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Evaluation of Wound Healing Effect of Bract Extracts of Musa Acuminata on Wistar Rats

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Abstract: The present investigation has been undertaken to investigate the wound-healing properties of methanol and aqueous bract extracts of Musa acuminata. The plant Musa acuminata has a long history in herbal medicine in many countries. Experiments were conducted following established procedures. Both bract extracts were evaluated for their in vitro antioxidant, as well as their total phenol and flavonoid content. The MBEMA & ABEMA ointments were administered topically to evaluate its wound healing potential in excision wound model for fourteen days. Povidone iodine ointment was the standard for the excision wound model. The extracts-treated group exhibited in vitro antioxidant comparable to that of the standard and control groups. MBEMA & ABEMA exhibited in vivo wound healing activity similar to that of the standard, but of lesser magnitude. The results may be attributed to the phytoconstituents, such as flavonoids and phenolics, present in MBEMA & ABEMA, which may have enhanced wound healing through their individual or cumulative effects. These findings suggest that bract of Musa acuminata could be used in the management of wound healing.

Keywords: Excision wound, Herbal ointment, Musa acuminata, Phytoconstituents, Povidone iodine ointment, Methanol bract extract of Musa acuminata (MBEMA), Aqueous bract extract of Musa acuminata (ABEMA)

Introduction

Wounds are physical injuries that break the skin, which require proper treatment to restore stability and functionality^[1]. The process of tissue repair includes hemostasis, inflammations, proliferation and remodeling of various cell types. The inflammatory phase involves vasoconstriction and the release of inflammatory mediators. During the proliferative phase, granulation tissue is formed as well as angiogenesis. The remodeling phase involves the improvement of collagen fibers ^[2-5]. Factors that contribute to chronic wounds include repetitive trauma, poor circulation, and oxidative stress. Scavenging reactive oxygen species may help in healing of chronic wound. People have relied on plants to treat various ailments, wounds and injuries, for centuries. This traditional approach to wound healing utilizes the natural properties of herbs and botanical ingredients to promote healing and prevent complications ^[6]. This study delves into the fascinating world of herbal wound care, exploring the reasons behind its popularity and the potential benefits that plants can offer. By incorporating these plant-based allies into wound care routines, alongside

conventional medical practices, individuals can potentially enhance their body's natural healing abilities. This holistic approach to wound healing offers a complementary strategy to support the body's recovery process [7].





Fig 1: Musa acuminata bract

Musa acuminata bract is a tree-like perennial plant native to many tropical and subtropical regions of the world. In the English language, it is commonly referred to as banana flower. Musa acuminata bract belongs to the family Musaceae (Fig 1). Although traditional claims and various in vitro studies are available, no scientific studies have been conducted on the bract's wound healing activity in animals. Therefore, it is necessary to perform a study on the wound healing activity of bracts in animals as bracts may be a potential source of safe, efficient, and cost-effective wound healing [8-9].

Materials and Methods

Collection and authentication of plant materials

Musa acuminata bracts were collected from the area surrounding Coimbatore in Tamil Nadu. The bracts were verified by the botanical survey of India, Southern circle, Coimbatore, Tamilnadan. The verification certificate number is BSI/SRC/5/23/2023/Tech/970. Bracts were cleaned soon after collection and shade dried. Once dried, bracts were ground to a coarse powder and stored in airtight plastic containers.

Preparation of extracts

99% of methanol and aqueous extracts of bract of Musa acuminata was obtained by maceration. 200g coarsely powder bracts were soaked in 1000 ml of each solvent for 48 hours at room temperature after extraction [10]. The raw extracts were then evaporated or concentrated by rotary evaporation and dried at room temperature to obtain crude extracts. The crude extracts were then weighed and stored at 4°C for further analysis.

Percentage Yield (%w/w) = Weight of extract obtained (g) x100

Weight of plant material used

Preliminary Phytochemical Screening

Various phytochemical screening assays were performed on methanol and aqueous bract extracts of Musa acuminata (MBEMA & ABEMA) to identify the phytoconstituents present in Musa acuminata bract using established techniques [11-14]

In Vitro Antioxidant Activity

Determination of total phenolics content

The phenolic content of the plant extract was measured using the Folin-Ciocalteu reagent, and the total phenolic content was reported as gallic acid equivalent/g extract^[15].

Determination of total flavonoid content

Plant extracts were tested for flavonoid concentration using an aluminium chloride colorimetrictechnique. Following a room temperature incubation period, a UV-VIS spectrophotometer wasused to detect the mixture's absorbance at 415nm. Quercetin equivalent milligrams per gram of flavonoid content was used [16].

DPPH free radical scavenging activity

The Blois method was used to measure the antioxidant capacity of an extract by its ability to scavenge the stable DPPH radical. Different concentrations of the extract and a reference compound were mixed with a DPPH solution, shaken, and left to stand before measuring absorbance. Quercetin served as a reference compound, and the percentage of inhibition was determined by comparing absorbance values. Free radical scavenging activity was measured as percentage inhibition [17].

Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100 ABTS free radical scavenging activity

The extract's ABTS radical-scavenging activity was assessed using the Rice-Evans method. ABTS was dissolved in water, and the ABTS radical cation was produced by reacting it with potassium persulphate. The extract remained stable for more than two days in the dark. The ABTS solution was diluted with PBS and equilibrated before adding the sample. The absorbance at 734nm was measured after incubating the reaction mixture. The percent ABTS inhibition was calculated using a specific formula. Tests were done in triplicate for accuracy^[18].

Percentage inhibition (I %) = (Abs control - Abs sample /Abs control) X 100

GC-MS method for identification of phytoconstituent in MBEMA and ABEMA

Gas Chromatography Mass Spectroscopy (GCMS) technique is used to identify the phytoconstituents present in the methanol and aqueous bract extracts of Musa acuminata (MBEMA & ABEMA). GC-MS analysis was carried out in Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. Perkin Elmer's Clarus

SQ8C Gas Chromatography - Mass Spectrometer was used for the extraction analysis^[19].

Preparation of herbal ointment

Hard paraffin and cetostearyl alcohol are taken in a china dish which is kept in a water bath at 70°C. Wool fat and white soft paraffin are added to this mixture and stirred until all the ingredients are melted. If necessary, decant or strain and mix cold and pack in a suitable container. For the preparation of methanolic and aqueous extracts of Musa acuminata ointment, 5g of active extracts were added to one part of simple sage base (5% w/w) to prepare the ointment [20].

Formula for the ointment

Table 1: Extracts were added to the base of the ointment

S. No	Ingredients	Master Formula	Reduced Formula	
1.	Wool fat	50g	4g	
2.	Hard paraffin	50g	3g	
3.	Cetosteary alcohol	50g	3g	
4.	White soft paraffin	850g	85g	
		1000g	95g	

Acute toxicity study

The dose was chosen based on a previously conducted study on Musa acuminata [21].

Animal models and experimental design

Protocols for the study using male 8-10 weeks old Wistar-albino rats were authorized by the Institutional Animal Ethical Committee (IAEC) KMCRET/ ReRc/ M. Pharm/ 73/2023 for Animal Care. Temperature (20–25°C), light/dark cycle, relative humidity (55–10%), and standard pellet diet were all maintained for the rats under standard settings. The research was carried out in compliance with authorized criteria and adhered to CPCSEA recommendations. Five groups with varying diets and treatments had been developed for the rats. To aid with histopathology research, blood samples were obtained.

Wound Healing Activities Experimental design

- Group I Control
- Group II Simple Ointment Base
- Group III Povidone Iodine (5%) Ointment Standard
- Group IV-5% Methanol Bract Extract of Musa Acuminata Ointment MBEMA
- Group V-5% Aqueous Bract Extract of Musa Acuminata Ointment ABEMA

Induction of excision wound

On the wounding day, rats were anaesthetized to create wounds on their dorsal fur area. The wound was outlined with methylene blue and a full thickness excision wound of 1.5cm was created. Ointments were applied daily after 24hrs until complete healing, with monitoring of wound area and contraction [22].

Wound Healing Evaluation Parameters

Percentage of wound closure

The healing process of wounds was monitored by assessing the contraction of excision wounds. This was done by tracing the outline of the wound and calculating the area on a graph sheet^[23]. All animals were monitored until complete healing and the mean of all healed wounds was determined.

% Wound closure = Initial wound area – wound area on a specific day \times 100

Initial wound area

Measurement of tensile strength

Wound Tensile Strength (WTS) is a measure of healing progress. WTS measures the restored tissue's ability to break under compression. This may partially indicate the quality of the regenerated tissue. On Day 14, all animals were excised from all tissues. Tissue tensile strength was measured on Day 14 using the ZWICK- ROELL [24]. The method used to test wound breaking strength was to calculate the weight of water for each area of the sample during the breaking process.

Estimation of hydroxyproline

Wound tissues were examined for hydroxyproline (a major component of collagen), fabrics were dried in hot-air oven (60–70°C) to constant weight and then hydrolyzed (6 N HCl) for 4 hours in sealed tubes at 130°C. The hydrolyzate is neutralised to pH 7 and then oxidized (Chloramine-T) for 20min. [25] The reaction is halted by the addition of 0.4 M perchloric acid. The color resulting with Ehrlich's reagent (60°C) is read at 557 nm UV spectrophotometer.

Estimation of uronic acid

Uronic acid 2.5mL of 0.025M Borax in concentrated sulphuric acid in stoppered tubes on a rack and cool to 4°C. There was 0.125mL of hydrolysate diluted 0.5mL with distilled water. Layer 0.5mL of hydrolysate on a Borax-sulphuric acid combination

and store in a rack at 4°C. Glass stoppers were used to close the tubes, which were then shaken slowly and forcefully. The tubes were kept chilly by placing them in an ice container ^[26]. The tubes were heated for 10min in a boiling water bath before cooling to room temperature. After shaking, each tube received 0.1mL of 0.125% carbazole reagent in absolute alcohol. The tubes were then heated in a boiling water bath for a further 15min before cooling to room temperature.

Estimation of total protein

0.1ml of homogenate is diluted to 1ml with distilled water and mixed with 5ml of alkaline solution. Stirring thoroughly, 0.5ml of Folin's reagent is added, thoroughly mixed and incubated at room temperature for 10min. Blue colour is evaluated at 660nm compared to blank [27]. The standard curve is based on 1mg/ml of cattle serum albumin. The protein content of the sample can be calculated in mg/100mg tissue.

Estimation of In vivo anti-oxidative activity

In granulation wound tissues, levels of both enzymatic and non-enzymatic antioxidants, including SOD, CAT, GSH were assessed [28-31]. Granulation wound tissues are used to test LPO as well [32].

Histopathology Analysis

At the conclusion of the study all animals were anaesthetized with ketamine and wound tissue samples were collected on Day 14 and stored in glass bottles containing 10% formulae solution for histology analysis. Microtomy was used to prepare sections of wound tissue (approximately 5μ m in thickness) stained with H&E dye for histology analysis [33]

Statistical analysis

Data were expressed as mean \pm SEM and one-way ANOVA followed by Dunnett's multiple comparison test. Differences were considered statistically significant when P<0.05. The obtained data were analyzed using Graph Pad Prism 9.5.1.

Results

Plant material yield

The extraction procedure yielded to Methanolic bract extract (MBEMA): 15.5 % w/w and Aqueous bract extract (ABEMA): 20 % w/w.

Preliminary Phytochemical Screening

Phytochemical screening of methanol and aqueous bract extracts Musa acuminata and solvent fractions showed the presence of different secondary metabolites (Table 2).

Table: 2 Phytochemical screening of bract extracts of Musa acuminata

SI. NO	Phytochemical Constituents	Mbema	Abema
1.	Carbohydrates	+	+
2.	Alkaloids	aloids +	
3.	Steroids and Terpenoids	+	+
4.	Glycosides	+	+
5.	Phenolic compounds and Tannins	+	+
6.	Saponins	+ +	
7.	Flavonoids	+	+

In Vitro Antioxidant Activity

Determination of total phenolics content

Total phenolic content of Musa acuminata bractextracts was calculated using standard curve of gallic acid (y=0.0047x+0.037, $R^2=0.9931$), which MBEMA was found to 77.25mg/g and ABEMA was found to be 57.75mg/g gallic acid equivalent (Table 3).

Determination of total flavonoids content

Total flavonoid content of Musa acuminata bractextracts was calculated using standard curve of quercetin (y=0.0047x+0.0408, $R^2=0.9932$), which MBEMA was found to 80.55mg/g and ABEMA was found to be 69.55mg/g quercetin equivalent (Table 3).

DPPH free radical scavenging activity

The 50% inhibitory concentration of DPPH radical scavenging activity of MBEMA was found to be 59.24µg/ml and ABEMA was found to be 57.70µg/ml (Table 3).

ABTS free radical scavenging activity

The 50% inhibitory concentration of ABTS radical scavenging activity of MBEMA was found to be 56.57µg/ml and ABEMA was found to be 56.24µg/ml (Table 3).

Table 3: In vitro antioxidant activity of bract extracts of Musa acuminata

Extracts	Total phenol (mg/g of gallic acid equivalent extracts)	Total flavonoid (mg/g of quercetin equivalent extracts)	DPPH IC ₅₀ (µg/ml)	ABTS IC ₅₀ (µg/ml)	
MBEMA	77.25mg/g	80.55mg/g	59.24µg/ml	56.57µg/ml	
ABEMA	57.75mg/g	69.55mg/g	57.70µg/ml	56.24µg/ml	

Gas chromatography–Mass Spectrometry (GC–MS) analysis

In the present study, the GC-MS analysis of methanol and aqueous bract extracts of Musa acuminata showed the presence of various phytochemicals activities, which may help in deriving the most inflammatory, antioxidant, antifungal properties of Musa acuminata bract. The number of common compounds found in both (methanol & aqueous) the extracts of bract was twenty chemical compounds. similarly, five chemical compounds are commonly found in bract extracts. As in the GC-MS analysis of methanol bract extract of Musa acuminata showed the presence of many phytoconstituents (Figure 2(A)). There are five compounds present, and they are 2,5-dihydroxyacetophenone, 2TMS derivative, 4-Propionyloxytridecane, Enterolactone, Phytol, n-Hexadecenoic acid shown in (Table 4(A)).

Fig 2(A): GC-MS analysis of methanol bract extract of Musa acuminata

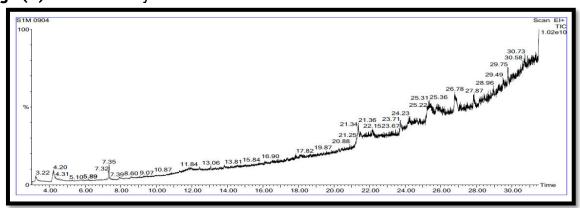


Table 4(A): Chemical composition of methanol bract extract of Musa acuminata

S.NO	Retention time	Compound name	Properties	
1.	30.680	2,5-dihydroxyacetophenone, 2TMS derivative	Anti-oxidant property	
2.	29.499	4-Propionyloxy tridecane	Anti-oxidant property	
3.	26.888	Enterolactone	Anti-oxidant,	
			Anti-inflammatory property	
4.	25.878	Phytol	25 979 Physical Anti-inf	Anti-inflammatory
4.	25.616		property	
5.	21.356	n-Hexadecenoic acid	Anti-inflammatory, Anti- microbial property	

As in the GC-MS analysis of aqueous bract extract of Musa acuminata showed the presence of many phytoconstituents (Figure 2(B)). There are five compounds present, and they are Glafenin, Squalene, 2-Cyclohexylpiperidine, Glycylsarcosine, d-Glycero-d-ido-heptose shown in (Table 4(B)). The methanol bract extracts of Musa acuminata shows the highest phytoconstituents as aqueous extract.

Fig 2(B): GC-MS analysis of aqueous bract extract of Musa acuminata

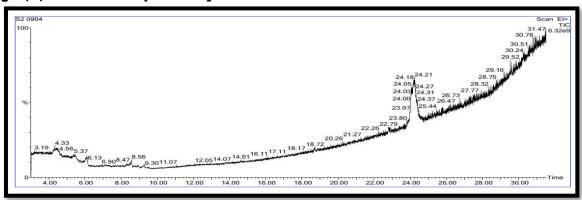


Table 4(B): Chemical composition of aqueous bract extract of Musa acuminata

S.NO	Retention time	Compound name	Properties	
1.	29.589	Glafenin	Anti-inflammatory property	
2.	24.047	Squalene	Anti-oxidant, Anti- inflammatory, Anti-bacterial property	
3.	8.285	2-Cyclohexylpiperidine	Anti-microbial property	
4.	7.160	Glycylsarcosine	Anti-inflammatory property	
5.	4.329	d-Glycero-d-ido- heptose	Anti-bacterial property	

Evaluation of Wound Healing Activity

Percentage of wound closure

Wound healing was significantly improved in MBEMA and ABEMA groups compared to control groups (Table 5). The reduction in wound area over the 14-day period was observed in different groups. Wound area was measured on Day 3, Day 5, Day 7, Day 9, Day 11 and Day 14 in all treatment groups (Fig 3). Very high significant wound closure rate was observed between Day 3 and Day 14.

Table 5: Effect of MBEMA & ABEMA on wound closure by excision wound model (N=6)

	% of Wound Closure					
Groups	3 rd Day	5 th Day	7 th Day	9 th Day	11 th Day	14 th Da y
Control	7.64±0.624	16.2±2.71	31.1±5.32	45.3±3.47	66.7±1.36	79.8±1.55
Simple						
ointment	14.8±1.46**	31.5±2.61 [*]	50.6±1.32**	61.7±7.09 [*]	68.7±1.64 [*]	84.2±0.48 ^{ns}
base						
Standard						
(Povidone	28.8±1.69***	42.3±.4.44***	68.4±3.99***	76.5±3.33***	91.6±1.53***	95.3±1.4***
iodine)						
MBEMA	***	***	***	***	***	***
ointment	27.8±1.88	40.6±2.36	59.1±3.67	72±2.52	80.3±2.05	93.2±0.86
ABEMA	***	**	***	**	***	***
ointment	26.5±1.23	39.1±6.31	58.8±3.07	71.5±4.18	77.6±1.92	90.9±1.18

Values are expressed in the mean \pm SD. Statistical significance (p) was calculated by using One-way ANOVA followed by Dunnett's multiple comparison test using prism 9.5.1. Level of significant shows ns- not significant *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001 calculated by comparing treated group with control group.

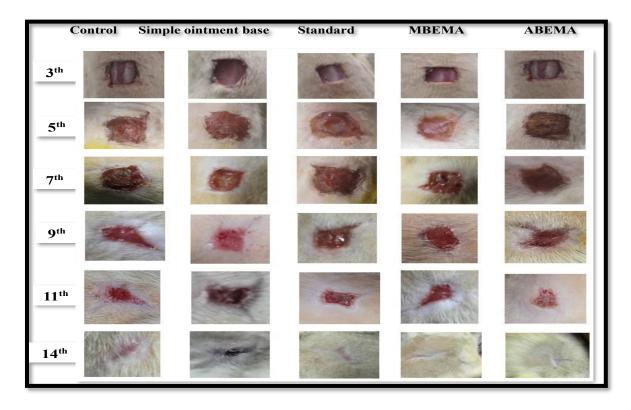


Fig 3: Percentage of wound closure on Excision wound model showing % wound closure area on different 3rd, 5th, 7th, 9th, 11th and 14th days.

Measurement of tensile strength

An optimal wound healing agent should have the ability to enhance collagen fibril viability around the wound site, resulting in an increase in wound tensile strength, which was measured by tensiometer. The MBEMA and ABEMA treatment groups demonstrated significant concentration-dependent action in increasing wound tensile strength compared to the control and treated groups (Fig 4)

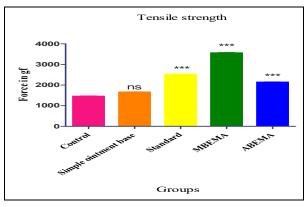
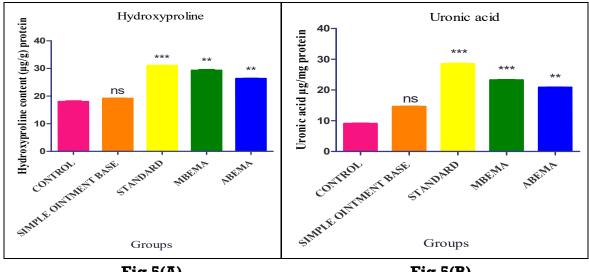


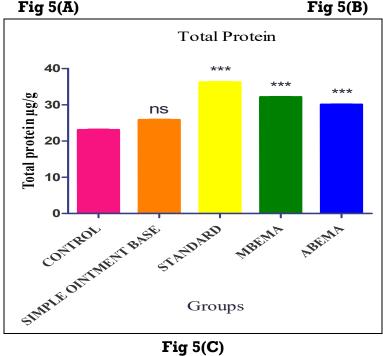
Fig 4

Values are expressed in the mean \pm SD. Statistical significance (p) was calculated by using One-way ANOVA followed by Dunnett's multiple comparison test using prism 9.5.1. Level of significant shows ns- not significant *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 calculated by comparing treated group with control group.

Estimation of Hydroxyproline, Uronic Acid and Total protein content

MBEMA and ABEMA treated groups increased hydroxyproline (HPR), Uronic Acid (UA) and Total protein content (TPR) by, respectively, compared with control groups. HPR, UA and TPR increased by, respectively, and ABEMA and MBEMA treated groups increased by, respectively. Results with MBEMA and ABEMA compared to control groups (Fig. 5(A), (B) and (C)).

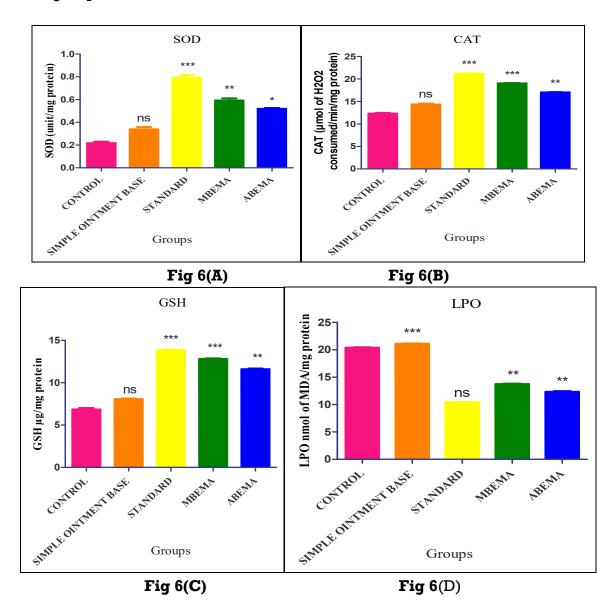




Values are expressed in the mean \pm SD. Statistical significance (p) was calculated by using One-way ANOVA followed by Dunnett's multiple comparison test using prism 9.5.1. Level of significant shows ns- not significant *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 calculated by comparing treated group with control group.

Estimation of In vivo anti-oxidative activity and Free radicals

MBEMA and ABEMA showed a significant increase in the levels of antioxidants, GSH, SOD and CAT (Fig 6(A), (B) and (C)), while free radicals in LPO decreased (Fig6(D)). The results of Musa acuminata bracts were compared with control groups.



Values are expressed in the mean \pm SD. Statistical significance (p) was calculated by using One-way ANOVA followed by Dunnett's multiple comparison test using prism 9.5.1. Level of significant shows ns- not significant *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 calculated by comparing treated group with control group.

Histopathology

Histopathological examinations of the excision wound tissue were performed on day 14 and the histopathological characteristics of the tissue of all animal groups are shown (Fig 7 (A), (B), (C), (D) and (E).

Group 1 - Control

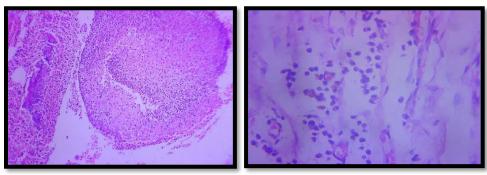


Fig 7 (A): Section studied from skin shows ulcerated epidermis. Dermis shows dense collections of neutrophils, lymphocytes and fibroblasts, surrounding area shows fibrocollagenous stroma with thin-walled congested vessels. Deep dermis also shows inflammatory infiltrates.

Group 2 - Simple Ointment Base

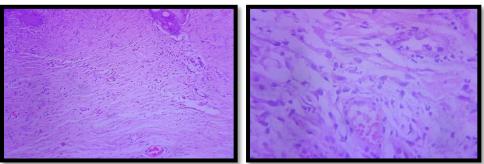


Fig 7 (B): Section studied shows skin with epidermis shows surface ulceration. Dermis shows moderate infiltration of lymphocytes and plasma cells with fibroblasts and proliferation of thin-walled capillaries forming granulation tissue.

Group 3 – Standard (Povidone Iodine)

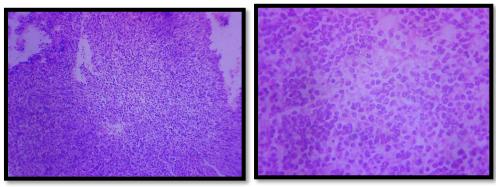


Fig 7 (C): Section studied shows skin with epidermis shows **Re-epithelialization**. Dermis shows infiltration of neutrophils, lymph plasmacytes, and macrophages in a fibrocollagenous stroma and proliferation of thin-walled capillaries.

Group 4 - Test 1 (Mbema)

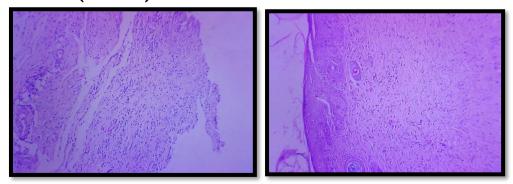


Fig 7 (D): Sections show skin with epidermis shows Re-epithelialization. Dermis shows dense fibrocollagenous stroma with scattered infiltration of lymphocytes and plasma cells and thin-walled congested vessels. Deep dermis shows scattered inflammatory infiltrates.

Group 5 - Test 2 (Abema)

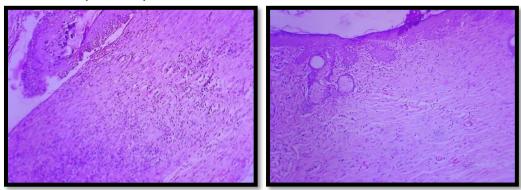


Fig 7 (E): Section shows skin with epidermis shows **Re-epithelialization**. Dermis shows granulation tissue composed of lymphocytes, neutrophils, plasma cells and thin-walled congested vessels in a fibrocollagenous stroma.

Discussion

This study investigated the wound healing potential of methanol (MBEMA) and aqueous (ABEMA) bract extracts of Musa acuminata in rats. The bracts contain various bioactive compounds including phenols and flavonoids, which exhibited significant free radical scavenging activity. Both bract extracts demonstrated wound healing activity comparable to a standard povidone-iodine ointment, accelerating wound closure and increasing tensile strength. MBEMA extract displayed superior wound healing activity compared to ABEMA, potentially due to its higher content of bioactive compounds. The extracts may promote healing by increasing antioxidant activity, collagen synthesis, and glycosaminoglycan synthesis, ultimately leading to faster tissue repair and regeneration. Histopathological analysis confirmed these findings, revealing a more advanced healing state in extract-treated groups with extensive collagen deposition and minimal inflammation. These findings suggest that Musa acuminata bract extracts hold promise as a therapeutic agent for promoting wound healing.

Conclusion

In conclusion, this study demonstrated that MBEMA and ABEMA extracts from Musa acuminata bract exhibit wound healing activity in a rat model. The bract extracts promoted wound healing by increasing epithelialization, tensile strength, and uronic acid content. These findings provide scientific evidence to support the ethnomedicinal use of Musa acuminata for wound healing and suggest the potential of these bract extracts for developing herbal wound healing therapies.

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