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Comparative Influence of Activated Charcoal on Surgical Wound-Healing Activity of Unripe and Ripe Banana (*Musa Sapientum*) Peel in Wistar Rats

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ABSTRACT: This research study was aimed to compare the influence of activated charcoal on wound healing activity of unripe and ripe banana *(Musa sapientum)* peel gel in wistar rats. Fifty two wistar rats with an average weight of (190 ± 0.08) g were divided into thirteen groups with four rats per group. For use in this research, 4 %, 8 %, 12 % and 16 % (w/w) creams were prepared from ground peels using paraffin base. 4 %, 8 %, 12 % and 16 % creams were supplemented with 1 g, 2 g, 3 g and 4 g of activated charcoal respectively. These creams were applied once daily for 20 consecutive days and the progress of wound healing was closely monitored using parameters such as rate of wound contraction, microbial load count, wound surface protein, Histology, scar area and tensile strength. These parameters were determined on post-wound days 4,8,12 and 16. Creams prepared from unripe banana *(Musa sapientum)* peel gel supplemented with activated charcoal showed greater wound healing activity when compared with the standard and creams prepared from ripe banana peel gel supplemented with activated charcoal. The highest rate of wound healing was observed in the groups of rats treated with 16% banana peel gel supplemented with 4 g of activated charcoal. The prophylactic effect elicited by *Musa sapientum* peel gel supplemented with activated charcoal in terms of wound contraction and related biochemical parameters strongly supports the positive influence of activated charcoal on wound-healing activity of *Musa sapientum* peel.

Keywords: Activated charcoal, wistar rat, *Musa sapientum,* lidocaine

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INTRODUCTION

Musa sapientum (Musaceae family) grows in humid lowland to upland tropical areas. Extensive investigations regarding anti-ulcerogenic and ulcer healing activities of banana have been carried out for the past 30 years. Multicentric trial has shown banana to be effective in healing of ulcer dyspepsia and delaying ulcer recurrences (Anhwange, 2015). The banana was selected with the promise that the ulcer healing activity could have effect on wound healing also (Atzingen *et al*., 2015). The *Musaceae* family is made up of three genera: Musa, Ensete and Musella. Musa is the largest group with about 35 species. Musa species are divided into different sections. According to recent DNA work (cite ref here), there are three sections: Musa (with 22 chromosomes), Callimusa (with 14 chromosomes) and Ignetimusa (with 14 chromosones). There are about seven Ensete species and one Musella species (Anhwange, 2015). For thousands of years products from natural sources have been used in caring for human health. Most of the drugs given even today are directly or indirectly from natural sources (Ibrahim, 2015). These medicines which are safe, free from side effects and eco-friendly are derived from

plants (Ibrahim, 2015). Recently, interest in local plants' research has increased significantly for a variety of reasons including an inability of many rural people and some governments to afford western-based pharmaceutical care, renewed interest in native resources and "traditional" health systems along with a greater appreciation for local and indigenous knowledge, international concerns for the conservation of biodiversity and their income-generating potential (Ibrahim, 2015). Limitations of synthesized compounds in the treatment of chronic diseases and the potential of plant-based medicine as a more effective and cheaper alternative was probably responsible for the fast growing industry of herbal medicine (Abu-Al-Basal, 2014). Medicinal plants of diverse species abound in Nigeria. In Nigeria, almost all the parts of banana plant *(Musa sapientum)* are used for the treatment of various diseases. It is suitable for consumption by people of any age group and so, is one of the world's most important food produce.

Banana is an excellent source of nourishment and a well-balanced diet to people of all ages around the globe and contributes to the income of individuals through crop production, processing and marketing. Banana is eaten in many ways and has plenty of nutritional and medicinal benefits (Gosain and Dipietro, 2016).

Wound care is constantly evolving with the advances in medicine but wounds are still a significant health problem worldwide, often having severe complications. In recent years, wound care professionals re-visited the ancient methods by using the traditional medicine in wound management (Omale and Isaac, 2013). Traditional medicines which are more effective, relatively non-toxic and cost-effective are gaining popularity throughout the world. The medicinal and biological effects of plants have urged scientists to examine these plants with a view to determine potential wound-healing properties (Thakur *et al*., 2016).

MATERIALS AND METHODS

Collection and preparation of banana peel

Fresh and matured fruits of *Musa sapientum* (ripe and unripe) were collected from a farm at Magongo in Ogori-Magongo Local Government Area of Kogi State. The banana fruits were taken to the Department of plant sciences and Biotechnology, Prince Abubakar Audu University, Anyigba, for identification and allotment of Voucher Number.

Preparation of banana peel gel

The gel used in the research was prepared from unripe and ripe banana (*Musa sapientum*) peels. First, the fruits were washed with water. After the first wash, these fruits were rinsed with distilled water and allowed to dry for another 20 minutes at room temperature. Next, the fruits were peeled and the pulps were discarded. The banana peels were diced into cubes of approximately $1mm^2$, transferred to a mortar and manually ground with a pestle until complete homogenization. 4 % gel was prepared by mixing 4 g of ground peel with 96 g of paraffin base (4 % cream), 8 % gel was prepared by mixing 8 g of ground peel with 92 g of paraffin base (8% cream), 12 % gel was prepared by mixing 12 g of ground peel with 88 g of paraffin base (12% cream) and 16 % gel was prepared by mixing 16 g of ground peel with 84 g of paraffin base (16% cream). For use in this research study, 4 %, 8 %, 12 % and 16 % creams were supplemented with 1 g, 2 g, 3 g and 4 g activated charcoal respectively. These formulations were applied once daily for 20 consecutive days. The wound surface is usually sterilized with methylated spirit before the application of these formulations.

Excision wound model

Full thickness wounds were created in the skin of the animal according to the model of Abu-Al-Basal (2014).

Wound surface protein

Granulation tissue was removed (by scrapping wound surface) for analysis of wound surface protein. Wound surface protein was determined using the method described by Association of Official Analytical Chemist, A.O.A.C (2017) Washington, DC.

Procedure

Samples of granulation tissue were weighed out into a conical flask and 5 g of anhydrous Sodium Sulphate was added. This was followed with the addition of 1g of Copper (II) tetraoxosulphate (VI) and 1 tablet of Kjeidahl catalyst (each tablet contains 1g of Na_2SO_4 + 0.05 g of Selenium). A portion of concentrated tetraoxosulphate (VI) acid and 5 glass beads (to prevent bumping during heating) was introduced into the mixture. The mixture was transferred into a fume cupboard where it was heated with occasional shaking till the solution turns green. The mixture was cooled and reheated gently until the green colour disappears. After cooling, the digest was transferred into a 250 ml volumetric flask and made up to mark with distilled water.

This was distilled using the Markham distillation apparatus. A 100 ml conical flask containing 5 ml boric acid was placed under a condenser such that the condenser tip was under the liquid. 5 ml of the digest was put into the condenser with a pipette via the small funnel

aperture, and this was followed by the addition of 4% sodium hydroxide solution. The distillation apparatus was steamed for about 5 to 7 minutes to collect enough ammonium sulphate. The solution in the receiving flask was filtered using 0.01N HCl and the nitrogen content was calculated.

Microbial load count

Wound surface microbial load was determined using the swab stick method as described by Aneda (2016).All glass wares which was used was thoroughly washed, rinsed with distilled water, dried and sterilized at 120^{0C} for 30 minutes. A portion (4.2 g) of nutrient agar was dissolved in 150 ml of distilled water and autoclaved at 121^{0C} for 15 minutes to remove all contaminants that may influence the growth of interfering microorganisms. The nutrient agar was left to cool to room temperature after which it was poured into a large Petri- dishes and left to solidify. Sterile swab stick was carefully unwrapped, dipped into sterile water, gently pressed and was used to swab the surface of wound. The sterile swab stick was streaked gently on the surface of the molten nutrient agar in zigzag manner to ensure an even distribution on the Petri- dishes. The culture plates were incubated at 37^{0C} for 24 hours and the microbial load count was done for the entire samples.

Percentage (%) wound contraction

Wound area was determined by tracing straight (perpendicular and horizontal) lines connecting the most external excision points in millimeter. Rate of wound contraction was determined on days 4, 8, 12 and 16 respectively using the formula;

$$\text{Wound contraction rate} = \frac{\text{Initial wound area - final wound area}}{\text{Initial wound area}} \times 100$$

Determination of the scar area

The scar area of all the groups in this study was measured on the 20^{th} day of the experiment. It was determined by tracing straight (perpendicular and horizontal) lines connecting the length and breadth of the scar area in millimeter square (mm^2).

Data analysis

Data obtained from the study was expressed as mean $\pm$ standard deviation. Different statistical tools were used to analyze the data obtained from the study. Analysis of variance (ANOVA) was applied at $P < 0.05$ to test for significant difference within and between groups (Graph pad software inc.2019).

DISCUSSION

The effects of unripe and ripe *Musa sapientum* peel gel (with or without activated charcoal) on the synthesis of wound surface protein is shown in (Table 1). There was a progressive increase in the synthesis of protein (Collagen) in all groups after the creation of wound. Animals in groups 7 had the highest concentration of protein on wound surface followed by animals in group 6. As shown in (Table 1), there was a sharp increase in protein content from days 4, 8, 12 and 16. The untreated group showed a slow gradual increase in protein content. Collagen is the predominant extra cellular protein in the granulation tissue of a healing wound (Omale and Emmanuel, 2013). There was a rapid increase in the synthesis of this protein in the wound area, soon after an injury, which provides strength and integrity to tissue matrix (Omale *et. al.,* 2013). This may be responsible for the progressive increase in the concentration of protein on days 4, 8, 12 and 16 respectively. There was a positive correlation (Table 2) between the mass of activated charcoal (AC) and the concentration of protein on wound surface. There is a significant difference ($p < 0.05$) between group 7 and the standard in (Table 2).

Wound-healing or repair is a natural process of regenerating dermal and epidermal tissue and may be categorized into three phases, viz, inflammation, proliferation and remodeling phase. In the inflammation phase, various growth factors such as tumor necrosis factor (TNF), and interleukins (IL) are released to initiate proliferation phase. The latter is characterized by angiogenesis, collagen deposition, granular tissue formation, epithelialization and wound contraction (Omale *et. al.,* 2013). In the last phase, the levels of collagen production and degradation equalize, after which disorganized fibres are rearranged thus increasing the tensile strength (Atzingen *et al.,* 2015). Biological activities in skin are due to its interaction with various binding proteins. In the tissue repair process, inflamed cells promote the migration and proliferation of endothelia cells leading to neovascularization of connective tissue cells which synthesize extracellular matrices including collagen and keratinocytes resulting in the re-epithelialization of the wound tissue (Hoppe and Granick, 2013). The significant increase observed in the group of animals treated with *Musa sapientum* peel gel supplemented with activated charcoal might be due to interactions between the activated charcoal and phytochemicals present in *Musa sapientum* peel gel. This influence of activated charcoal is even more pronounced in unripe *Musa sapientum* peel gel than ripe peel gel.

Table 1: Effects of *Musa sapientum* peel gel (with or without activated charcoal) on wound surface protein.

Group	Day 4	Day 8	Day 12	Day16
1 (Control)	13.0 ± 3.88^{a}	27.9 ± 0.25^{a}	66.4 ± 3.31^{a}	82.1 ± 0.99^{a}
2 (Standard)	19.4 ± 2.50^{c}	34.7 ± 0.31^{c}	77.5 ± 3.15^{de}	87.8 ± 1.43^{b}
3 (AC Alone)	16.2 ± 4.05^{b}	31.6 ± 2.62^{b}	74.6 ± 2.29^{c}	86.7 ± 0.15^{b}
4 (4% unripe)	28.0 ± 2.65^{f}	49.3 ± 4.83^{f}	80.0 ± 0.26^{f}	94.9 ± 0.19^{d}
5 (8% unripe)	29.1 ± 4.29^{fg}	52.2 ± 2.29^{g}	83.3 ± 0.12^{g}	96.7 ± 1.68^{e}
6 (12% unripe)	30.7 ± 4.05^{gh}	53.8 ± 1.69^{gh}	85.6 ± 2.26^{h}	97.8 ± 0.19^{e}
7 (16% unripe)	32.3 ± 5.76^{h}	56.0 ± 0.25^{h}	88.9 ± 1.51^{i}	98.2 ± 0.99^{e}
8 (4% ripe)	20.8 ± 4.70^{c}	38.7 ± 2.54^{d}	75.6 ± 0.30^{cd}	92.0 ± 2.74^{c}
9 (8% ripe)	22.7 ± 1.48^{d}	39.9 ± 2.89^{d}	76.8 ± 0.80^{d}	92.0 ± 0.19^{c}
10 (12% ripe)	23.3 ± 3.31^{de}	44.4 ± 3.43^{e}	77.8 ± 0.20^{d}	94.1 ± 2.65^{c}
11 (16% ripe)	25.0 ± 1.43^{e}	46.7 ± 0.14^{ef}	78.9 ± 5.10^{ef}	94.2 ± 0.30^{c}
12 (unripe)	17.5 ± 4.25^{b}	31.0 ± 3.31^{b}	73.5 ± 2.42^{bc}	84.3 ± 0.18^{a}
13 (ripe)	15.8 ± 3.89^{b}	30.6 ± 1.80^{ab}	72.4 ± 2.5^{4b}	83.0 ± 0.29^{a}

Values with different superscripts on the same column are significantly different at $p < 0.05$; (n = 4). AC- Activated charcoal.

Table 2: Effects of *Musa sapientum* peel gel (with or without activated charcoal) on rate of wound contraction.

Group	Day 4	Day 8	Day 12	Day16
1 (Control)	13.0 ± 3.88^{a}	27.9 ± 0.25^{a}	66.4 ± 3.31^{a}	82.1 ± 0.99^{a}
2 (Standard)	19.4 ± 2.50^{c}	34.7 ± 0.31^{c}	77.5±3.15^{de}	87.8 ± 1.43^{b}
3(AC Alone)	16.2 ± 4.05^{b}	31.6 ± 2.62^{b}	74.6 ± 2.29^{c}	86.7 ± 0.15^{b}
4 (4% unripe)	28.0 ± 2.65^{f}	49.3 ± 4.83^{f}	80.0 ± 0.26^{f}	94.9 ± 0.19^{d}
5 (8% unripe)	29.1± 4.29^{fg}	52.2 ± 2.29^{g}	83.3 ± 0.12^{g}	96.7 ± 1.68^{e}
6(12% unripe)	30.7±4.05^{gh}	53.8±1.69^{gh}	85.6 ± 2.26^{h}	97.8 ± 0.19^{e}
7 (16% unripe)	32.3 ± 5.76^{h}	56.0 ± 0.25^{h}	88.9 ± 1.51^{i}	98.2 ± 0.99^{e}
8 (4% ripe)	20.8 ± 4.70^{c}	38.7 ± 2.54^{d}	75.6±0.30^{cd}	92.0 ± 2.74^{c}
9 (8% ripe)	22.7 ± 1.48^{d}	39.9 ± 2.89^{d}	76.8 ± 0.80^{d}	92.0 ± 0.19^{c}
10 (12% ripe)	23.3±3.31^{de}	44.4 ± 3.43^{e}	77.8 ± 0.20^{d}	94.1 ± 2.65^{c}
11 (16 % ripe)	25.0 ± 1.43^{e}	46.7± 0.14^{ef}	78.9± 5.10^{ef}	94.2 ± 0.30^{c}
12 (unripe)	17.5 ± 4.25^{b}	31.0 ± 3.31^{b}	73.5±2.42^{bc}	84.3 ± 0.18^{a}
13 (ripe)	15.8 ± 3.89^{b}	30.6±1.80^{ab}	72.4 ± 2.5^{4b}	83.0 ± 0.29^{a}

Values with different superscripts on the same column are significantly different at $p < 0.05$. (n=4). AC- Activated charcoal.

Recent studies with plant extracts have shown that phytochemical constituents, like flavonoids, are known to promote wound healing process mainly due to its astrigent and microbial properties which appears to be responsible for wound contraction and increased rate of epithelialization (Omale and Emmanuel, 2013). Wound contraction is a process that occurs throughout the healing period commencing from the fibroblastic stage whereby the area of the wound undergoes shrinkage (Hoppe and Granick, 2013). Granulation tissue facilitates epithelia migration which helps to close the wound by contraction thereby reducing the area requiring epithelia coverage (Atzingen *et al.,* 2015).

Table 2 shows the effects of unripe and ripe *Musa sapientum* peel gel (with or without activated charcoal) on rate of wound contraction. Wound contraction was faster in groups of animals treated with unripe *Musa sapientum* peel gel supplemented with activated charcoal. The lowest area of opened wound was observed in the group (group 7) treated with 16 % unripe *Musa sapientum* peel gel supplemented with 4 g of activated charcoal. The wound-healing property (wound contraction) observed in this group might be attributed to the interaction of activated charcoal with phytochemical constituents contained in unripe *Musa sapientum* peel. The prophylactic effect elicited by a mixture of activated charcoal and *Musa sapientum* peel gel in terms of wound contraction is a strong indication that activated charcoal can be used to boost its wound-healing potential.

Table 3 shows the result of antimicrobial characteristics of unripe and ripe *Musa sapientum* peel gel mixed with activated charcoal. The cream is potent on microorganism reducing their population on the wound surface. On day 8, the wound surface was still wet and attracts microorganisms but as the healing progresses, there was a decrease in microbial population. The unripe *Musa sapientum* peel gel, supplemented with activated charcoal, was more potent than the ripe *Musa sapientum* peel gel supplemented with activated charcoal.

Preliminary phytochemical analysis of *Musa sapientum* revealed the presence of alkaloids, terpenes and flavonoids (Shi *et al.,* 2010). Flavonoids are known to promote the wound-healing process mainly due to their

Table 3: Effects of *Musa sapientum* (with or without activated charcoal) on microbial load counts.

Group	Day 4	Day 8	Day 12	Day16
1 (Control)	98^{g}	86^{i}	70^{h}	58^{j}
2 (Standard)	85^{bc}	62^{ab}	49^{c}	38^{f}
3 (AC Alone)	92^{d}	78^{g}	57^{e}	42^{g}
4 (4% unripe)	89^{d}	71^{e}	54^{d}	31^{d}
5 (8% unripe)	86^{c}	67^{c}	48^{c}	28^{c}
6(12% unripe)	84^{b}	63^{b}	45^{b}	24^{b}
7 (16% unripe)	82^{a}	60^{a}	42^{a}	21^{a}
8 (4% ripe)	91^{d}	75^{f}	66^{g}	47^{h}
9 (8% ripe)	93^{e}	70^{de}	58^{e}	39^{f}
10 (12% ripe)	96^{f}	65^{bc}	54^{d}	35^{e}
11 (16% ripe)	86^{c}	68^{d}	53^{d}	36^{e}
12 (unripe)	94^{e}	64^{b}	57^{e}	39^{f}
13 (ripe)	97^{fg}	81^{h}	62^{f}	51^{i}

Values with different superscripts on the same column are significantly different at $p < 0.05$.; (n = 4). AC- Activated charcoal.

Table 4: Effects of *Musa sapientum* (with or without activated charcoal) on scar area.

Group	Day 21
1 (Control)	9.0^{d}
2 (Standard)	6.0^{a}
3 (AC Alone)	8.0^{c}
4 (4 % unripe)	7.0^{b}
5 (8 % unripe)	7.0^{b}
6(12 % unripe)	8.0^{c}
7 (16 % unripe)	6.0^{a}
8 (4 % ripe)	6.0^{a}
9 (8 % ripe)	8.0^{c}
10 (12 % ripe)	8.0^{c}
11 (16 % ripe)	6.0^{a}
12 (unripe)	7.0^{b}
13 (ripe)	8.0^{c}

Values with different superscripts on the same column are significantly different at $p < 0.05$.; (n=4). AC- Activated charcoal.

antimicrobial property. (Atzingen *et al.,* 2015). This might be responsible for a progressive decrease in microbial load on wound surface. The microbial load at the four time points was lower for unripe *Musa sapientum* peel gel supplemented with activated charcoal when compared with ripe *Musa sapientum* peel gel supplemented with activated charcoal. The lowest microbial load was observed in the group of animals treated with 16% unripe *Musa sapientum*peel gel and 4g of activated charcoal (Table 3).

Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils (Hoppe and Granick, 2013). Figures of Tensile (Wound) strength is acquired from both remodeling of collagen and formation of stable intra- and intermolecular crosslinks. The collagen molecules synthesized are laid down at the wound site and become cross-linked to form fibres (Hoppe and Granick, 2013). In excision wound model, an increase in tensile strength was observed at the four time points. This might be due to the increase in collagen concentration and stabilization of the fibres. Since wounds treated with unripe *Musa sapientum* peel gel supplemented with activated charcoal showed greater tensile strength, it might be inferred that the addition of activated charcoal did not only increase collagen synthesis per cell but also aided in cross-linking the protein. The scar area decreased with an increase in the mass of activated charcoal as shown in (Table 4). Group 7 and 8 have the lowest scar area on day 21.

Conclusion

This research study has revealed that activated charcoal can be used to boost wound-healing potential of banana (*Musa sapientum*) peel. Wound-healing activity of *Musa sapientum* peel increased with an increase in concentration of activated charcoal. The research study

also revealed that unripe banana (*Musa sapientum*) peel exhibits greater wound-healing activity compared to ripe banana (*Musa sapientum*) peel. The prophylactic effects elicited by a mixture of activated charcoal and *Musa sapientum* peel gel in terms of wound contraction and other related biochemical parameters is a strong indication that activated charcoal can be used to boost the wound healing potential of *Musa sapientum* peel.

Conflict of interest

The authors declared that there is no conflict of interest.

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