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BIOLOGICAL PROPERTIES OF EXTRACTS FROM LOCALLY GROWN BANANA LEAVES INDICATE THEIR POSSIBLE USE FOR WOUND DRESSING IN ARUSHA, TANZANIA

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ABSTRACT: This 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. Screening for phytoconstituents in extracts from studied banana plant species, ijuhi inkundu (IJ) mlelembo (ML) and kimalindi (KIM), revealed the presence of various 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 ($p < 0.05$). Antioxidant activity was evaluated by measuring the ability of extracts to scavenge DPPH free radicals. Scavenging of DPPH was in the order of > kimalindi > ijuhi inkundu > mlelembo. Brine shrimp results for toxicity showed that almost all extracts were non-toxic to the shrimps, exhibiting mild toxicity by giving the LC^{50} values higher than 100 µg/mL.

INTRODUCTION: Wound dressings are local therapeutic agents intended to create an optimal environment for wound healing, with specific properties according to type and physiologic healing stage of the wounds [1]. Preferably, wound dressing material should combine the following features: a moist environment at the wound interface, antiseptic properties to microorganisms and non-adherent to the wound surface, non-toxic, non-allergenic, and non-sensitizing properties [2, 3]. Modern wound dressing materials comply with

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Most of these requirements, however, they are expensive to the extent that their use is limited in developing countries like Tanzania. Banana trees (*Musa Sapientum*) grow throughout many countries. Their leaves are limitless sources of biomaterial in these countries. They have been used in many ways, including wrappings, plate mats, decorations, cooking and writing surfaces. Furthermore, they have been used in wound dressings as described in early reports; for instance, due to their non-adherent and cooling properties, smallpox patients were treated while lying on a base of banana leaves in India [4].

For the first time, leaves from banana trees were reported in the literature as a wound dressing material for burn patients in 1980 [5]. Gore and Akolekar extended these preliminary reports and published several clinical trials in which they

compared the use of banana leaves on burn patients with Vaseline gauze [6]. Banana leaf dressing resulted in rapid epithelization and was associated with less pain during dressing changes than Vaseline gauze. Moreover, wounds treated with banana leaves had fewer infections and healed simultaneously as wounds treated with Vaseline gauze dressings. These studies provided important baseline knowledge on applying banana leaves as wound dressing material because they had never been studied before from a scientific point of view. Further studies were therefore urgently needed to assess the safety profile and biological properties of banana leaves as alternative wound-dressing materials.

Therefore, this current study assessed the biological properties and safety profile of extracts from locally grown banana leaves in Arusha (Tanzania) to affirm their possible use in wound dressing. This, to our knowledge, is the first of its kind study in Sub-Saharan Africa. The following biological properties of locally grown banana leaves extracts from Arusha were studied herein; phytochemical properties, antimicrobial properties, antioxidant and cytotoxicity properties.

MATERIALS AND METHODS:

Chemicals and Tested Microorganisms: Ethyl acetate (EA), n-Hexane (Hex), and methanol (MeOH) were procured from Fisher Scientific (UK), whilst, Dimethyl sulfoxide (DMSO), para-Iodonitrotetrazolium and Phosphate buffered saline pH 7.4 (PBS) were procured from Sigma (Poole, Dorset, UK). 2, 2-diphenylpicrylhydrazyl (DPPH) was borrowed from the school of Pharmacy, the Muhimbili University of Health and Allied Sciences (MUHAS). 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 obtained from the Department of microbiology (MUHAS). Brine shrimps' eggs were procured from Aquacultures innovations (Grahams town 6140), and sea salt was locally prepared by evaporating ocean water, collected along the Koko coast of the Indian Ocean, Dar es salaam.

Preparation of Plant Materials: Banana leaves were collected from three banana species; two species were of indigenous Mchare bananas *viz*; ijihi inkundu and mlelembo and the remaining species were a Musa Cavendish, locally known as Kimalindi. They were all collected in 2019 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 [7]. The collected banana leaves were dried under shade and finally pulverized into a fine powder for extraction.

Extraction of Banana Leaves Extract: The dried powder of each banana leaves, viz. Ijuhi 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 selected banana leaves powder were first soaked into n-hexane solvent for 48 hours. The solvent-containing extracts were then filtered by a cotton wool plug, followed by a filter paper (Whatman No 1). The filtrate was concentrated by a rotary evaporator 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 h, followed by filtration and concertation as prior. The remaining n-hexane residues were re-soaked into ethyl acetate solvent for 48 h, followed by filtration and concentration by a rotary evaporator at 60 °C and reduced pressure. The obtained residues were re-soaked again into ethyl acetate solvent for an additional 24 h, followed by filtration and concentration as prior. The remaining residues of ethyl acetate were re-soaked into methanol solvents for 48 h, followed by concentration by using rotavapor at 60 °C and reduced pressure. The obtained residues were further subjected to methanol for an additional 24 h, followed by concentration as prior done.

Methanol residues were re-soaked into a mixture of ethyl acetate and methanol (1:1) solvents for 48 h, followed by concentration and filtration as prior, thereafter the obtained residues were then re-soaked again into ethyl acetate and methanol (1:1) solvents for 24 h 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, whilst the plant residues were stored for future use.

Qualitative Phytochemical Screening: A study on major plant extracts phytoconstituents was carried out by using standard qualitative methods for phytochemical screening as defined by various authors with some modifications. A total of seven secondary metabolites, phytoconstituents *viz.* saponins, alkaloids, flavonoids, phenols, tannins, terpenoids, and anthraquinones, were screened.

Test for Saponins (Foam Test): The presence of saponins was detected according to the foam test procedures [8]. Briefly, 5.0 mL of distilled water was mixed with 250 mg of banana leaves methanolic extract in a test tube. Thereafter, a reaction mixture was constantly shaken vigorously. The appearance of foam, which persisted for about 10 min indicated the presence of saponins.

Test for Terpenoids: The presence of terpenoids was detected by the method described by 9 with some modification. About 4 mL of chloroform was mixed with 100 mg methanolic extract. A reaction mixture was then vigorously mixed, followed by the addition of 4 mL of concentrated H_2SO_4 to form a layer. The development of grey coloration at the interface indicated the presence of terpenoids.

Test for Tannins (Braemer's Test): The presence of tannins was detected according to 10 with minor modifications. Briefly, 250 mg methanolic extract was mixed with 5 mL of 45% ethanol. A reaction mixture was thereafter vigorously mixed and boiled. A reaction mixture then filtered, followed by the addition of 1 mL of distilled water and a few drops of 1% ferric chloride. A greenish grey to black coloration indicated the presence of tannins.

Test for Phenols (Ferric Chloride Test): The presence of phenols in the extract was detected by adopting the methods described by 11 with little modifications. Briefly, the ferric chloride test involved 250 mg of methanolic extract, which was mixed with a few drops of 10% $FeCl_3$. The formation of bluish-black color indicated the presence of phenols.

Test for Anthraquinones (Borntrager's Test): Anthraquinones were detected following the method described by 12, with some modification. Briefly, 2 mL of a sample extract was mixed with 4 mL of chloroform in the test tube and lasted for 5 min. A reaction mixture was thereafter mixed with 10 mL of 10% ammonia solution. Violet, pink to red color at the lower layer of the mixture (ammonia), indicated anthraquinones.

Test for Flavonoids (Alkaline Reagent Test): The presence of flavonoids was detected according to the methods described by [9, 11, 12]. Briefly, a small amount of crude extracts was mixed with 2 mL of 2% NaOH. The formation of an intense yellow color that turned colorless upon the addition of dilute HCL solution indicated the presence of flavonoids.

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;

Meyer's Reagent: A creamy-white colored precipitate was considered for the presence of alkaloids.

Wegner's Reagent: A reddish-brown precipitate was taken as an indicator for the presence of alkaloids.

Dragendorff's Reagent: A formation of red precipitate was considered as evidence for the presence of alkaloids.

Antimicrobial Activity: The antimicrobial susceptibility exhibited by the tested microorganisms against plant extracts was determined by broth microdilution assay [13, 14].

The method involves the determination of minimum inhibitory concentrations (MIC) by using 96-microtitre plates.

Experimental Material and Procedures: To address this activity, the following materials, *viz,*

100 mg of plant extract, 1:1 ratio of 100 mg of plant extract mixed in 50:50 mg of plant extracts for synergies, Ketoconazole and ciprofloxacin tablets, SDB and NB, INT-dye, sterile micropipettes and tips, 96-micro-titer plates, digital beam balance and tested microorganisms were used.

Experimental Procedures: The 96-microtiter plates were pre-loaded with 50 µl of either NB for bacterial testing, or SDB for fungal testing, followed by 50 µl of the plant extract (100 mg/mL) into the first well of each tested raw, to reach a maximum volume of 100 µl in the first well. Thereafter, the mixture was in-depth mixed by pipetting up and down within a well, followed by pipetting out 50 µl of a mixture in the first-row wells to the succeeding row wells.

The processes were progressively repeated till the last bottom-row wells remained, and 50µl was discarded. To each well, 50 µl of bacteria suspension of 0.5 Mac Farhl and standard (equivalent to 1.5 × 108 approximate bacterial/fungal load) was added into each well to make a final volume of 100 µl per well. The wells with ciprofloxacin served as a positive control, whilst DMSO was used as a negative control and the wells with bacteria/fungi and broth were left to validate the experiment by monitoring the growth of the tested microorganisms. After that, plates were incubated at 37 °C for 24 h. 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 h. Bacteria/ fungi growth was indicated by the change of INT-dye color into a purple color whilst the persistence of INT color indicated live bacteria/fungi.

Microbial 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, where data were considered as statistically significant at a $P<0.05$. Wherever there was a statistical difference between groups, Tukey and Bonferroni post hoc tests were used to confirm the existence of differences among groups.

Antioxidant Activity of Banana Leaves Extract: Antioxidant activity of banana leaves was determined by the scavenging ability of methanolic extracts of banana leaves on DPPH (1, 1-diphenyl-2-picryl-hydrazy) free radical. Scavenging of DDPH by plant extracts involves the reduction of nitrogen-free radical by hydrogen atom from the phenolic group (s) of plant extracts [15]. 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 [16, 17].

Experimental Materials and Procedures: To address this objective, the following materials were used; DHHP, 80% methanol, 1 g of methanolic extract from each plant, Ascorbic acid as a standard antioxidant compound. A stock solution of 1 g/mL from each sample was diluted into a final working concentration of 200, 400, 600, 800, and 1000 µl/mL in methanol. 1 mL of 0.3 mmol /L of DPPH in methanol was thereafter 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.

After that, the absorbance of the reaction mixture was recorded at 517 nm, whereby decoration of DPPH color indicated scavenging of DPPH free radical, which was marked by decreased optic density (OD). Scavenging inhibition of DPPH by either extract/ ascorbic acid was expressed from the equation (1) below, where Ao stands for OD of control (DPPH in methanol), Ai stands for OD of sample or standard. All experiments were performed in triplicate, and results were expressed as a mean ± SEM.

$$[(Ao-Ai) \div Ao] \times 100$$

DPPH Scavenging Data Analysis: Percentage inhibition by banana leaves extracts, and standard/ ascorbic acid was expressed as a mean ± SEM. A student t-test was used to compare the mean interrelation between two groups (control and banana leave extract). The one-way ANOVA was used for the mean interrelation among groups. Data were considered as statistically significant at a $P<0.05$. Tukey and Bonferroni post hoc tests were used to confirm the existence of differences among groups.

Brine Shrimp Lethality Test (BST): The brine shrimp's lethality assay serves as a hint for toxicity and cytotoxicity of plant extracts [18, 19]. BST is further used for the establishment of important bioactive compounds sourced from plant extracts, with their potential pharmacological importance and half inhibitory/effective concentration [20]. In the present study, toxicity and cytotoxicity of native banana leave extracts, as well as their half inhibitory concentration (IC50), were evaluated by BST. Briefly, the assay involves using Brine shrimp larva (Artemia Salina) to predict active bioactive compounds capable of producing a lethal pharmacological response to the shrimps [18].

Preparation of Brine Shrimp Larva: The brine shrimps were prepared according to [18, 19] with minor modification. 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. After that, 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.

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.

Cytotoxicity Assay: 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 10 brine shrimps larvae each, and volumes were adjusted into 5 mL by addition of 3.8 g/L of brine solutions. All experiments were done in duplicate, where DMSO served as a negative control whilst cyclophosphamide served as a standard anticancer control drug. After 24 h, toxicity and cytotoxicity were evaluated by counting out the dead larvae.

BST Data Analysis: Percentage mortality was plotted against their logarithmic concentrations by using MS. Excel, whereby a linear regression model was used to calculate the concentration that resulted in the killing of the half of the larvae; LC^{50}, LC16, LC84 and 95 confidence interval (95% CI), whereby, the LC^{50} > 100 µg/mL are considered as safe [21].

RESULTS AND DISCUSSION:

Phytochemical Screening of Plant Extracts: Preliminary screening for phytoconstituents in both plants (ijuhi inkundu, mlelembo, and kimalindi) revealed the presence of various secondary metabolites *viz.* anthraquinones, alkaloids, flavonoids, tannins, terpenoids, phenols, phytosterol, and saponins in their methanolic extracts **Table 1.** Such findings agree with previous work by [22, 23] that revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, and steroids on the methanolic extracts of *Musa Sapientum* var.

Sylvestris and Musa *X Paradisiaca*. Studies on banana species have shown the presence of different pharmacologically bioactive compounds from bananas as reviewed by [24]. Such compounds have been reported to present in different parts of banana plants including, leaves, flowers, pulps and banana fruit. The phytoconstituents reported in the present study are known to have medicinal values.

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACTS OF THREE BANANA LEAVES VARIETIES

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

+ = presence of phytoconstituent screened.

Antimicrobial Assay: Antimicrobial activities of all three banana varieties were tested in this study against six pathogenic microorganisms. A total of 12 leaves extracts from kimalindi (KIL), mlelembo (MLL), and ijuhi inkundu, coded as IJL Hex, IJLEA, IJL MeOH, IJLAE: MeOH (1:1), MLL Hex, MLLEA, MLL MeOH MLLEA: MeOH (1:1), KIL Hex, KILEA, KIL MeOH and KILEA: MeOH (1:1 **Table 2.** were used to study the antimicrobial activities of these local banana leaves. The p-INT dye was employed in distinguishing the wells with living microorganisms from the wells with dead microorganisms, of which the wells with the living microorganisms turned p-INT dye into a purple color, whilst the wells with dead microorganisms didn't.

According to [13], antimicrobial susceptibility to the plant crude extract is expressed in minimum inhibitory concentration (MIC), with crude extracts effectiveness grouped into three categories *viz:* strong activity (MIC = 0.05- 0.5 mg/mL), moderate activity (MIC = 0.6 – 1.5 mg/mL) and weak activity (MIC > 1.5 mg/mL). To exhaust more bioactive compounds, four solvents of different polarity *viz.* non-polar (Hex), intermediate polar (EA) and polar (MeOH and EA: MeOH (1:1)) were used. Antimicrobial susceptibility was influenced by both solvents and varieties of banana leaves ($P<0.05$). Depending on the nature of solvent, antimicrobial susceptibility was in the order of EA: MeOH > EA> MeOH > Hex, with average MIC 1.14 ± 0.15, 2.60 ± 0.43, 6.34 ± 1.96 and 6.49 ± 2.59 mg/mL respectively ($p<0.05$).Susceptibility of microorganisms to banana varieties were in the order of KIM > ML > IJ, with average MIC of 1.51 ± 0.17, 4.65 ± 1.25, 6.27 ± 2.36 mg/ mL respectively, with kimalindi being more effective than the rest of plants ($p < 0.05$). This finding suggests that kimalindi leaves present the best option when used to dress wounds, as it has better antimicrobial property compared to the other two studied leaf extracts (mlelembo and ijuhi inkundu). Perhaps this is also another reason why kimalindi leaves are not always frequently infected with bacterial/fungal infections, more research on this is thus warranted.

Antimicrobial Activity of Extracts: A total of twelve extracts from 3 banana leaves' varieties were screened for their antimicrobial activity. KILEA: MeOH and IJLAE: MeOH extracts had the lowest MIC value against *C. albicans, C. Neoformans,* and *P. aeruginosa* (0.1953 mg/mL). IJLEA extract had a promising activity on E. coli, *C. ablicans, and C. neoformans* with the MIC values of 0.391 mg/mL, followed by *S. typhi*, S. *aureus,* and *P. aeruginosa* which were all inhibited to the concentrations of 1.563, 6.25, and 12.50 mg/mL respectively **Table 2.**

KILEA extract showed promising activity against P. aeruginosa and *C. neoformans* (MIC = 0.3963), whereby *E. coli, S. typhi, S. aureus* and *C. ablicans* were all inhibited by the KILEA extract up to the MIC values of 3.125, 3.125, 1.563 and 0.781 mg/ mL respectively **Table 2.** KIL Hex extract had a promising activity against *P. aeruginosa*, with MIC value of 0.391 mg/mL, followed by *C. ablicans* (MIC = 0.781 mg/mL), *C. neoformans* and S. *aureus* (MIC = 1.563 mg/mL). Both *E. coli* and *S. typhi* were all inhibited up to the concentrations of 3.125 mg/ mL **Table 2.** KIL MeOH extract had apromising activity against *P. aeruginosa* (MIC = 0.391 mg/mL), followed by *S. aureus, C. ablicans*, and *C. neoformans* (MIC =1.563 mg/ mL), whereby, both *E. coli* and *S. typhi* were least inhibited with the MIC values of 3.125 mg/mL and 6.25 mg/ mL respectively.

Both IJL Hex and IJL Me OH extracts exhibited activity against the tested organisms. IJL Hex extract had a promising activity against *C. Ablicans* and *C. Neoformans*, with the MIC value of 1.563 mg/mL, followed by *S. aureus* (MIC = 3.125 mg/mL), while *E. coli* and *S. typhi* were all inhibited ata concentration of 6.25 mg/mL. IJL Hex extract was less effective against *P. aeruginosa* with the MIC value of 12.5 mg/mL. Methanolic extract of IJL had antimicrobial activity against at least all of the tested microorganisms **Table 2.** of which *C. neoformans* and *E. coli* were more inhibited (MIC= 1.563 mg/mL), followed by *C. ablicans* (MIC=3.125 mg/mL), *S. typhi* (MIC = 6.25 mg/mL), and *S. aureus* (MIC= 12.5 mg/mL). The least activity was exhibited by *P. Aeruginosa* with a MIC value of 12.50 mg/mL. Extracts from mlelembo exhibited the least activity among all extracts, whereby Hex extracts had weak antimicrobial activity among all the three extracts compared to the MeOH and EA extract *viz. E. coli*

(MIC= 25 mg/mL), *S. typhi* (MIC= 12.5 mg/mL), S. aureus (MIC= 12.5 mg/mL), *P. aeruginosa* (MIC= 12.5 mg/mL), (*C. ablicans* MIC= 6.25 mg/mL), *C. Neoformans* (MIC= 6.25 mg/mL). EA extract of mlelembo had promising activity against tested organisms by inhibiting *C. Ablicans* at a MIC value of 0.781 mg/mL, whilst both *E. coli*, *S. aureus*, and *C. neoformans* had MIC values of 1.563 mg/mL. The least MIC values were exhibited by *S. Typhi* (3.125 mg/mL) and *P. aeruginosa* (6.25 mg/mL). Moreover, Methanolic extract from mlelembo exhibited strong activity against C. ablicans and *C. neoformans*, with MIC values of 1.563 3.125 mg/mL respectively, while *E. coli*, *S. typhi*, *S. aureus* and *P. aeruginosa* had the same MIC values of 12.5 mg/mL.

TABLE 2: 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	0.390625	1.5625	6.25	12.5	0.390625	0.390625
IJLMeOH	1.5625	6.25	12.5	25	3.125	1.5625
IJLAE: MeOH (1:1)	0.390625	0.390625	1.5625	6.25	0.1953125	0.1953125
MLLHex	25	12.5	12.5	12.5	6.25	6.25
MLLEA	1.5625	3.125	1.5625	6.25	0.78125	1.5625
MLLMeOH	12.5	12.5	12.5	12.5	1.5625	3.125
MLLEA: MeOH (1:1)	0.78125	1.5625	0.78125	1.5625	0.390625	0.78125
KILHex	3.125	3.125	1.5625	0.390625	0.78125	1.5625
KILEA	3.125	3.125	1.5625	0.390625	0.78125	0.390625
KILMeOH	3.125	6.25	1.5625	0.390625	1.5625	1.5625
KILEA:MeOH (1:1)	0.78125	1.5625	1.5625	0.1953125	0.78125	0.78125
Ketoconazole	*NA*	*NA*	*NA*	*NA*	0.78125	0.39
Ciprofloxacin	0.78125	0.390625	0.78125	0.390625	*NA*	*NA*

Key: IJLHex -Ijuhi inkundu leaves n-hexane extract, **IJLEA** -Ijuhi inkundu leaves ethyl acetate extracts, IJLMeOH-Ijuhi inkundu leaves methanolic extract, and **IJLAE:** MeOH- Ijuhi 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, NA- not applicable.

TABLE 3: MEAN COMPARISON OF THE 12 EXTRACTS (N=72)

Plant extracts	Average MIC (mg/mL)
KILEA: MeOH	0.94 ±0.22
MLLEA: MeOH	0.98 ±0.22
IJLAE: MeOH	1.50 ±0.97
KIL MeOH	1.56 ± 0.52
KIL Hex	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, Anova p<0.05.

One-way analysis of variance (1-way ANOVA) revealed a statistical difference of mean among all 12 extracts ($p < 0.05$) **Fig. 1.** Effectiveness of plant extracts against tested microorganisms were in the order of KILEA: MeOH (1:1) > MLLEA: MeOH (1:1) > IJLAE: MeOH (1:1)> KIL MeOH> KILEA = KIM Hex > MLLEA > IJLEA > IJL Hex > IJL MeOH > MLL MeOH > MLL Hex **Table 3.** Generally, 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.

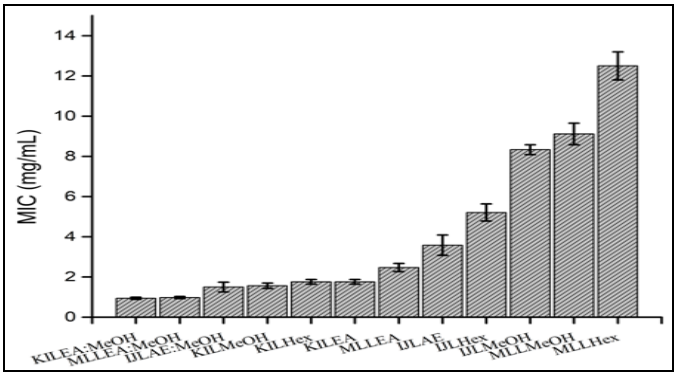

FIG. 1: BAR PLOT INDICATING THE EFFECTIVENESS OF PLANT EXTRACTS IN TESTED ORGANISMS. ANOVA REVEALED A STATISTICAL DIFFERENCE OF MEAN AMONG ALL NINE EXTRACTS (P < 0.05).

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.

Fig. 2 and **Table 4.** These results suggest that the studied leaves (especially kimalindi) may be used in the dressing of wounds involving fungal infections (*C. Albicans* and *C. neoformans*) and also *S. aureus*, all of which are common wound infections, which may include surgical wounds, burns and pressure/bed sores.

This finding, therefore, 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 ijuhi inkundu, mlelembo, and kimalindi extracts **Fig. 2.**

TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY TO THE EXTRACTS (N=72)

Microorganisms	***C. ablicans***	***C.neoformans***	***S. aureus***	***S. typhi***	***E. coli***	***P. aeruginosa***
Average MIC (mg/mL)	1.45 ±0.49	1.64 ± 0.48	4.59±1.41	4.75 ± 1.18	4.88 ± 2.08	7.54 ± 2.22

Results are presented as the minimum inhibitory concentration (mg/mL). Each value is a mean (n=72) ± SEM, ANOVA ($p<0.05$).

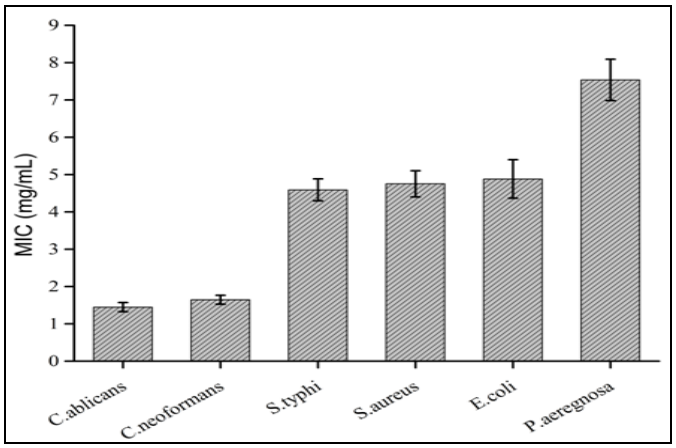

FIG. 2: MEANS SUSCEPTIBILITY OF TESTED MICROORGANISMS TO THE EXTRACTS (N=72), ANOVA P>0.05.

Antimicrobial Susceptibility as Influenced by the Combined Effect of Both Plant Varieties and Solvents: In an attempt to study the co-influence of both solvents and banana leaves' varieties in influencing antimicrobial susceptibility, a two-way analysis of variance (2-way ANOVA) was used.

Results from the two-way ANOVA revealed a significant interaction between solvents and the banana leaves' varieties in prompting the antimicrobial susceptibility of the studied micro-organisms ($p < 0.05$), with kimalindi extract showing best effect in combined solvents (EA: MeOH) and also when used in individual solvents **Fig. 3.** This result further confirms the observed individual effect of studied banana leaves extracts (susceptibility of tested microorganisms to tested banana leaves extracts), further affirming our above conclusion.

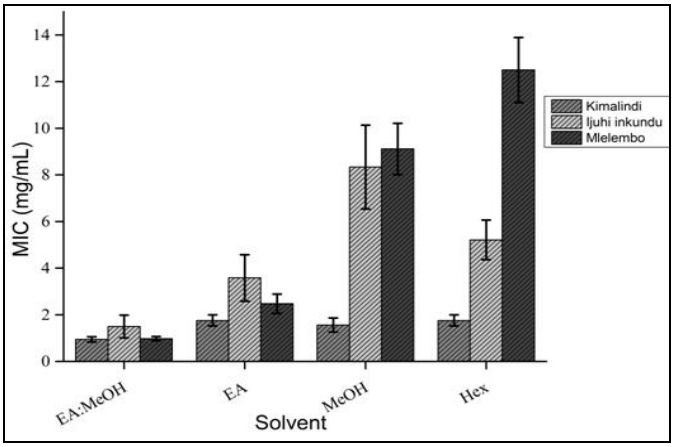

FIG. 3: BAR GRAPH REPRESENTING AN INTERACTION BETWEEN SOLVENT AND BANANA LEAVES' VARIETIES IN INFLUENCING MIC AMONG THE TESTED MICROORGANISMS. RESULTS ARE PRESENTED AS A MEAN ± SEM (N=72), 2-WAY ANOVA P<0.05.

Antioxidant Activity of Extracts: Antioxidant activity was evaluated by measuring the ability of the methanolic extracts from both kimalindi, mlelembo and ijuhi inkundu to scavenge DPPH free radicals. DPPH free radical scavenging ability of methanolic extract was recorded by measuring the decrease in the absorbance of the DPPH in methanol. Following antioxidant assay, all extracts exhibited promising antioxidant activity by reducing the absorbance value of the methanolic DPPH solution.

There was a statistically significant difference in the scavenging of methanolic DPPH solution, of which ascorbic acid scavenged the methanolic-DPPH solution more than the crude extracts ($p<0.05$). The scavenging of methanolic DPPH solution was in the order of ascorbic acid > kimalindi > ijuhi inkundu > mlelembo **Fig. 4** and **Table 5.** These results show that kimalindi extract had better scavenging of methanolic DPPH solution, and hence better antioxidant activity compared to the other two leaves extracts (ijuhi inkundu and mlelembo). This result suggests that kimalindi leaves present a better option when used for wound dressing compared to the other two studied leaves. This also needs to be further tested in a trial involving infected wounds.

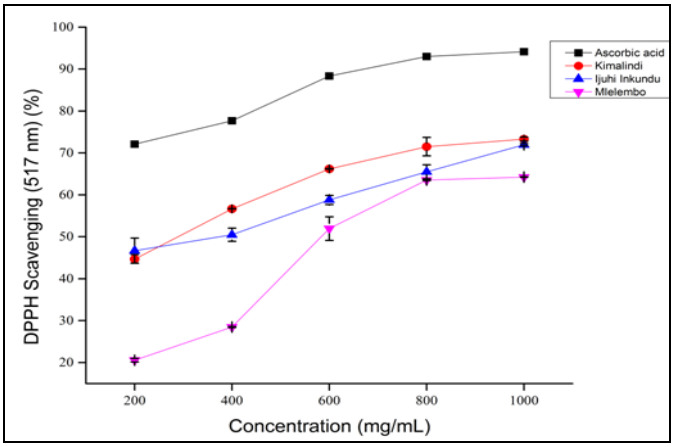

FIG. 4: DPPH FREE RADICAL OF SCAVENGING OF ASCORBIC ACID, KIMALINDI EXTRACT, IJUHI INKUNDU EXTRACT, AND MLELEMBO. VALUES ARE REPRESENTED AS MEAN ± SEM.

TABLE 5: SCAVENGING OF MEOH DPPH SOLUTION EXHIBITED BY BOTH CONTROL AND EXTRACTS

Extract/Control	Ascorbic acid	Kimalindi	Ijuhi inkundu	Mlelembo	Probability P<0.05
DPPH inhibition (%)	85.05±4.35	62.47±5.30	58.68±4.66	45.75±9.01	2.97E-03

The results are presented as the % scavenging of MeOH- DPPH free scavenging by extracts. Each value is presented as mean ± SEM (n=20), ANOVA p<0.05.

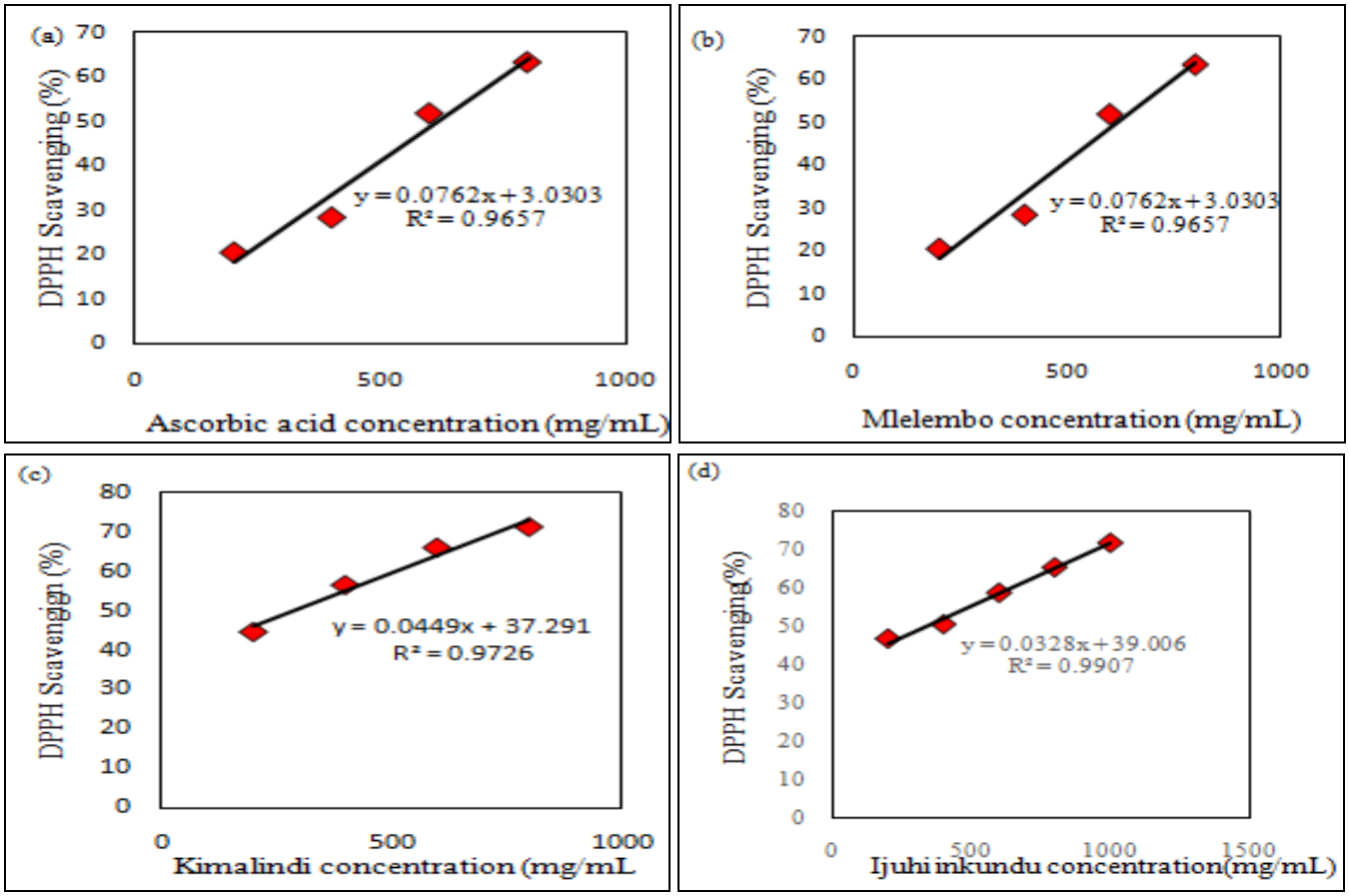

FIG. 5: LINEAR REGRESSION FITTING FOR DETERMINATION OF IC50, (A)-ASCORBIC ACID, (B)-MLELEMBO MEOH EXTRACT, (C)-KIMALINDI-MEOH EXTRACT AND (D)-IJUHI INKUNDU-MEOH.

Antioxidant Activity Expressed by Plant Extracts: All tested extracts had a promising antioxidant activity by scavenging the methanolic DPPH free radical; however, among all the tested

extracts, KIL MeOH exhibited the most scavenging activity by reducing the percentage of DPPH free radical up to 62.47 ± 5.30%, followed by IJL MeOH (58.68 ± 4.66%) and MLL MeOH (45.75 ± 9.01%) **Table 5.** Therefore, this finding has led to our above conclusion that kimalindi leaves present the best option when used for wound dressing. When compared with control (ascorbic acid), either extract revealed a statistical difference in scavenging of DPPH free radical $p<0.05$ **Table 5**, whereby ascorbic acid exhibited the most scavenging ability.

Half Scavenging of MEOH-DPPH Solution Exhibited by Extract: Linear regression was used to compute the half scavenging concentration exhibited by plant extracts on the methanolic DPPH solution (IC50) **Fig. 5**, whereby the IC^{50} was in the order of KIL MeOH> IJL MeOH >ascorbic acid > MLL MeOH **Table 6.** Again, kimalindi extract proved to have better half scavenging of MeOH-DPPH solution compared to the other extracts. Even though ascorbic acid was more effective in scavenging.

DPPH free radicals, still, it's IC^{50} was a little lower compared to that of KIL MeOH and IJL MeOH, further affirming our conclusion that kimalindi leaves present the best option when used in wound dressing.

TABLE 6: IC^{50} FOR TESTED EXTRACTS AND CONTROL.

Extract/control	Ascorbic acid	KILMeOH	MLLMeOH	IJLMeOH
IC_{50} (mg/mL)	393.41	283.05	616.40	335.18
Regression equation	y = 0.0367 × + 64.438	y = 0.0449 × + 37.291	y = 0.0762 × + 3.0303	y = 0.0328 × +39.00
R^2	0.9769	0.9726	0.9657	0.9907

The concentration that inhibits (scavenge) half of MeOH- DPPH solution, R2 Coefficient of determination

Brine Shrimp's Toxicity Activity:

TABLE 7: BRINE SHRIMP ACTIVITY OF AN EXTRACT

Sample code	LC_{50} (µg/mL)	95% CI	Regression equation	(R^2)
KIL Hex	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
KIL MeOH	607.4043	351.0967 -1050.822	y = 44.171logx - 72.949	0.9553
MLL Hex	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
MLL MeOH	874.8601	473.1148 - 1617.7471	y = 39.44 logx - 66.033	0.9430
IJL Hex	249.5970	174.6335-356.7393	y = 78.386logx - 137.91	0.8686
IJLAE	539.5235	316.8582 - 918.6654	y = 45.554logx - 74.454	0.9876
IJL MeOH	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

Results are expressed as LC^{50} (µg/mL) and 95% confidence interval. Linear regression was used to calculate LC50, LC16 and LC^{84} while the regression coefficient explained the validity of the model.

Brine shrimp results obtainable in **Table 7.** This showed that almost all banana leaves extracts, with the exception of KIL Hex and MLLEA, were practically non-toxic to the shrimps. They exhibited mild toxicity by giving the LC^{50} values higher than 100 µg/mL, in which KIL Hex and MLLEA extracts exhibited the LC^{50} value of 94.39 µg/mL (89.3965- 186.5158, 95% CI) and 78.07213 µg/mL (45.1609-134.9676, 95% CI) respectively. However, we are not sure whether the observed toxicities in KIL Hex and MLLEA resulted from used hexane and ethyl acetate solvents or kimalindi and mlelembo extracts per se. Moreover, methanolic, hexane and ethyl acetate extracts from ijuhi inkundu represented moderate toxicity to the shrimps, with the LC^{50} values of 249.5970 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 and hexane extracts from mlelembo (MLL MeOH and MLL Hex) were nontoxic to the shrimps, with the LC^{50} of 874.8601 µg/mL (473.1148 - 1617.7471 95% CI) and 292.4156 µg/mL (196.0747- 436.4303, 95% CI) respectively. Methanolic and ethyl acetate extracts from kimalindi (KIL MeOH and KILEA) exhibited weak toxicity to the shrimps by having LC^{50} value of 607.4043 µg/mL (351.0967 -1050.822, 95% CI), and 129.1273 µg/mL (89.3965- 186.5158, 95% CI) respectively.

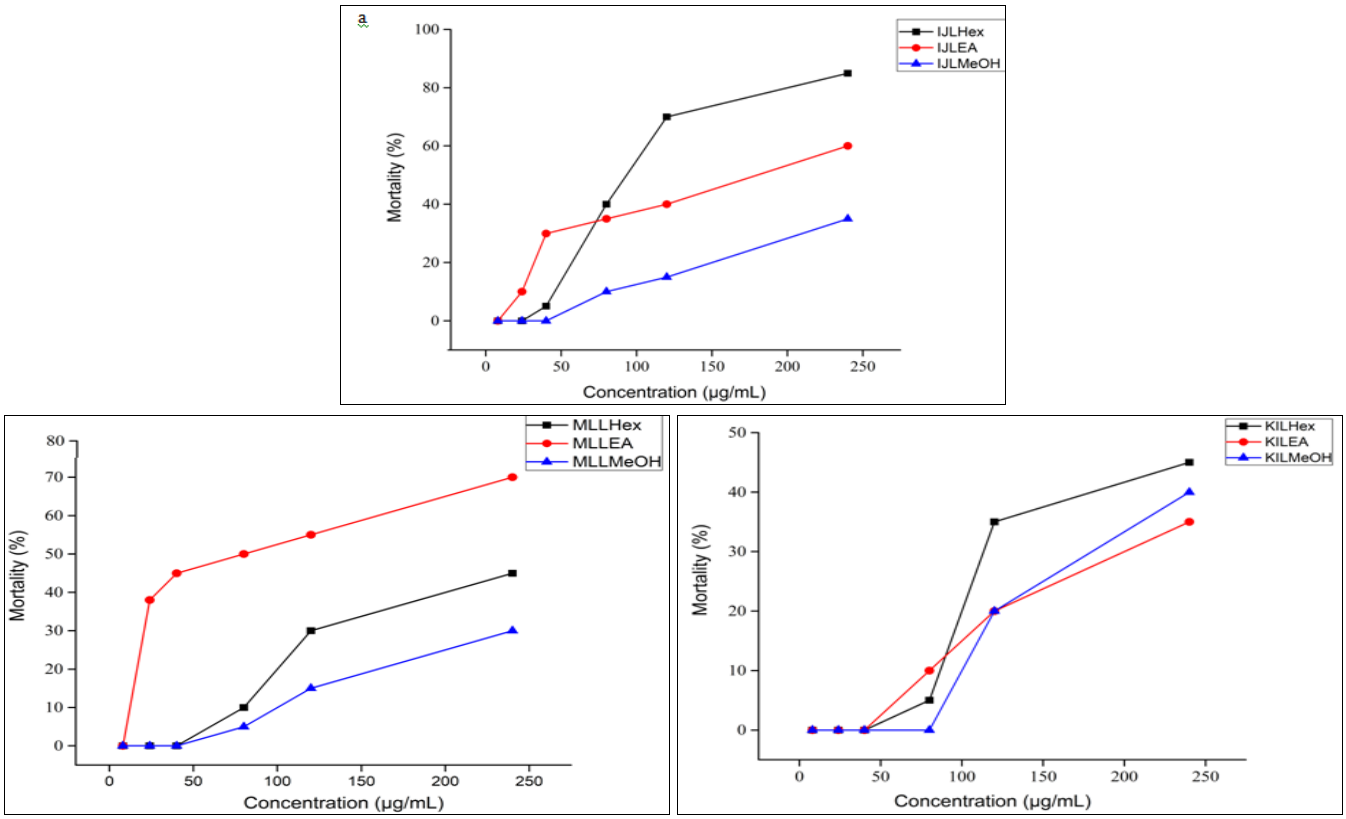

FIG. 6: RESULTS ARE PRESENTED AS THE PERCENTAGE MORTALITY OF SHRIMPS INFLUENCED BY EXTRACTS CONCENTRATION. FIGURES A, B, AND C REPRESENTING THE % MORTALITY OF SHRIMPS FOLLOWING TREATMENT WITH RESPECTIVE EXTRACTS

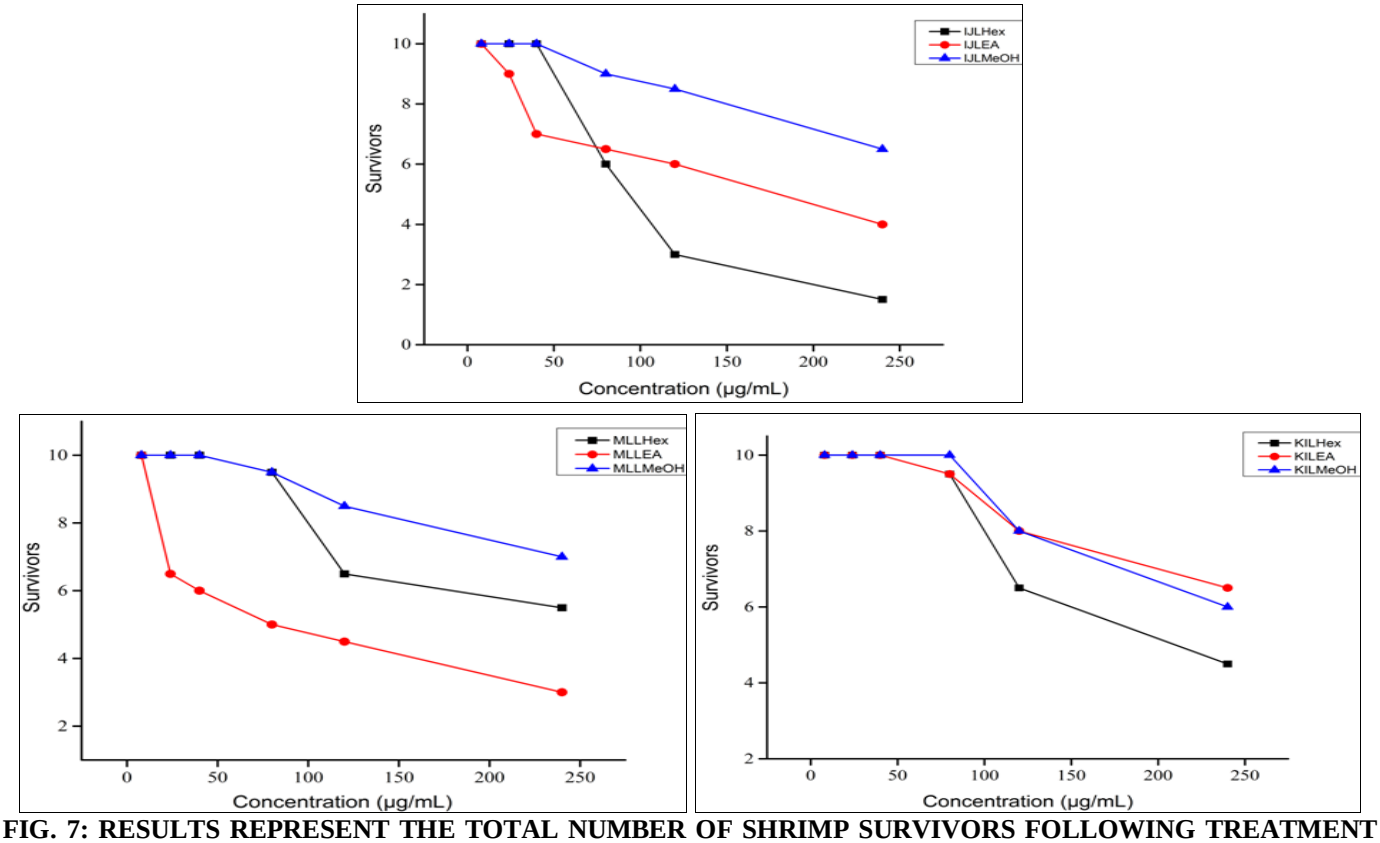

FIG. 7: RESULTS REPRESENT THE TOTAL NUMBER OF SHRIMP SURVIVORS FOLLOWING TREATMENT WITH THE RESPECTIVE EXTRACTS. SURVIVORS ARE IN DUPLICATES PER EACH OF THE TESTED CONCENTRATIONS.

Fig. 6 and **Fig. 7.** Show the percentage of shrimp mortality and survival versus extract concentration, all of which showed that mortality increased with increased extract concentration and survival increased with decreased extract concentration.

CONCLUSION: The current study assessed the biological properties and safety profile of extracts from locally grown banana leaves (ijuhi inkundu, mlelembo and kimalindi) in Arusha. Results from antimicrobial, antioxidant, and toxicity studies affirmed their possible use in wound dressing. Findings from this study also 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 results from antimicrobial, anti-oxidant, and toxicity studies. 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.

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COFLICTS OF INTEREST: The authors declare no competing interest

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