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# Wound healing and antioxidant capacity of *Musa paradisiaca* Linn. peel extracts

[Cicatrización de heridas y capacidad antioxidante de extractos de cáscara de *Musa paradisiaca* Linn.]

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**Abstract**

*Context*: *Musa paradisiaca* has several biological activities within them wound healing, hypoglycemic, hepatoprotective, antimicrobial, antioxidant, among others. However, these properties in peel have been poorly explored.

*Aims*: Evaluate the wound healing activity induced by an incision wound model using methanolic, hexanoic and chloroformic extracts from *M. paradisiaca* peel.

*Methods*: Dehydrated *M. paradisíaca* peel was mixed with methanol, hexane, and chloroform. The presence of bioactive substances of the *M. paradisiaca* peel extracts was carried out by the Trease and Evans methods. Antioxidant capacity was evaluated by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. Acute toxicity was realized according to up and down OECD procedure in BALB/c mice. Wound healing activity was evaluated in male Wistar rats. Histological analyses of tissues were made by microscopy using staining methods of hematoxylin and eosin and Masson-trichrome.

*Results*: Treated groups with methanolic and hexanoic extracts of *M. paradisiaca* peel showed better wound healing activity in comparison with the group treated with chloroformic extract, with an inhibition of DPPH radical bleaching of 89-90%. It may be due to the presence of alkaloids, tannins, saponins and phenols as principal constituents by conferring antioxidant capacity. The extract did not induce any toxicity.

*Conclusions*: The findings showed the wound healing and antioxidant capacity of *M. paradisiaca* peel extract. It was observed that depending on the extraction solvent; there is a variation in the antioxidant capacity that also affects the effectiveness of the restoration of tissue, suggesting that the antioxidant capacity could play a major role in the process of wound healing.

*Keywords*: Antioxidant capacity; banana peel; *Musa paradisiaca*; wound healing activity.

**Resumen**

*Contexto*: *Musa paradisiaca* tiene diversas actividades biológicas como la cicatrización de heridas, hipoglicemiante, hepatoprotector, antimicrobiano, antioxidante, entre otros. Sin embargo, estas propiedades en la cáscara han sido poco exploradas.

*Objetivos*: Evaluar la actividad de cicatrización en un modelo de herida inducida por incisión usando extractos metanólico, hexanoico y clorofórmico de la cáscara de *M. paradisiaca*.

*Métodos*: La cáscara deshidratada de *M. paradisiaca* se mezcló con metanol, hexano y cloroformo. La presencia de sustancias bioactivas se realizó de acuerdo a los métodos reportados por Trease y Evans. La capacidad antioxidante se evaluó por el método de 2,2-difenil-2-picrilhidrazil (DPPH). La toxicidad aguda se realizó de acuerdo al método arriba y abajo de la OCDE, en ratones BALB/c. Los extractos se evaluaron en ratas Wistar. El análisis histológico de tejidos se realizó por microscopía, utilizando tinción de hematoxilina-eosina y tricrómica de Masson.

*Resultados*: Los grupos tratados con el extracto metanólico y hexanoico de cáscara de *M. paradisiaca* mostraron una mejor cicatrización de la herida en comparación con el grupo tratado con extracto clorofórmico, con una inhibición de la decoloración del radical DPPH del 89-90%. Esto puede deberse a la presencia de sustancias antioxidantes como alcaloides, taninos, saponinas y fenoles. El extracto no indujo toxicidad.

*Conclusiones*: Estos hallazgos muestran la capacidad tanto de cicatrización como antioxidante del extracto de la cáscara de *M. paradisiaca*. Dependiendo del solvente para la extracción, existe variación de la capacidad antioxidante que afecta la eficacia de restauración del tejido, mostrando que la capacidad antioxidante desempeña un papel importante en el proceso de cicatrización de la herida.

*Palabras Clave*: Actividad de cicatrización de heridas; cáscara de plátano; capacidad antioxidante; *Musa paradisiaca*.

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## INTRODUCTION

*Musa paradisiaca* is cultivated in the tropical and subtropical countries around the world. Banana is the name commonly used to refer to the fruit of the species of *Musa* genre. The *M. paradisiaca* fruit is a good source of nutrients such as potassium, phosphorus, calcium, nitrogen, iron, vitamins C and E (Alabi et al., 2013).

Different parts of *M. paradisiaca* have been used in traditional medicine (Swathi et al., 2011), and also several biological activities have been reported, such as antiulcerogenic, antidiarrhoeal, hypoglycemic, hepatoprotective, antimicrobial, wound healing, hypocholesterolemic and antioxidant (Pannangpetch et al., 2001; Ojewole et al., 2003; Mallick et al., 2006; Vijayakumar et al., 2008; Fagbemi et al., 2009; Nirmala et al., 2012; Yakubu et al., 2015). However, the biological activities of the *M. paradisiaca* peel have been poorly explored. To date only is known that *M. paradisiaca* peel shows protective role in atherosclerosis disease, regulation of thyroid function and bactericidal activity (Parmar and Kar, 2007; 2008; Alisi et al., 2008).

The process of wound healing is promoted by active principles such as triterpenes, alkaloids, and biomolecules, which are in several plant natural products. These agents usually influence one or more phases of the healing process (Suguna et al., 2002).

Previous studies have shown the antiulcerative activity of *M. sapientum* pulp (Pannangpetch et al., 2001; Imam and Akter, 2011), and the wound healing effect of *M. paradisiaca* stems (Amutha and Selvakumari, 2014), therefore the aim of this study was to evaluate the wound healing activity induced by an incision wound model using methanolic, hexanoic and chloroformic extracts of *M. paradisiaca* peel.

## MATERIAL AND METHODS

### *Musa paradisiaca* peel extracts

*M. paradisiaca* Linn. fruits were obtained in the market from Jalisco state in Mexico. The banana peel was treated with 2% citric acid for 15 min, then was dried at 37°C until constant weight in a stove and pulverized by a blender. Subsequently, it was passed through a 0.6 mm mesh sieve. Dehydrated peels of *M. paradisíaca* were mixed with methanol, hexane and chloroform solvents in a ratio 1:2 w/v. The mixtures were stirred at 100 rpm for 3 h, and then were filtered, and the solvent was evaporated under reduced pressure by a rotary evaporator (Büchi R-210, Flawil, Switzerland). The yield was calculated with the weights of dry fruit and extract. The extracts were stored at 4°C until further analysis.

### Phytochemical analysis

The presence of bioactive substances of the *M. paradisiaca* peel extracts was carried out by determining the qualitative analysis of alkaloids, tannins, saponins and phenols by the Trease and Evans (2002) methods.

Briefly, for alkaloids, the extract was stirred with 1% HCl on a water bath. Mayer's reagent was added to the mixture. The turbidity of the precipitate was taken as an indication of the presence of alkaloids.

For tannins, the extract was mixed with distilled water, filtered, and ferric chloride was added to register the presence of blue-green precipitate.

Saponins were determined boiling extract with distilled water in a tube and observing the formation of stable foam.

For the determination of phenols, ferric chloride solution was added to the filtered mixture of extract and distilled water. The green-blue color was indicative of a phenolic hydroxyl group presence.

### Animals

All studies were conducted in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (National Institute of Health, 1985) and they were handled following the animal care guidelines in accordance with regulations enacted by the Federal Government of Mexico (NOM-062-ZOO-1999). An internal committee reviewed the protocol for the care of laboratory animals.

Male Wistar strain rats of three months of age and 180-200 g weight and BALB/c mice (eight-week-old male, 23 ± 2 g) were purchased from the Zooterio of the University of Guadalajara. They were housed under standard conditions (3 rats and

5 mice per cage at 23 ± 2°C at relative humidity 44–55% and light and dark cycles of 10 and 14 h, respectively) with rodent diet and water *ad libitum* during the experiment.

### Wound healing activity

Rats were divided into five groups containing four animals each. The first was the negative control group treated with saline solution as vehicle, the second was the positive control treated with a commercial healing (Recoveron diluted in saline solution) at dose of 10 mg/kg, the third, fourth and fifth groups were treated with methanolic, hexanoic and chloroformic extracts of *M. paradisiaca* peel at a dose of 100 mg/kg, by topical application respectively.

Rats were anaesthetized by droperidol (2 mg/kg, i.m) and ketamine (50 mg/kg, i.m). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal incision of 2 cm in the skin, along the dorsal region, was made using a bistoury assuring the injury in the skin package (epidermis, dermis, and hypodermis) as described by Ehrlich and Hunt (1968). Care was taken that the incisions were made at least 1 cm lateral to the vertebral column. A point closed the wounds with a 3-0 braided silk (Teleflex, Illinois, USA). Recovery of the animals was allowed on a warm and wet blanket; then they were returned to their normal conditions. All was made under aseptic conditions. The wounds were left undressed, and the treatments were applied topically once a day (24 h after post-lesion) for 21 consecutive days. All rats, 24 h after the last application were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and then were sacrificed using carbon dioxide atmosphere.

### Tissue collection

The wounds and the skin specimens of the treated and untreated control group were excised by a 1 cm biopsy punch (Ferreira et al., 2008). The tissue specimens were immersed in a fixing solution (4% paraformaldehyde in 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH 7.34) for 6 h, then the specimens were placed in a cryoprotectant solution (30% sucrose, 0.5% Arabic gum) for 3 days at 4°C to make cuts of 15 µm thick with cryostat (Leica CM1950, Federal Republic of Germany) and stored in cryoprotectant solution (phosphate buffer/ethylene glycol/glycerol) at –20°C until used (Kelly et al., 2015). The collagen fibers analysis of the tissue cuts were stained with Masson-trichrome [HT15 Trichrome Stain (Masson) kit, Sigma-Aldrich, Toluca, MX] and for general morphological observation were stained with hematoxylin and eosin. Histopathological changes in the section of the skin were observed under the microscope (Leica 090-135 with camera DFC-290, Federal Republic of Germany) at 50 or 100X magnification.

### Antioxidant capacity

Antioxidant capacity was evaluated by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. Aliquots of 200 µL of each *M. paradisiaca* peel extract (methanolic, hexanoic and chloroformic) at a concentration of 10 mg/mL, were mixed with 2 mL of DPPH (125 µM) in 80% methanol and then was incubated in the dark for 30, 60 and 90 min to ensure complete neutralization of the radical. The absorbance was measured at 520 nm by a spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). An aliquot of 200 µL of 125 µM butylated hydroxytoluene (BHT) was used as a standard measured at 30 min. The total antioxidant capacity (%TAC) was expressed as the percentage inhibition of DPPH radical and determined with the following equation:

$$\%TAC = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where Abs is absorbance.

### Acute toxicity test

The acute toxicity of the *M. paradisiaca* peel extract was carried out according to OECD 425 method as described by Padilla-Camberos et al. (2013). Five groups of five female mice each were orally administered by cannula at different doses consisting of 125, 250, 500, 1000, and 2000 mg/kg b.w. Mortality was recorded 24 h after the administration of the extracts. Animals were observed during two weeks to detect signs of delayed toxicity like aggressiveness, piloerection, tremors, convulsions, salivation, diarrhea, and lethargy. At the end of the study, all animals were anesthetized with sodium pento-

barbital (50 mg/kg, i.p.), then were euthanized using carbon dioxide chamber.

### Statistical analysis

Statistical comparison was performed by one-way analysis of variance followed by Duncan *posthoc* analysis ($p<0.05$) using Statgraphics XVI software (Statpoint Technologies, Inc. Virginia USA). Data were performed in triplicate.

## RESULTS AND DISCUSSION

Medicinal plants are widely used in several countries as complementary and alternative medicine due its low cost and easy access. Biological activities of *Musa paradisiaca* peel are not completely known, recently has been reported its protective role in atherosclerosis disease, regulation of thyroid function and its activity against *Staphylococcus* and *Pseudomonas* species (Parmar and Kar, 2007; 2008; Alisi et al., 2008). Now in this study, it is reported its wound healing activity.

### Phytochemical analysis of *M. paradisiaca* peel

The phytochemical analysis of the *M. paradisiaca* peel extracts qualitatively revealed the presence of alkaloids and tannins in appreciable amounts, while saponins and phenols were present in moderate quantities. These results are similar with the reported for the methanolic *M. paradisiaca* stem extract, except by the presence of flavonoids and glycosides (Amutha and Selvakumari, 2014).

### Wound healing activity of *M. paradisiaca* peel

The healing effect of each extract was compared with the normal tissue of the untreated control. In this normal tissue the four layers of the epidermis can be identified (stratum basale, spinosum, granulosum, and lucidum), they are composed of keratinized stratified squamous epithelium. After epidermis the two layers of the dermis are found, the papillary and reticular, the first dermis layer is composed of loose connective tissue as the collagen and blood vessels, while the reticular layer is composed of dense irregular connective tissue (Fig. 1).

In the treatment performed with methanolic and hexanoic *M. paradisiaca* peel extract was observed a process wound healing (Figs. 2 and 3), complete epithelialization (Figs. 2A and 3A), thickening by the increase of collagen fibers (Figs. 2A and 3B), and the presence of cellular infiltration of fibroblasts (Figs. 2B and 3C). Besides, the methanolic *M. paradisiaca* peel extract showed more proliferating blood capillaries (Fig. 2B).

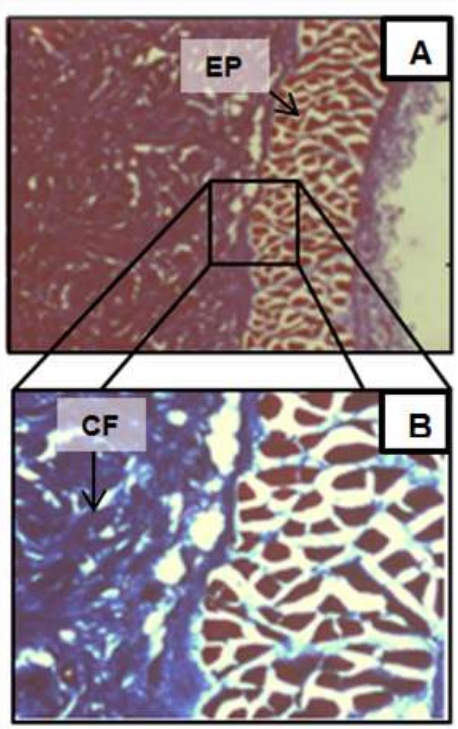


**Figure 1.** Histopathological evaluation of skin in control Wistar strain rat treated with the vehicle by topical application.

Picture show the normal structure of skin composed of epithelium (stratified squamous epithelium keratinized, EP), collagen fibers stained in color blue (CF). [Masson-Trichome, 50X (A) 100X (B)].

Regarding the treatment with chloroform *M. paradisiaca* peel extract (Fig. 4) the wound healing could be observed by the presence of collagen fibers and cellular infiltration (Fig. 4A), but the epithelialization was incomplete and hemorrhagic foci was seen in the dermis (Fig. 4A). Tissue remodeling was more disorganized in comparison to the other extracts (Fig. 4B).

The positive control (Fig. 5) showed a wound healing and, remodeling of the tissue, with a complete epithelialization and thickening of the epidermal layer (Fig. 5A), the presence of collagen fibers. Also, it is observed the presence of cellular

infiltration of fibroblasts and proliferation of blood vessels (Fig. 5B-C).

The methanol and hexane extracts of *M. paradisiaca* peel promotes healing in the wound; these results are consistent with those reported by Amutha and Selvakumari (2014), where it was observed that the methanolic stem extract of *M. paradisiaca* had healing activity. Also, Atzingen et al. (2013) observed increasing of collagen fiber and major inflammatory cell infiltration when *M. sapientum* peel gel on surgical wounds in rats was applied. However, they did not use any type extraction of bioactive compounds. In this study, the extraction of bioactive molecules was performed with specific solvents such as methanol and it was observed that the use of this organic solvent favors the wound regenerative process (Agarwal et al., 2009; Atzingen et al., 2013; Amutha and Selvakumari, 2014). It is supported by the poor wound healing capacity showed by chloroformic *M. paradisiaca* peel extract that is clearly reflected in their limited regenerative activity (Fig. 4).

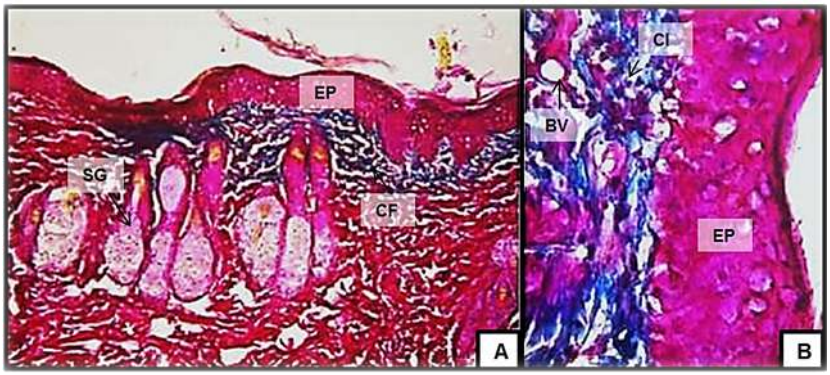


**Figure 2.** Histopathological evaluation of Wistar strain rat skin treated with *Musa paradisiaca* methanolic extract (100 mg/kg) by topical application.

The picture shows the epithelium (EP), collagen fibers (CF), sebaceous glands (SG), blood vessels (BV) and cellular infiltration of fibroblasts (CI). [Masson-Trichome, 50X (A) 100X (B)].

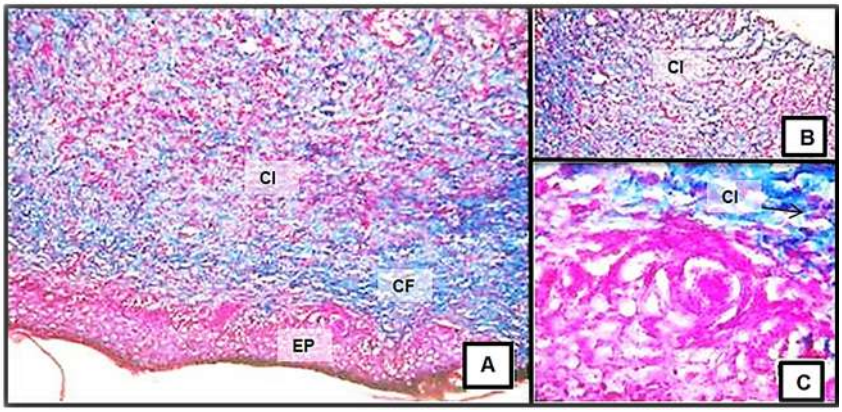


**Figure 3.** Histopathological evaluation of Wistar strain rat skin treated with *Musa paradisiaca* hexanic extract (100 mg/kg) by topical application.

The picture shows the epithelium (EP), collagen fibers (CF), and cellular infiltration of fibroblasts (CI). [Masson-Trichome, 50X (A and B) 100X (C)].

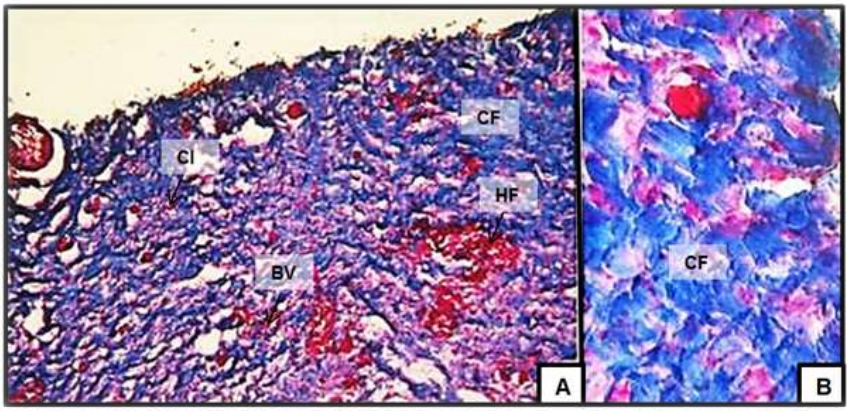


**Figure 4.** Histopathological evaluation of Wistar strain rat skin treated with *Musa paradisiaca* chloroform extract (100 mg/kg) by topical application.

The picture shows the presence of collagen fibers (CF), blood vessels (BV), the focus of hemorrhage (HF) and cellular infiltration of fibroblasts (CI). [Masson-Trichome, 50X (A) 100X (B)].

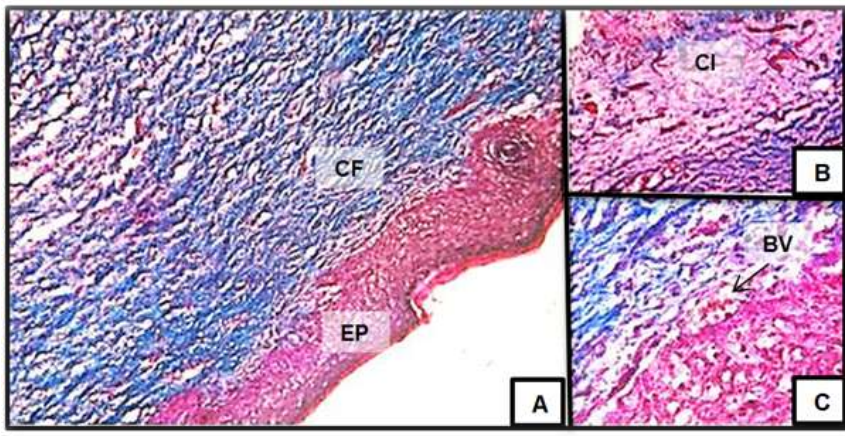


**Figure 5.** Histopathological evaluation of positive control (Recoveron 10 mg/kg) applied to the skin of Wistar strain rat.

The picture shows the epithelium (EP), collagen fibers (CF), blood vessels (BV) and cellular infiltration of fibroblasts (CI). [Masson-Trichome, 50X (A) 100X (B and C)].

Wound healing is a response to tissue injury that includes molecular and cellular processes for tissue repair. In a wound healing process, the collagen is the predominant extracellular protein in the granulation tissue. After an injury is increased the collagen synthesis to provide the integrity and strength to tissue matrix. It is reflected by the intensity of blue color in Masson trichrome staining (Albaayit et al., 2015). In the regenerative process, also is carried out the processes of reepithelization, angiogenesis, proliferation and remodeling. The cells that perform those processes are the keratinocytes, fibroblasts, endothelial and inflammatory cells (Agarwal et al., 2009; Atzingen et al., 2013).

## Antioxidant capacity of *M. paradisiaca* peel

To know whether a relation exists between the antioxidant capacity and the wound healing effect, antioxidant test for the three extracts was conducted at minute 30 the hexanoic extract was statistically significant higher in relation with the others extracts. The methanolic and hexanoic extracts from *M. paradisiaca* peel showed higher antioxidant capacity regarding chloroformic extract it was statistically significant higher with 89% for methanol, 90% for hexane and 34% for chloroform at minute 90, where the highest antioxidant capacity of the three extracts was observed. Hexanoic extract had the highest antioxidant capacity of 84% at 60 min for this extract was statistically significant higher, while the chloroform extract showed lowest antioxidant capacity with a percent of 16% at 60 min (Fig. 6). It observed that there is an interrelation between antioxidant capacity and wound healing activity since the tissues treated with the methanolic and hexanoic extract showed a better healing unlike to chloroform extract. The results of this study indicate that the antioxidant capacity could play a major role in the process of wound healing.

It is known that antioxidants play a role in the removal of inflammation products, and they are beneficial in wound healing (Pereira and Maraschin, 2015). In this study the presence of the tannins, saponins and alkaloids are reported, these compounds promote the wound healing process due to their antioxidant activities which could explain the wound healing capacity of the *M. paradisiaca* peel extracts (Kim et al., 2011; Senthil et al., 2011).

Taking into account that banana peels are discarded or used for the animal feeding and as organic fertilizer (Charrier et al., 2004) and skin wounds affect a large part of the population (Atzingen et al., 2013). The use of the *M. paradisiaca* peel could be a good alternative for the treatment of skin wounds.

## Acute toxicity test

The safety of plant extracts is crucial when it will be used clinically; therefore, we tested the acute oral toxicity in mice finding that the use of *M. paradisiaca* peel extract would be safe because the study did not show toxic effects. This result coincides with the reported by Agarwal et al. (2009), where the pulp of *M. sapientum* was non-toxic by the oral route.

The wound healing activity of the *M. paradisiaca* peel reported in this study provides the basis for the

development of topical pharmaceutical formulations. Further studies are required to determine the optimal concentration for the wound healing in a reduced time, identify the compound or compounds responsible for the wound healing activity and determine the antioxidant activity *in vivo* of the *M. paradisiaca* peel.

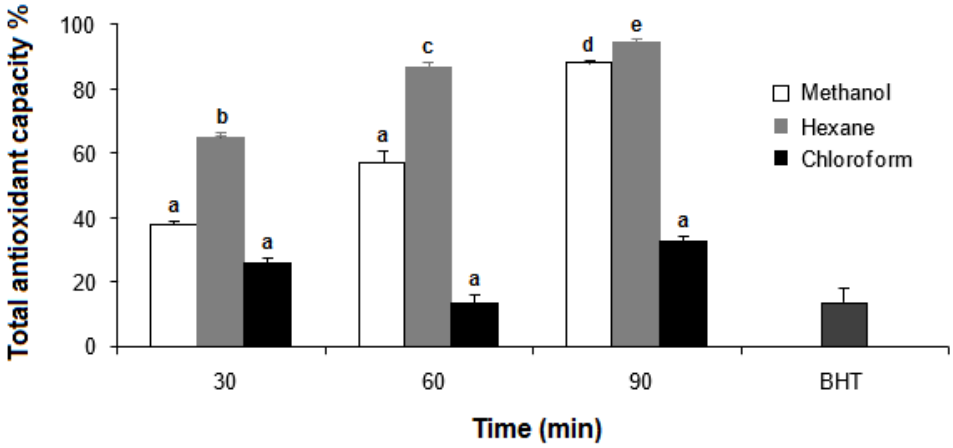


**Figure 6.** Total antioxidant capacity at different times of *Musa paradisiaca* peel extracts at a concentration of 100 mg/mL.

Bars accompanied by different letter(s) indicate significant differences ($p<0.05$) using Duncan *posthoc* analysis.
Data are expressed as mean ± SD (n = 3). BHT (butylated hydroxytoluene) was used as a standard antioxidant compound.

## CONCLUSIONS

The finding showed the wound healing activity and antioxidant capacity of *M. paradisiaca* peel extract. The methanolic and hexanoic extracts of *Musa paradisiaca* peel showed wound healing activity in Wistar rats; it may be due to the presence substances that confer antioxidant capacity, contributing to accelerating the healing process of the wound healing. It shows that the antioxidant capacity could play a major role in the process of wound healing. The formulation of a pharmaceutical form of subcutaneous application could be a good alternative for the treatment of wounds.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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|---|---|---|---|---|---|---|
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| Definition of intellectual content | | x | | | | |
| Literature search | x | x | | x | | |
| Clinical studies | | | | | | |
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