* were weakly inhibited (MIC > 1.5 mg/mL). The recorded MIC values correlate with the toxicity result from the brim shrimps' assay, which demonstrated that most of the extracts were practically safe, highlighting their potential for banana leaves as a dressing material.

Several studies on the medicinal values of different parts of banana, including leaves, pseudostem, fruits, peels, and pulps, have demonstrated the antimicrobial potential of bananas to one of the factors underlying their medicinal values as reviewed by Singh *et al.* (2016). Their antimicrobial potential is due to their secondary metabolites such as tannins (Javed *et al.*, 2020; Shohayeb *et al.*, 2013), saponins (Benzidia *et al.*, 2019). A study by Asuquo and Udobi (2016) has reported the potential antimicrobial activity against the gram-positive and gram-negative bacterial of clinical importance, whereby *S. aureus* and *Shigella dysenteriae* were susceptible against the aqueous leaf extracts to the concentration of 3.125 mg/ mL of the (Asuquo & Udobi, 2016).

Jouneghani *et al.* (2020) have shown the leaf extracts of ten different studied banana cultivars to be more active against eight human pathogens than the extracts from the pseudostems and corn waste (Jouneghani *et al.*, 2020). They further demonstrated the ethanol and acetone extract to be more effective than the aqueous and n-hexane extracts. Karuppiah and Mustaffa (2013) have shown that ethyl acetate extracts of M. paradisiaca leaf exhibited the highest antimicrobial activity against *E. coli*, *P. aeruginosa,* and *Citrobacter sp* compared to n-hexane and methanol extracts (Karuppiah & Mustaffa, 2013). Thus, both studies have pinpointed the potential health

benefits associated with banana leaves and the role of solvent in enhancing the extraction of potentially bioactive compounds of medicinal importance.

In conclusion, the aforementioned results on the antimicrobial activity of the studied 12 extracts from the banana leaves have pinpointed their antimicrobial potential, which affirms their possible use in wound management, particularly, wound infected with fungi (*C. ablicans and C. neoformans*) as well as *S. aureus*, all of which are commonly occurring wound infections. Application of studied banana leaves may thus possibly reduce the period required for complete wound healing, hence reducing the wound dressing cost. The extracts from ethyl acetate and ethyl acetate: methanol that showed promising activity gives a possibility of the fractionation for further studies.

4.3 Antioxidant of banana leaves extracts

The studied banana leaves had their extracts evaluated for their antioxidant property by evaluating the ability of the methanolic, ethyl acetate: methanol (1:1), ethyl acetate, and n-hexane extracts from *kimalindi, mlelembo,* and *ijihu inkundu* leave to scavenge DPPH free radical. Scavenging of the free radical by the banana extracts was determined by measuring the decrease in the reaction mixture's absorbance. Following antioxidant assay, all extracts exhibited promising antioxidant activity by reducing the absorbance value of the methanolic DPPH solution.

For the case of methanolic extracts, *kimalindi* extracts had the best scavenging activity with an IC_{50} value of 62.47 ± 5.30%, followed by the extracts from the *ijihu inkundu* and *mlelembo*, which had the average scavenging activity values of 58.68±4.66%, and 45.75 ± 9.01% respectively (Fig. 6). The ethyl acetate: methanol (1:1) extracts had a promising antioxidant activity, like for the case of methanolic extracts, the scavenging activity of ethyl acetate: methanolic extracts was in according to kimalindi > ijihu inkundu > mlelembo with the average scavenging activity of 86.36 ± 4.24 %, 70.97 ± 5.60% and 56.25 ± 6.42 % respectively (Fig. 7).

Among the ethyl acetate extracts, the best scavenging activity was on the *kimalind*i ethyl acetate extracts (% scavenging value = 68.82 ± 8.51%), followed by the ethyl acetate extracts of ijihu inkundu and mlelembo, which had the % scavenging values of 64.81 ± 7.11% and 54.65 ± 7.45% respectively (Fig. 8). The least scavenging activity was exhibited by the extracts

of n-hexane as follows: kimalindi (65.18 ± 3.32 %), ijihu inkundu (40.47 ± 5.39 %), and mlelembo (35.31 ± 4.5 %) ((Fig. 9).

The results above show that among all extracts from the three banana varieties, the *kimalindi* extract had better scavenging activity against the DPPH free radicals, and hence better antioxidant activity than the other extract *(ijihu inkundu* and *mlelembo).* Thus, the *kimalindi* leaves present a better option, when choosing better wound dressing material, than the other two studied leaves.

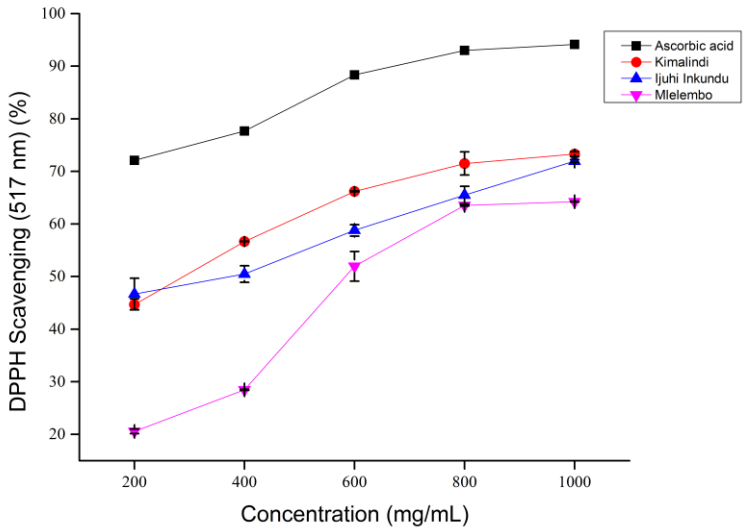

Figure 6: Percentage inhibition of DPPH free radicals exhibited by methanolic from the three banana leaves varieties

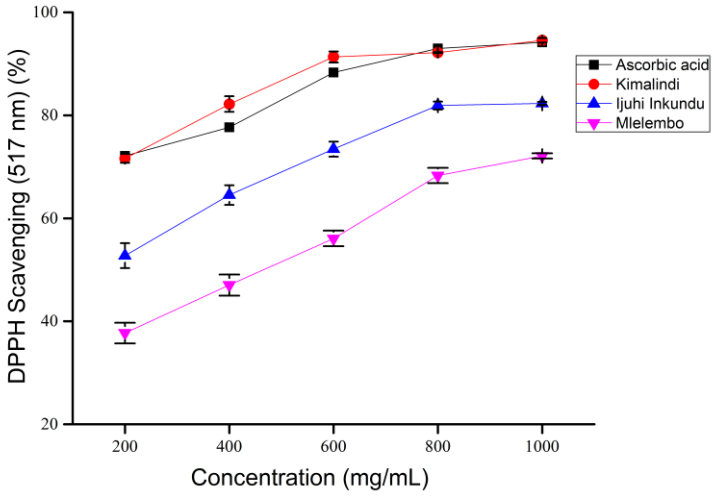

Figure 7: Percentage inhibition of DPPH free radicals exhibited by ethyl acetate: methanol (1:1) extracts from the three banana leaves varieties

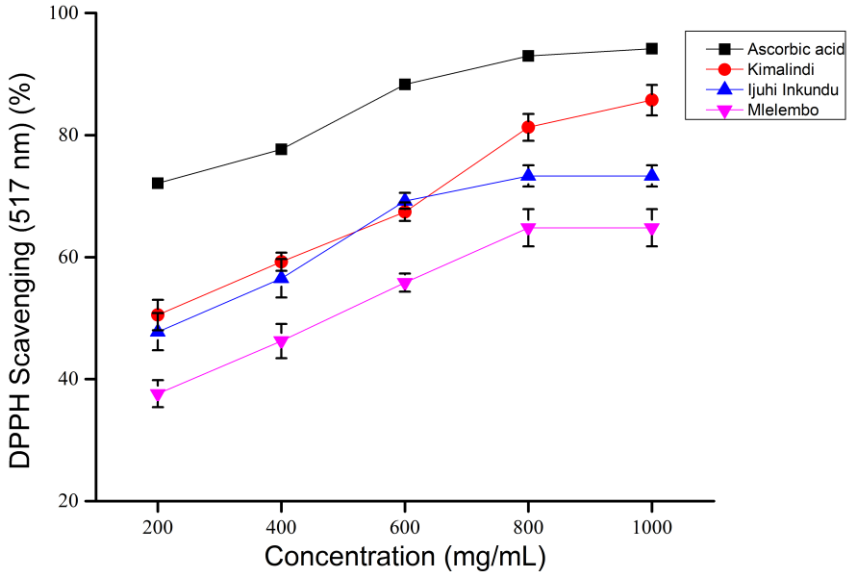

Figure 8: Percentage inhibition of DPPH free radicals exhibited by ethyl acetate extracts from the three banana leaves varieties

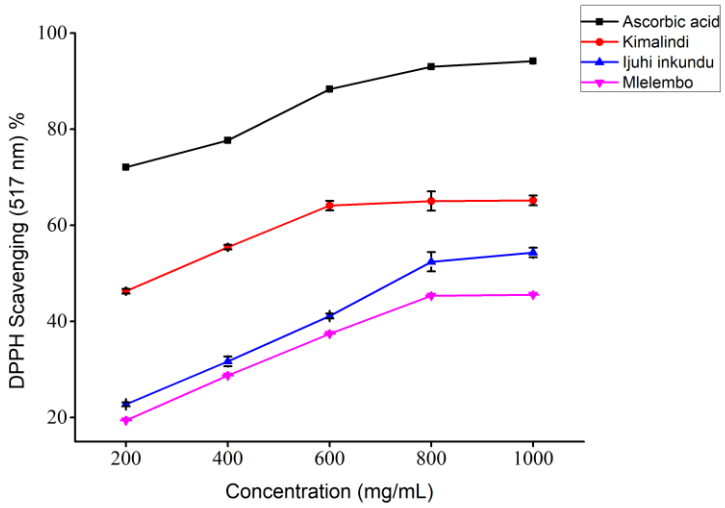

Figure 9: Percentage inhibition of DPPH free radicals exhibited by n-hexane extracts from the three banana leaves varieties

4.3.1 Half scavenging activity exhibited by banana leaves extracts

The concentration providing the half scavenging inhibitory (IC_{50}) of DPPH was calculated from the standard curve's linear equation (scavenging activity versus concentrations). For the case of the methanolic extracts, an IC_{50} was in the order of KILMeOH > IJLMeOH > MLLMeOH, with the IC_{50} values of 253.61mg/mL, 335.18 mg/ml, and 616.40 mg/mL of the methanolic extracts, respectively (Fig. 10). These results are parallel with % inhibition of which the *kimalindi* extracts exhibited the most % scavenging than their counterparts extracts. Among the ethyl acetate: methanol (1:1) extracts, the low IC_{50} value was shown by kimalindi (IC_{50} = 123.49 mg/mL), followed by ijihu inkundu (IC_{50} = 459.29 mg/mL), the least half scavenging activity was on mlelembo, which scavenge the 50% of the free radicals at a concentration of 645.16 mg/mL of the ethyl acetate: methanol (1:1) extracts (Fig. 11). In all ethyl acetate extracts, kimalindi extracts of ethyl acetate had the most scavenging activity whereby its IC_{50} value was at 193.85 mg/mL of ethyl acetate extracts, while the ethyl acetate extracts of ijihu inkundu and mlelembo had the half scavenging activity at 209.31 mg/mL and 478.76 mg/mL of ethyl acetate extracts respectively (Fig. 12).

For the case of *n*-hexane extracts, the best half scavenging concentrations were on the *n*-hexane extracts of *kimalindi* (IC_{50} = 210.59 mg/mL), whereby the extracts of *ijihu inkundu* and *mlelembo* required a high concentration to scavenge half of the free radicals at a concentration of 828.04 mg/mL and 1025.22 mg/mL of *n*-hexane extracts respectively (Fig. 13).

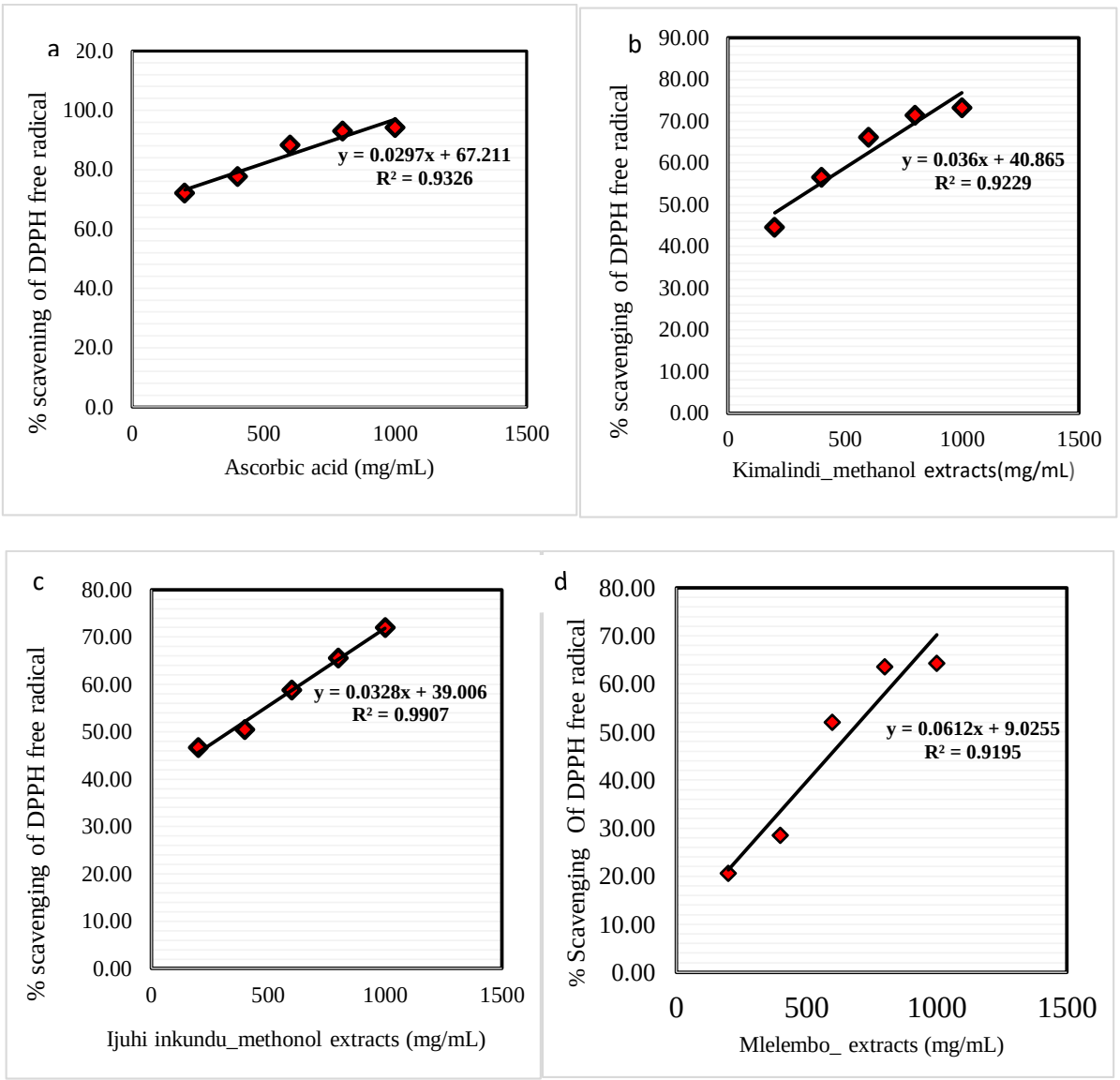

Figure 10: Linear fitting, (a) - standard ascorbic acid, (b)-methanolic extracts of kimalindi, (c) -methanolic extract of *ijihu inkundu*, and (d)-for the methanolic extract of *mlelembo*

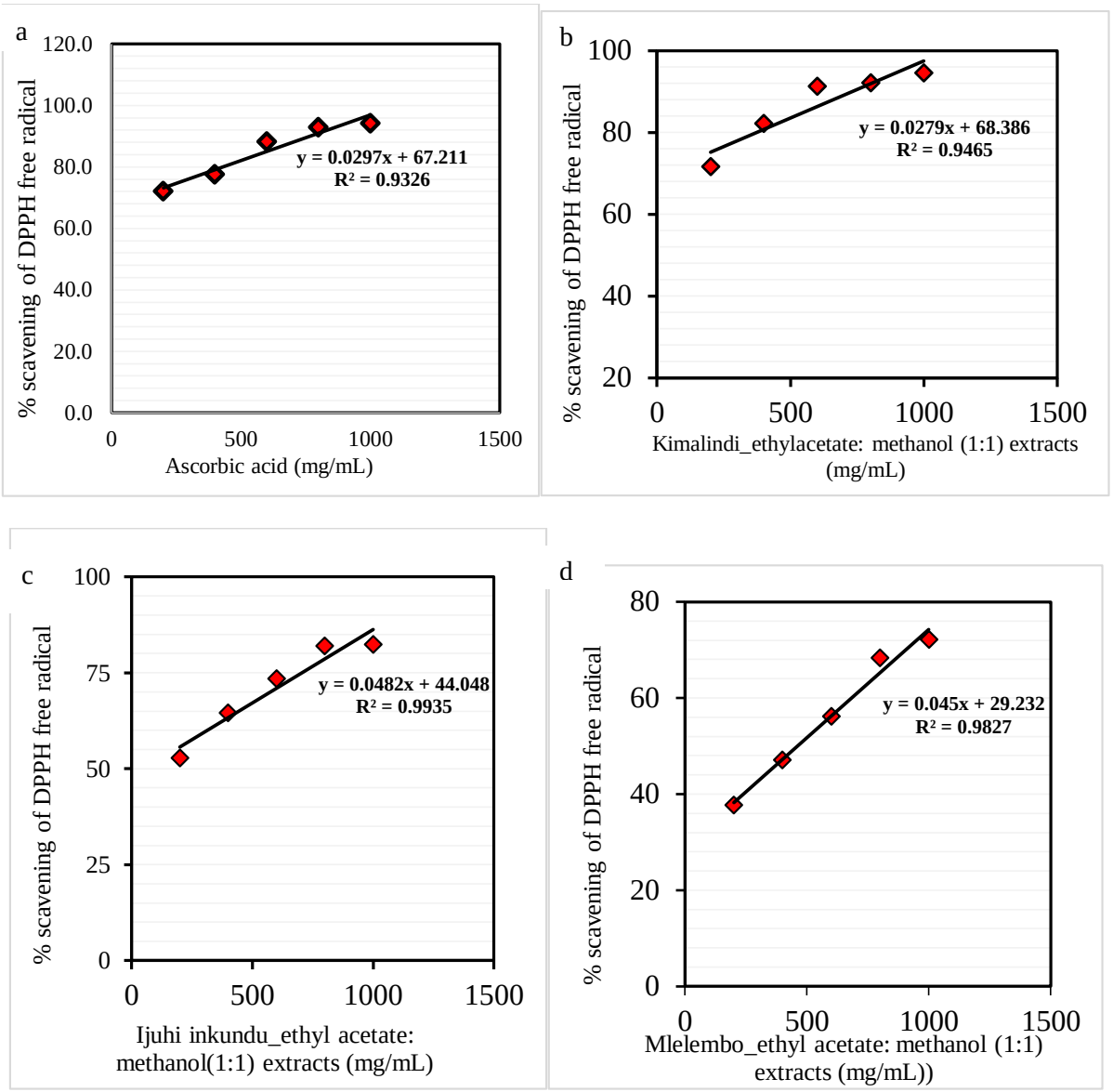

Figure 11: Linear fitting (a) standard ascorbic acid, (b) ethyl acetate: methanolic extracts of kimalindi, (c) ethyl acetate: methanolic extract of ijihu inkundu, and (d) for ethyl acetate: methanolic extract of mlelembo

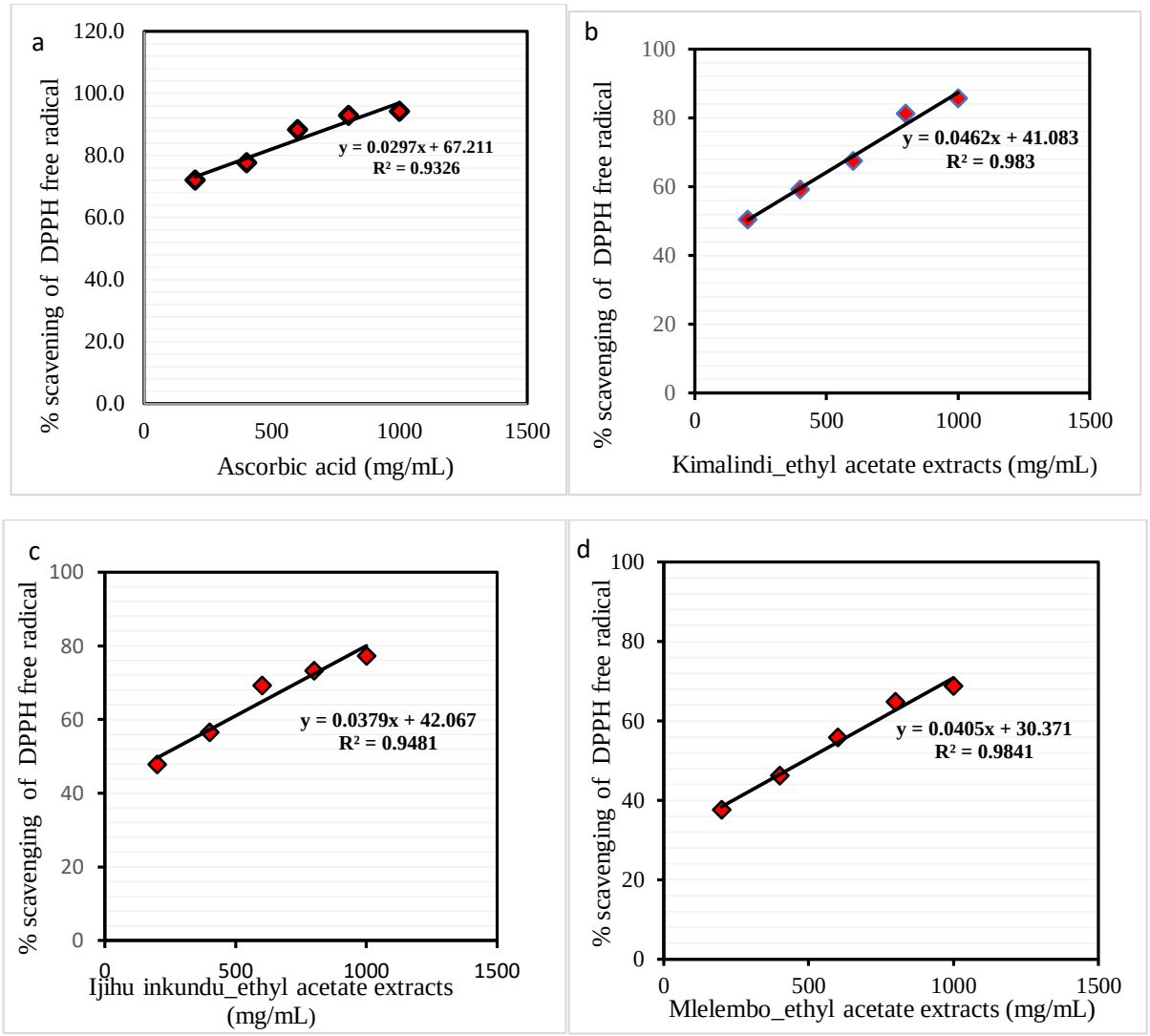

Figure 12: Linear fitting (a) standard ascorbic acid, (b) ethyl acetate extracts of kimalindi, (c) ethyl acetate extract of ijihu inkundu, and (d) for ethyl acetate extracts of mlelembo

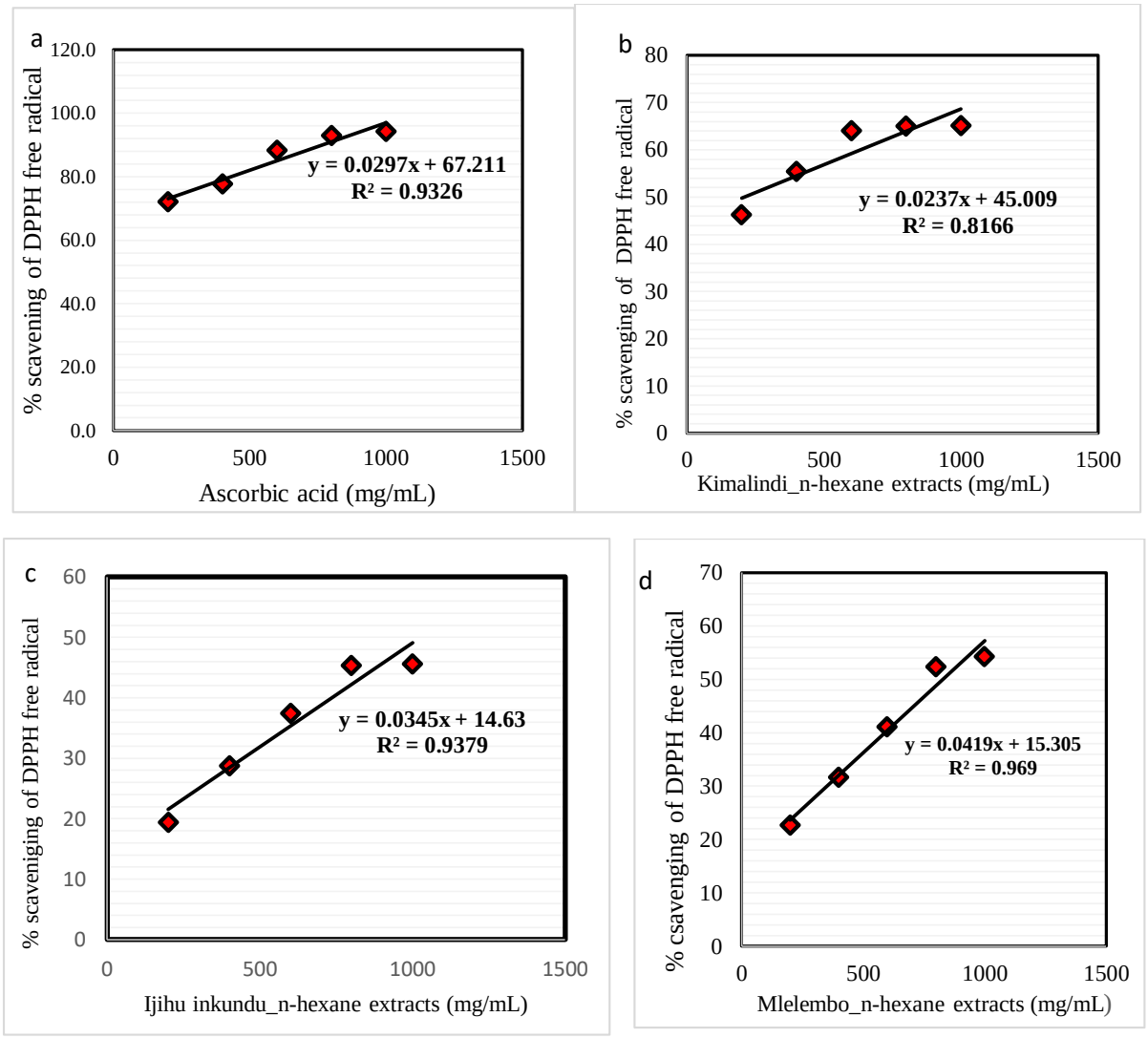

Figure 13: Linear fitting (a) standard ascorbic acid, (b) *n*-hexane extracts of kimalindi, (c) *n*-hexane extracts of ijihu inkundu, and (d) for *n*-hexane extracts of mlelembo

4.3.2 General discussion on antioxidant activity of studied banana leaf extracts

The obtained antioxidant results from the leaf extracts have pinpointed the reducing potential of the three locally grown bananas (*Kimalindi, Mlelembo,* and *Ijihu inkundu*) in Arusha, Tanzania. DPPH was used as an oxidants/ reactive oxygen species (ROS) to study the scavenging ability of the three studied banana leaves; the scavenging power was studied by comparing the % inhibition exhibited by the plant extracts. Ascorbic acid with % scavenging values of 85.05 ± 4.35 was used as a standard to validate the scavenging potential of the studied extracts. Among the three studied banana leaves, the best scavenging activity was exhibited by the kimalindi leaves in all solvents used.

Among the methanol extracts, the methanol extracts from *kimalindi* had the best scavenging activity, inhibited DPPH free radical to 62.47 ± 5.30 %, followed by the methanolic extracts from mlelembo and *ijihu inkundu*, which had % scavenging values of 58.68 ± 4.66% and 45.75±9.01% respectively. Furthermore, *kimalindi* extracts scavenging half of the free radical at a concentration of 235.61 mg/mL of methanolic extracts, followed by the *ijihu inkundu* (IC_{50} = 335.18 mg/mL) and *mlelembo* (IC_{50} = 669.51 mg/mL).

For the case of ethyl acetate: methanol (1:1) extracts, the most scavenging activity was on *kimalindi* (86.36 ± 4.24%), while both *ijihu inkundu* and *mlelembo* had % scavenging of DPPH values of 70.98 ±5.6 and 56.25 ± 6.42, respectively. Unlike for the methanolic extracts, the best half scavenging activity was recorded on *ijihu inkundu* (IC_{50} = 123. 49 mg/mL), followed by *mlelembo* (IC_{50} = 459.29 mg/mL) and *kimalindi* (IC_{50} = 645.16 mg/mL). The results tell that despite its high scavenging, it is threefold less effective and fivefold less effective in scavenging half of DPPH free radicals than the ethyl acetate: methanol (1:1) extracts from *mlelembo* and *ijihu inkundu,* respectively.

Among the ethyl acetate extracts, kimalindi extracts exhibited the most scavenging activity (68.82 ± 8.51%), followed by *ijihu inkundu* and *mlelembo*, which had the % scavenging of DPPH free radicals' values of 64.81 ± 7.11 and 54.65 ± 4.66, respectively. Subsequently, *kimalindi* extracts from ethyl acetate required a concentration of 193.85 mg/mL of extracts to scavenge half of all DPPH free radicals followed by *ijihu inkundu* (IC_{50} = 209.31 mg/mL) and *mlelembo* (IC_{50} = 484.67 mg/mL), indicating that the *kimalindi* ethyl acetate extracts required much lower concentration than its counterparts ijihu inkundu and mlelembo extracts to scavenge half of all the DPPH oxidants.

The *n*-hexane extracts exhibited the least scavenging activity among the four solvents; however, *kimalindi* extracts had a potent scavenging activity (65.18 ± 3.32%), followed by the *n*-hexane extracts of ijihu inkundu and mlelembo, which had the % scavenging values of 40.47 ± 5.39 and 35.31 ± 4.50 respectively. Like for the methanol and ethyl acetate extracts, the *n*-hexane extracts from kimalindi required low concentration to scavenge the half of all DPPH free radicals (IC_{50} = 210.59 mg/mL) followed by *ijihu inkundu* (IC_{50}= 828.04 mg/mL) and *mlelembo* (IC_{50} = 1025.22 mg/mL).

The above-discussed results are parallel with the presence of various phenolic phytoconstituents screened in this study (Table 2). Several studies have indicated the role of phenolic compounds from the banana leaf in ROS scavenging as reviewed by Kumar *et al.* (2012) and Singh *et al.* (2016). In an attempt to find the correlation between phenolic compounds and IC_{50} of extracts against DPPH and (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS), (Fidrianny *et al*., 2015) revealed a negative and high correlation between the phenolic contents from the leaves and peduncle extracts and their DPPH and ABTS IC_{50} where the increase in phenolic contents had a significant influence in lowering the IC_{50} of the studied plant extracts (Fidrianny *et al.*, 2015).

Ideally, the antioxidant potent is one of the features that is required for any wound dressing materials (Fitzmaurice *et al.*, 2011). For rapid wound repair, an equilibrium between the ROS and the antioxidants molecules should be maintained (Dunnill *et al.*, 2017; Roy *et al.*, 2006; Schäfer & Werner, 2008). Excess ROS results in tissue damages and eventually hamper the biological process of wound healing; antioxidants may interfere with the chemotaxis movement of immune cells that are cleaning agents of cell debris and microorganisms during the inflammation phase (Fitzmaurice *et al.*, 2011). Therefore, the obtained results should clinically assess the various antioxidant parameters activity in a study involving wound model.

4.4 Brine shrimp's lethality test

The brine shrimps lethality results indicated that most of the extracts, except for the *n*-hexane extracts of *kimalindi* and ethyl acetate extracts of mlelembo, were practically non-toxic to the shrimps (Table 5). Clarkson toxicity index classified the toxicity profiles of the studied extracts. Most banana extracts exhibited mild toxicities by giving the LC_{50} values of > 100 µg/mL of extracts except for the *n*-hexane extracts of kimalindi (KILHex) and ethyl acetate extracts of mlelembo, which had LC_{50} values of 94.39 µg/mL and 78.072 µg/mL respectively. However, we are unsure whether the observed toxicities in KILHex and MLLEA resulted from the used hexane and ethyl acetate solvents or *kimalindi* and *mlelembo* extracts *per se*.

Moreover, methanolic, hexane and ethyl acetate extracts from *ijihu inkundu* represented medium toxicity to the shrimps, with the LC_{50} values of 249.597 µg/mL (174.6335-356.7393, 95% CI), 539.5235 µg/mL (316.8582 - 918.6654, 95% CI), and 306.7989 µg/mL (218.0268-431.7133,95% CI), respectively. Moreover, methanolic extracts from *mlelembo* (MLLMeOH) had low toxicity to the shrimps, with the LC_{50} of 874.8601 µg/mL (473.1148 - 1617.7471 95% CI), while its counterpart extracts of *n*-hexane exhibited a low toxicity to the shrimps with LC_{50} value of 292.4156 µg/mL (196.0747- 436.4303, 95% CI). Methanolic extracts from kimalindi (KILMeOH) had a low toxicity to the shrimps (LC_{50} = 607.4043 µg/mL, 351.0967 -1050.822, 95% CI), while its fellow ethyl acetate extracts (KILEA) had a medium toxicity to the shrimps 129.1273 µg/mL (89.3965- 186.5158, 95% CI) respectively.

Table 5: Brine shrimps' activity of banana leaves extracts

Plant extracts	LC_{50} (µg/mL)	95% CI	Regression line	R^2
KILHex	94.38985	75.0091-123.8115	y = 94.358logx -136.35	0.9551
KILEA	129.1273	89.3965- 186.5158	y = 46.623logx - 48.422	0.9307
KILEA: MeOH	0	0	0	0
KILMeOH	607.4043	351.0967 -1050.822	y = 44.171logx - 72.949	0.9553
MLLHex	292.4156	196.0747- 436.4303	y = 60.545logx - 99.304	0.9573
MLLEA	78.07213	45.1609-134.9676	y = 50.733logx - 46.012	0.9999
MLLEA: MeOH	0	0	0	0
MLLMeOH	874.8601	473.1148 - 1617.7471	y = 39.44 logx - 66.033	0.9430
IJLHex	249.5970	174.6335-356.7393	y = 78.386logx - 137.91	0.8686
IJLEA	539.5235	316.8582 - 918.6654	y = 45.554logx - 74.454	0.9876
IJLEA: MeOH	0	0	0	0
IJLMeOH	306.7989	218.0268-431.7133	y = 81.963logx - 153.83	0.9777
Cyclophosphamide	16.365	12.006 - 22.305	Y = 69.968logx 34.936	0.995

Keynote: 0 = No mortality recorded at all level of concentration **IJLHex** = Ijihu inkundu leaves n-hexane extract, **IJLEA** = Ijihu inkundu leaves ethyl acetate extracts, **IJLMeOH** = Ijihu inkundu leaves methanolic extract, and IJLAE: MeOH = Ijihu inkundu leaves ethyl acetate: methanol extract (1:1). **MLLHex** = mlelembo leaves n-hexane extracts**, MLLEA**= mlelembo leaves ethyl acetate extracts, **MLLMeOH** - Mlelembo leaves methanolic extract, and **MLLEA: MeOH** (1:1) = Mlelembo leaves ethyl acetate: methanol extracts. **KILHex** = kimalindi leaves n-hexane-extracts, **KILAE** = kimalindi leaves ethyl acetate extract, **KILMeOH** = kimalindi leaves methanolic extracts, and **KILEA: MeOH (1:1)** = Kimalindi leaves ethyl acetate: methanol extracts.

4.4.1 General discussion on brine shrimps lethality assay

The brine shrimps results were interpreted by considering Clarkson toxicity index t as follows: LC_{50} < 100 µg/mL as moderate toxic and LC_{50} > 100 µg/mL as non-toxic, Cyclophosphamide with LC_{50} = 16.3 µg/mL was used as a positive control to monitor the potential yield of some anticancer drugs. There was no mortality at all levels of the tested concentration against ethyl acetate: methanol (1:1) extracts of *kimalindi*, *mlelembo,* and *ijihu inkundu;* hence acute exposure is well tolerated.

Toxicity results indicated that 58% of all extracts had LC_{50}> 100 µg/mL, suggesting that they are practically non-toxic. The obtained toxicity results from methanolic extracts: KILMeOH

(607.404 µg/mL), MLLMeOH (874.86 µg/mL), and IJLMeOH (306.799 µg/mL), supports the possible applicability of using alcohol as a means of preparing banana leaves extracts for the topical appliance in wounds, at Nkoroanga hospital, Arumeru district.

These results further affirm the fact that the use of banana leaves from the locally grown banana should be well utilized as the cheaply available dressing biomaterial among the community of Arusha, since the incidence of burns and cutting wounds is high among the community that cannot afford daily wound management medications. Several studies have demonstrated bananas' safety profile as a backup for their clinical utility; most of those studies have focused their attention on the other parts of bananas, such as fruits, pulps, peels, and flowers. Therefore, this is the first study within a region to demonstrate the safety profile of locally grown banana leaves using brine shrimps modes to affirm their possible property of wound dressings materials.

The study that involved leaves from dessert banana showed that the cytotoxicity activity of hexane, acetone, ethanol and water extracts was 5 to 10 fold lower than the standard antiviral drugs, thus suggesting their therapeutical potential (Panda *et al.*, 2020). Few studies on the *in vivo* safety profiles of banana leaves have shown that the plants are practically non-toxic. In one study, the aqueous extracts from the leaves of *Musa paradisiaca* and its fractions had mild toxicity on Swiss Albino mice with the median lethal dose of 489.9 mg/kg body weight (Asuquo & Udobi, 2016). A study on methanolic extracts from the flower parts of *Musa paradisiaca* has reported an absence of acute toxicity in Albino Wister rats with an LD_{50} value of > 5000 mg/kg body weight (Jawla *et al.*, 2012).

Even though the brine shrimps results signify the locally grown banana's applicability as wound dressing materials, further *in vivo* studies are required to correlate these results.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Wound healing is an essential aspect of the quality of life of the patients. This study has highlighted the potential use of banana leaves from the locally grown bananas commonly grown in Arusha, Tanzania (*kimalindi*, *mlelembo*, and *Ijihu inkundu*). Results from the antimicrobial and antioxidant properties and toxicity profiles affirmed their possible use in wound dressing. Findings from this study suggest that *kimalindi* leaves present a better option when choosing which banana leaves among the studied three is to be used in wound dressing, based on antimicrobial, anti-oxidant, and toxicity results. Ethyl acetate and methanol solvents gave extract which exhibited the best antioxidant and antimicrobial properties. Two extracts gave the LC_{50} of < 100 µg/mL, indicating their potential of having anticancer activity; the rest of the extracts had LC_{50} > 100 µg/mL, indicating their safety upon acute exposure when applied as wound dressing materials. Finally, this alternative wound dressing biomaterial needs to be tested in a controlled clinical trial and compared with modern wound dressing material to get them licensed as medical devices.

5.2 Recommendations

(i) There is a need to conduct fractionation studies against the crude extracts that exhibited the potent activity against the tested microorganisms.

(ii) As this is the first study to assess the antioxidant potential of the extracts from locally grown bananas by observing their ability to scavenge DPPH free radicals: other assays for determination of the antioxidant ability of plant extracts such as hydrogen peroxide, ABTS, and ferric reducing assay should be incorporated in the future studies of the same kind.

(iii) The extracts that gave the LC_{50} < 100 µg/mL should be fractionated for assessing their possible anticancer activity.

(iv) A further trial of the studied banana leaves' extracts on animals/humans is warranted. These should include comparing them with currently used conventional wound dressing methods in the study area.

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APPENDICES

Appendix 1: Anova for MIC for 12 extracts

Univariate (one factor/ plant extracts) Test signified statistical difference among 12 extracts in inhibiting tested microorganisms.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	969.8884	11	88.17167	4.937266	2.08E-05	1.952212
Within Groups	1071.504	60	17.8584			
Total	2041.392	71				

Appendix 2: Descriptive statistics for the extracts MIC

Banana leaves extracts	Count	Average	Variance	SE of Mean	Standard deviation
IJLHex	6	5.208333	17.2526	1.6971	4.15363
IJLAE	6	3.580729	24.25639	2.01065	4.92508
IJLAE: MeOH	6	1.497396	5.686442	0.97	2.38463
IJLMeOH	6	8.333333	83.65885	3.73405	9.14652
MLLHex	6	12.5	46.875	2.79508	6.84653
MLLEA	6	2.473958	4.007975	0.81731	2.00199
MLLEA: MeOH	6	0.976563	0.228882	0.19531	0.47842
MLLMeOH	6	9.114583	27.75065	2.16061	5.2789
KILHex	6	1.757813	1.327515	0.4704	1.15218
KILEA	6	1.757813	1.327515	0.4704	1.28372
KILEA: MeOH	6	0.94401	0.281016	0.21642	0.53011
KILMeOH	6	1.5625	1.647949	0.52408	2.07314

Appendix 3: Anova for microorganism's susceptibility

Univariate (one factor/ microorganisms) Test signified statistical difference among six tested microorganisms

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	313.6237	5	62.72475	2.396058	0.04658	2.353809
Within Groups	1727.769	66	26.17831			
Total	2041.392	71				

Appendix 4: Descriptive statistic for the tested microorganism's susceptibility

Tested organisms	Count	Average	Variance	SE of Mean	Standard deviation
E. coli	12	4.882813	51.81052	2.07787	7.19795
S. typhi	12	4.589844	16.76039	1.18182	4.09395
P. aeruginosa	12	4.752604	23.79909	1.40828	4.87843
S. aureus	12	7.535807	59.05469	2.21838	7.68471
C. ablicans	12	1.448568	2.902349	0.4918	1.7033
C. neoformans	12	1.64388	2.742825	0.47809	1.65615

Appendix 5: Anova for interaction between solvent and banana variety for MIC

Multivariate (two factors with replication/ banana leaves and solvents) Test signified statistical difference among 12 extracts in inhibiting tested microorganisms

	Source of Variation	SS	df	MS	F	P-value	F crit
	Plant	281.4388	2	140.7194	7.8797328	0.000915	3.150411
	Solvent	390.6944	3	130.2315	7.2924492	0.0003	2.758078
	Interaction	297.7551	6	49.62586	2.7788524	0.018847	2.254053
	Within	1071.504	60	17.8584			
	Total	2041.392	71				

Appendix 6: Descriptive statistic for interaction between banana leaves and solvent

Banana	Solvent	Count	Average	Standard deviation	SE of Mean	Variance
Ijihu Inkundu	Hex	6	5.20833	4.15363	1.69571	17.2526
	AE	6	3.58073	4.92508	2.01065	24.25639
	MeOH	6	8.33333	9.14652	3.73405	83.65885
	AE: MeOH	6	1.4974	2.38463	0.97352	5.68644
Mlelembo	Hex	6	12.5	2.38463	2.79508	46.875
	AE	6	2.47396	6.84653	0.81731	4.00798
	MeOH	6	9.11458	2.00199	2.15061	27.75065
	AE: MeOH	6	0.97656	5.26789	0.19531	0.22888
Kimalindi	Hex	6	1.75781	1.15218	0.47037	1.32751
	AE	6	1.75781	1.15218	0.47037	1.32751
	MeOH	6	1.5625	1.28372	0.52408	1.64795
	AE: MeOH	6	0.94401	0.53011	0.21642	0.28102
N		72				

Appendix 7: Anova for % scavenging of DPPH free radical by banana leaves extracts

Univariate (one factor/ banana leaves extracts) Test, signified statistical difference in scavenging of DPPH free radical.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	10701.336	11	972.8487	5.774075	7.53E-06	1.99458
Within Groups	8087.3107	48	168.4856			
Total	18788.646	59				

Appendix 8: Descriptive statistic for % scavenging of banana leaves extracts

Groups	Count	Average	Variance	SE of Mean	Standard deviation
IJLHex	5	40.466	181.48	6.024615838	13.47145055
IJLAE	5	64.81045	151.5445	5.505352482	12.31034239
IJLAE: MeOH	5	70.97688	156.97	5.60303456	12.52876616
IJLMeOH	5	58.68447	108.5806	4.660056709	10.42020358
MLLHex	5	35.306	126.6091	5.032078696	11.25207003
MLLEA	5	54.6528	166.4326	5.769447889	12.90087767
MLLEA: MeOH	5	56.2478	206.299	6.42337902	14.36311213
MLLMeOH	5	45.74569	407.3266	9.025813645	20.18233286
KILHex	5	59.22129	68.70337	3.70684156	8.28874971
KILEA	5	68.82346	217.4618	6.594873289	14.74658498
KILEA: MeOH	5	86.36379	89.89596	4.240187835	9.481348237
KILMeOH	5	62.47278	140.5242	5.301399087	11.85428873
N	60				

Appendix 9: Anova for the interaction between banana leaves and solvents

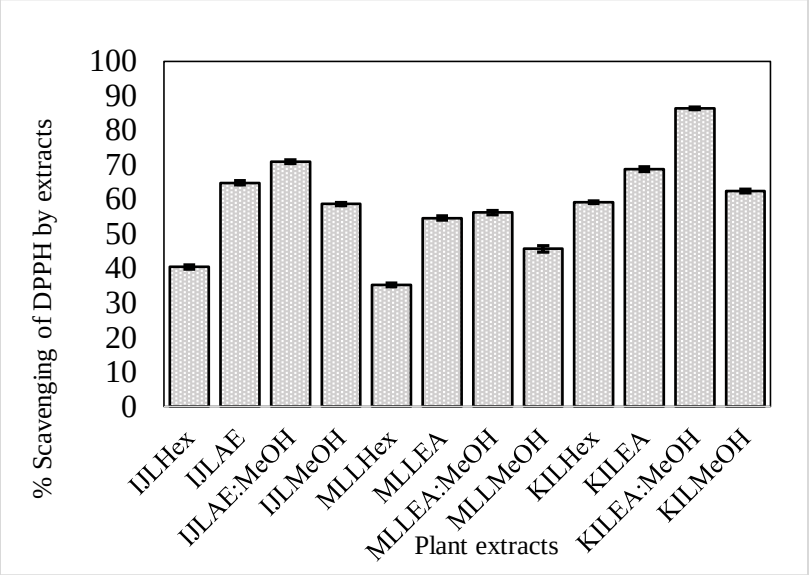
Multivariate (two factors with replication/ banana leaves and solvents) test, signified statistical difference among 12 extracts in DPPH scavenging

Source of Variation	SS	df	MS	F	P-value	F crit
Plant	4508.313	2	2254.156	13.37892	0.0000241	3.190727
Solvent	5546.917	3	1848.972	10.97406	0.0000133	2.798061
Interaction	646.1059	6	107.6843	0.639131	0.6983203	2.294601
Within	8087.311	48	168.4856			
Total	18788.65	59				

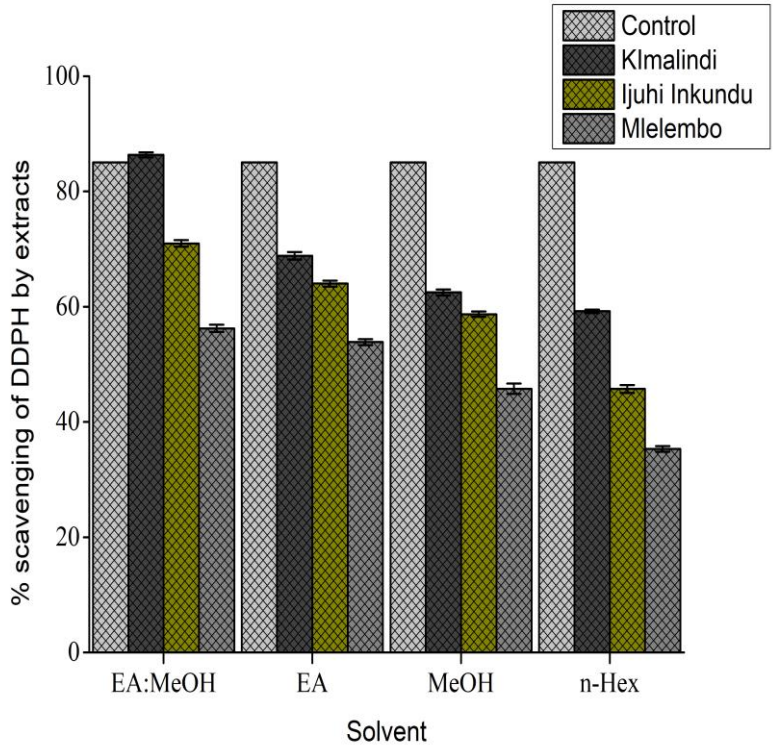
Appendix 10: Descriptive statistics for interaction between banana leaves and solvents in scavenging of DPPH free radical

Solvent	Banana	Count	Average	Standard deviation	SE of Mean	Variance
AE: MeOH	Kimalindi	5	86.36379	9.48135	4.24019	89.89596
	Ijihu inkundu	5	70.97788	12.5299	5.60354	156.99829
	Mlelembo	5	56.2478	14.36311	6.42338	206.29899
AE	Kimalindi	5	68.82346	14.74658	6.59487	217.46177
	Ijihu inkundu	5	64.01045	11.39297	5.09509	129.79982
	Mlelembo	5	53.8528	11.88883	5.31685	141.34424
MeOH	Kimalindi	5	62.47278	11.85429	5.3014	140.52416
	Ijihu inkundu	5	55.3638	8.44166	4.22083	71.26159
	Mlelembo	5	45.74569	20.18233	9.02581	407.32656
Hex	Kimalindi	5	59.22129	8.28875	3.70684	68.70337
	Ijuhi inkundu	5	45.7162	17.62304	7.19457	310.57136
	Mlelembo	5	35.306	11.25207	5.03208	126.60908
N		60				

Appendix 11: Percentage scavenging of DDPH free radical by banana leaves extracts



Appendix 12: Interaction of banana leaves and solvent in scavenging of DPPH free radical



Appendix 13: Percentage mortality of shrimps

	Concentration (µg/mL)/ % Mortality					
	240	**120**	**80**	**40**	**24**	**8**
Plant extracts						
KILHex	45	35	5	0	0	0
KILEA	35	20	10	0	0	0
KILEA: MeOH	¤	¤	¤	¤	¤	¤
KILMeOH	40	20	10	0	0	0
MLLHex	45	30	10	0	0	0
MLLEA	70	55	50	45	38	0
MLLEA: MeOH	¤	¤	¤	¤	¤	¤
MLLMeOH	30	15	5	0	0	0
IJLHex	85	70	40	5	0	0
IJLEA	60	40	35	30	10	0
IJLEA: MeOH	¤	¤	¤	¤	¤	¤
IJLMeOH	35	15	10	0	0	0

RESEARCH OUTPUTS

Accepted research paper for publication

Telesphory, A., Grosche S., Elingarami, S., Vianney, J. M., & Swai, H. (2021). Biological properties of extracts from locally grown banana leaves indicate their possibleuse for wound dressing in Arusha, Tanzania. *International Journal of Pharmaceutical Sciences and Research, 12*(10), 10-11. http://dx.doi.org/10.13040/IJPSR.0975-8232.12(10). 1000-13

Poster presentation

Biological properties and safety profile of extracts from locally grown banana leaves in Arusha, Tanzania: For their possible use in wound dressing