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ORIGINAL ARTICLE

GC-MS Profiling and Wound Healing Potential of *Musa paradisiacal L.* latex in L929 Fibroblast Cells

R. Rakkimuthu*, U. Sivaranjani, A.M. Anandakumar, P. Sathishkumar and D. Sowmiya
Dr. Mahalingam Center for Research and Development, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi-642001, Tamil Nadu, India
Corresponding Author: Email: rakkimuthu@ngmc.org

ABSTRACT
The banana, or Musa paradisiaca L., is a member of the Musaceae family, which is grown extensively in tropical as well as subtropical climates. Although bananas are mostly prized for their nutritional content, different plant components have been utilized for many years to manage inflammation in traditional medicine, burns, and wounds. Nevertheless, there is still little scientific proof of its capacity to cure wounds. The main focus of the study was investigation of phytochemical composition of M. paradisiaca latex methanolic extract using GC-MS and in vitro fibroblast cell migration assay to evaluate the latex's ability to promote wound healing. GC-MS analysis revealed 20 bioactive components in the methanolic extract. The primary components were beta-sitosterol (21.49%), Cycloheptane,4-methylene-1-methylene-1-methyl -2-(2-methyl-1-yl)-1-vinyl-(20.79%), 17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexa -decahydrocyclopenta [a] -phenanthren-3-ol (16.64%) and minor compounds were [a]10alpha-Eremophilane (7.93%) and Benzo[h]quinoline,2,4-dimethyl (5.98%). The wound healing experiment showed that a concentration of 100 µg/ml produced 91.2% wound closure within 24 hrs, indicating that the extract significantly boosted fibroblast cell migration in contrast to the untreated control group. Beta-sitosterol and other bioactive ingredients in latex are probably responsible for the considerable promotion of fibroblast cell migration and wound closure. Additional mechanistic and in vivo research is required to validate its potential in medicines and regenerative medicine.
Keywords: *Musa paradisiaca, GC-MS analysis, Beta-sitosterol, wound healing, L929 cells, cell migration, therapeutic potential*

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INTRODUCTION

Banana (*Musa sp.*), a monocotyledonous perennial plant belonging to the order Zingiberales and family Musaceae, are among the most popular fruits grown and eaten worldwide. About forty species make up the genus Musa, which is found in Australia, New Guinea, India, and Southeast Asia. Bananas hold significant economic and nutritional value [1], being the fourth most important staple food and the agricultural product with the highest value, after chocolate, coffee, sugar, and grains. Bananas (*Musa* sp.) are grown across more than 120 nations on 10 million hectares, producing 88 million metric tons annually, making them essential to the world's fruit economy [2]. Banana latex, a watery sap exuded from the plant, is a complex emulsion containing proteins, gums, oils, tannins, sugars, starches, alkaloids, and resins. The majority of plants generate white latex, while some produce yellow, orange, or crimson latex. Latex forms through the laticiferous system, which develops in plants through rows of cells in the meristem or by the dissolution of cell walls, creating continuous tubes called latex vessels. *M. paradisiaca* latex has been numerous antidiabetic substances, some of the substances that have been found include rutin, catechin/epicatechin, nicotiflorin, p-hydroxybenzoic acid, p-coumaric acid, gallic acid, caffeic acid, trans-cinnamic acid, lupeol, ferulic acid, vanillic acid, and chlorogenic acid [3]. Wound healing, a critical physiological process, involves tissue repair and restoring normal function following injury. Chronic wounds, which fail to heal properly, can result in significant morbidity, disability, and socioeconomic burdens [4]. Wounds may be classified as open or closed and undergo several stages of healing, including

hemostasis, inflammation, proliferation, granulation, epithelialization, and remodeling. Oxidative stress and free radicals often complicate wound healing, necessitating effective local therapies to minimize systemic effects, prevent excessive bleeding, and reduce inflammation [5]. Generally, banana fruits contribute to a healthy diet and offer remarkable medicinal benefits. Bananas promote the retention of calcium, nitrogen, and phosphorus, supporting the regeneration of healthy tissues. Additionally, bananas are among the few fruits suitable for ulcer patients due to their ability to neutralize gastric acids. Despite the documented benefits of bananas, the therapeutic potential of *M. paradisiaca* latex remains largely unexplored. Traditional uses of *M. paradisiaca* latex suggest its efficacy in treating various illnesses, but scientific validation is limited. This study aims to bridge this gap by scientifically evaluating wound-healing properties of *M. paradisiaca* latex. The research seeks to confirm traditional claims, identify bioactive compounds responsible for these activities, and establish the potential of *M. paradisiaca* latex as a valuable medicinal resource. By addressing these objectives, the study highlights the underutilized potential of banana latex, traditionally considered a waste product and emphasizes its importance in therapeutic applications.

MATERIAL AND METHODS

Collection of plant

M. paradisiaca plants were gathered from its surrounding area of Pollachi, Coimbatore district, Tamilnadu. The latex from the leaf and stem of *M. paradisiaca* was collected aseptically and extracted using methanol as the organic solvent. After that, the mixture underwent a 10-minute centrifugation at 2000 RPM. The extracted material that was produced was carefully collected along with stored in an airtight container.

GC-MS Analysis

The methanol extract from *M. paradisiaca* leaf as well as stem latex was subjected to Agilent MS-5975C mass spectrometer and Agilent GC-7890A gas chromatograph, configured to run in electron ionization mode at 70 eV, are employed in GC-MS analysis [6]. Helium has been employed as the carrier gas in an Agilent DB5MS capillary column (30 mx0.25mm internal diameter x 0.25micron film thickness) with a flow rate of 7.6522 PSI. The temperature program began at 50°C with a 1:100 split ratio and rose by 12°C per minute to 300°C. The retention indices mass spectra within these, constituents were identified have also compared to its NIST library version 2.0 and real sample spectra version 2.0.

In Vitro wound scratch assay

In 24-well plates, L929 cells were cultivated to approximately 80% confluency 1×102 cells/ml is the density. Using a sterile cell, a tiny linear cut was made in the confluent monolayer scraper employing the technique outlined by Liang *et al* [7]. To get rid of cellular debris, cells were thoroughly washed with 1 × PBS before being exposed to varying concentrations of test substances. At various intervals, cell growth was observed: at 0, 4, 18, and 24 hours, pictures taken using an inverted phase contrast microscope of the moving cells. (Radical Instruments, India) connected to a digital camera (Nikon, Tokyo, Japan).

$$\text{Wound Closure (\%)} = \frac{Ti - Tf}{Ti} \text{X } 100$$

Where *Ti* represents the the wound area at 0 hours.Tf represents the wound area at 4, 18, and 24 hours of therapy with relation to the total studied area.

Statistical Analysis

The SPSS 16 program was used to do the statistical analysis. The data on wound healing percentage was statistically analyzed employing a one-way analysis of variance (ANOVA). The results were presented with a significance threshold of $p<0.05$ and presented as mean ± standard deviation (SD). A value has been deemed statistically significant if P was less than 0.05.

RESULT AND DISCUSSION

GC-MS Analysis

The methanolic extract of *M. paradisiaca* displayed twenty peaks on the GC-MS Chromatogram (Figure 1). The components Beta-sitosterol (21.49%), Cycloheptane,4-methylene-1methylene-1-methy-2-(2-methyl-1-vinyl-) (20.79%), 17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta [a] phenanthran-3-ol (16.64%) and minor compounds were 10 alpha.-Eremophilane (7.93%), Benzo[h]quinoline,2,4-dimethyl (5.98 %) (Figure 2) along with trace amounts of other compounds, as listed in Table 1. The methanolic extract *M. paradisiaca* included 20 bioactive components and these compounds possess pharmacological properties that may contribute to the plant's therapeutic potential. Beta-sitosterol and Cycloheptane,4-methylene-1methylene-1-methy-2-(2-methyl-1-vinyl-) has anti-inflammatory and anti-cancer qualities [8], Because of the existence of the methylene and vinyl groups,

this compound could be used as an intermediate in the synthesis of other organic compounds or materials, particularly those requiring alkenyl (vinyl) groups. Additionally, the minor compound Dimethyl tetrasiloxane has demonstrated antioxidant and anti-inflammatory properties [9].

Table 1. GC-MS Identified Compounds in the Methanolic Extract of *Musa paradisiaca* latex.

S. No	RT	Compound name	Area %	Structure	Molecular weight
1	12.863	Tetracosanoic acid,methyl ester	1.07	$C_{25}H_{50}O_2$	382.7 g/mol
2	13.086	2-cyclopentene-1-undecanoic acid	1.75	$C_{16}H_{28}O_2$	252.39 g/mol
3	14.008	7,11-Dihydroxytomatidine tetraacetate	1.53	$C_{26}H_{39}NO_6S$	493.7 g/mol
4	14.252	Eta.-pentamethylcyclopentadienyl Ethylisonitril-(N,N,N,N-tetramet hylethin-1,2-diamin)-molybdaeniodi	1.53	$C_{10}H_{15}Cl_3Ti$·	289.4 g/mol
5	15.174	2(1H)-Naphthalenone,octahydro-4a-methyl-7-(1-methylethyl)-,(4a.alpha.,7.beta.,8a.beta.)	2.57	$C_{14}H_{24}O$	208.34 g/mol
6	16.885	Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)	3.06	$C_{11}H_{13}NO_3$	207.23 g/mol
7	17.085	Benzo[h]quinoline,2,4-dimethyl	5.98	$C_{15}H_{13}N$	207.27 g/mol
8	17.274	5-Methyl-2-phenylindolizine	1.25	$C_{15}H_{13}N$	207.27 g/mol
9	17.963	Tetrasiloxane,decamethyl	1.04	$C_{10}H_{30}O_3Si_4$	310.68 g/mol
10	18.463	1H-indole-2-carboxylic acid ,6(4--ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropyl ester	1.64	$C_9H_9NO_3$	179,17 g/mol
11	19.396	Cyclotrisiloxane,hexamethyl	1.72	$C_6H_{18}O_3Si_3$	222.46 g/mol
12	19.596	Heptasiloxane,1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl	3.55	$C_{14}H_{42}O_6Si_7$	503.07 g/mol
13	19.796	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	16.64	$C_{29}H_{50}O$	414.7 g/mol
14	19.907	Cycloheptane,4-methylene-1-methylene-1-methyl -2-(2-methyl-1-yl)-1-vinyl	20.79	$C_{15}H_{24}$	204.35 g/mol
15	20.151	10alpha-Eremophilane	7.93	C15H28	208.38 g/mol
16	20.462	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]	1.68	$C_{12}H_{14}ClN$	20770 g/mol
17	21.140	Cyclotrisiloxane, hexamethyl	1.74	$C_6H_{18}O_3Si_3$	222.46 g/mol
18	21.385	4-Dehydroxy-N-(4,5-methylenedioxy- 2-nitrobenzylidene)tyramine	2.13	$C_{16}H_{14}N_2O_4$	298.29 g/mol
19	21.696	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl	1.15	$C_{14}H_{44}O_6Si_7$	505.09 g/mol
20	22.484	beta.-Sitosterol	21.49	C29H50O	414.71 g/mol

This table1 presents the GC-MS analysis identified the chemical constituents of the banana latex methanolic extract. The information includes peak area percentages, chemical formulae, and retention durations (RT), which show how abundant each molecule is in relation to the others. A naturally occurring Beta-sitosterol, a plant sterol, is existing in a range of meals made from plants, including nuts, seeds, and vegetables. Its anti-inflammatory, antidiabetic, and lipid-lowering properties have all been thoroughly studied. Beta-sitosterol is a viable option for treating diabetes-related complications, such as DFUs, because it has been shown to improve insulin sensitivity and decrease blood glucose levels. Prolonged inflammation is a primary feature of diabetic wound healing, and beta-sitosterol has anti-inflammatory properties are thought can help reduce the long-term inflammatory reaction that prevents tissue healing. Additionally, beta-sitosterol has been demonstrated to modulate lipid metabolism, which can further support wound healing by improving cell membrane integrity and reducing oxidative stress in the wound bed [10].

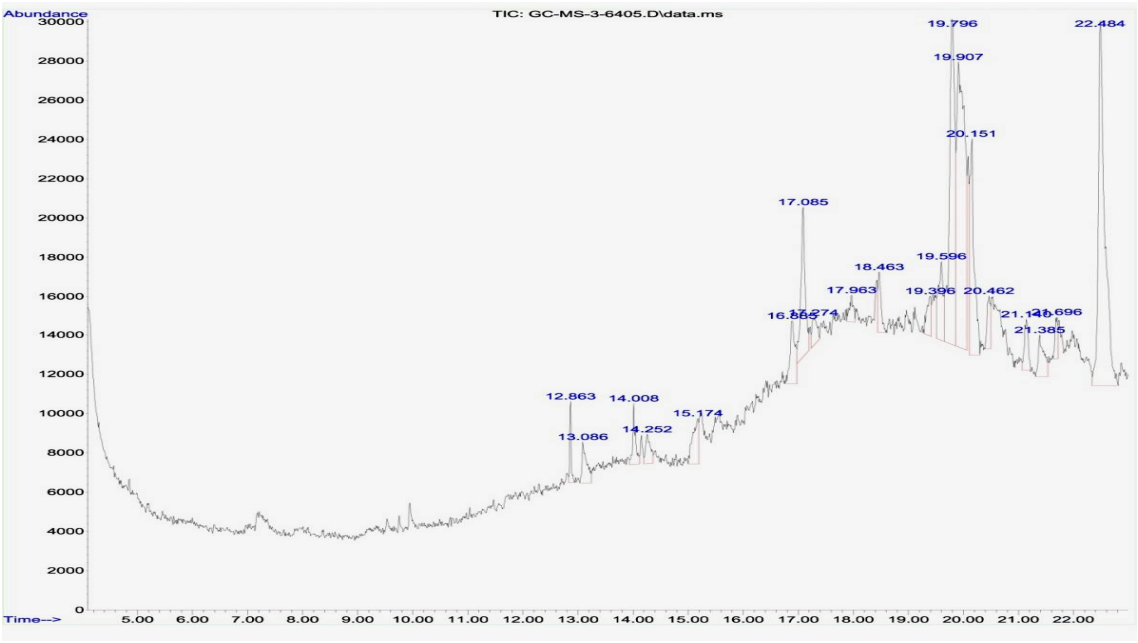

Figure-1 GC-MS chromatogram of methanolic extract of *Musa paradisiaca* latex.

GC-MS chromatogram showed the identified chemical constituents with their respective retention times and peak intensities

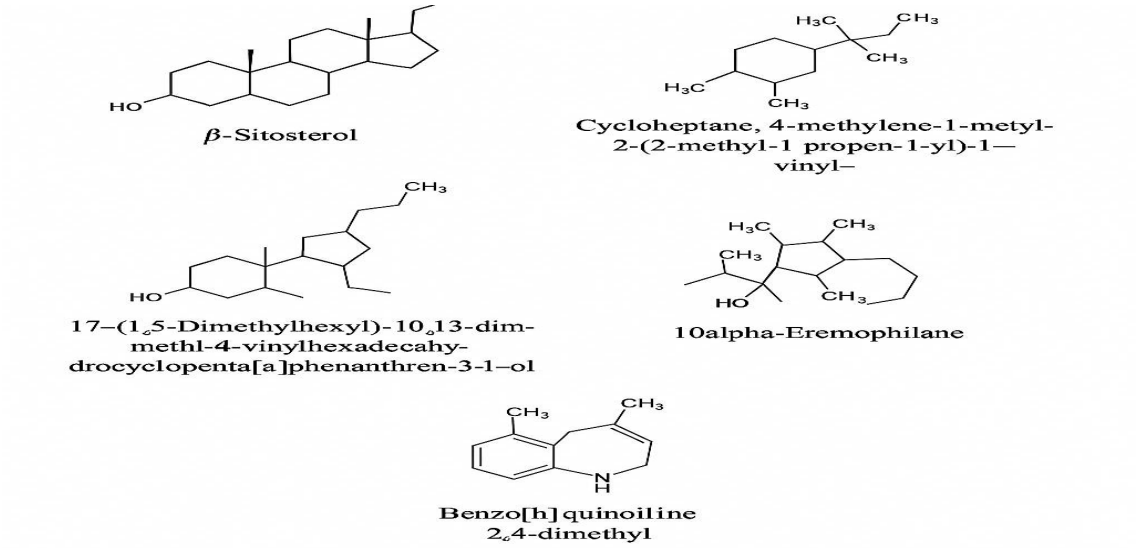

Figure: 2 Structures of Beta-sitosterol Beta-sitosterol , Cycloheptane,4-methylene-1methylene-1-methy-2-(2-methyl-1-vinyl , 17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadeca hydro -cyclopenta[a] phenanthran-3-ol, 10 alpha-Eremophilane and Benzo[h]quinoline,2,4-dimethyl

Wound Healing Activity

A popular *in vitro* technique for researching wound healing[7] is the scratch assay, which was carried out on the L929 fibroblast cell line. The effectiveness of the treatment in healing wounds was evaluated at different amounts (25, 50, 75, and 100 µg/ml). This wound closure percentage significantly increased with increasing concentration. At 25 µg/ml concentration, the wound closure was 44.1 ± 0.91%, which increased to 65.33±0.76%. At 50 µg/ml. A further increase to 75 µg/ml led to 84.03 ± 0.80% wound closure, while the highest closure (91.2 ± 0.64%) was observed at 100 µg/ml concentration.

Table 2. Effect of Different Concentrations on Wound Closure (%) in 24 Hours

S. No.	Concentration (%)	Wound Closure (%) (Mean ± SD)
1.	Control	3.67 ± 2.08
2.	25	44.1 ± 0.91*
3.	50	65.33 ± 0.76*
4.	75	84.03 ± 0.80*
5.	100	91.2 ± 0.64*

N=3

The mean ± standard deviation (SD) represents the data. Statistical significance have been determined by calculating a p-value of less than 0.05 using a one-way ANOVA. Treatment with *Musa paradisiaca* latex extract demonstrated enhanced cell migration toward the artificially created wound, indicating the extract's ability to promote wound healing by stimulating fibroblast migration. Similarly, a study on another plant species, *Aerva bracteolata*, shown that the methanolic extract of the plant not only improved fibroblast and keratinocyte migration, but also raised the expression of genes related to wound healing [11].

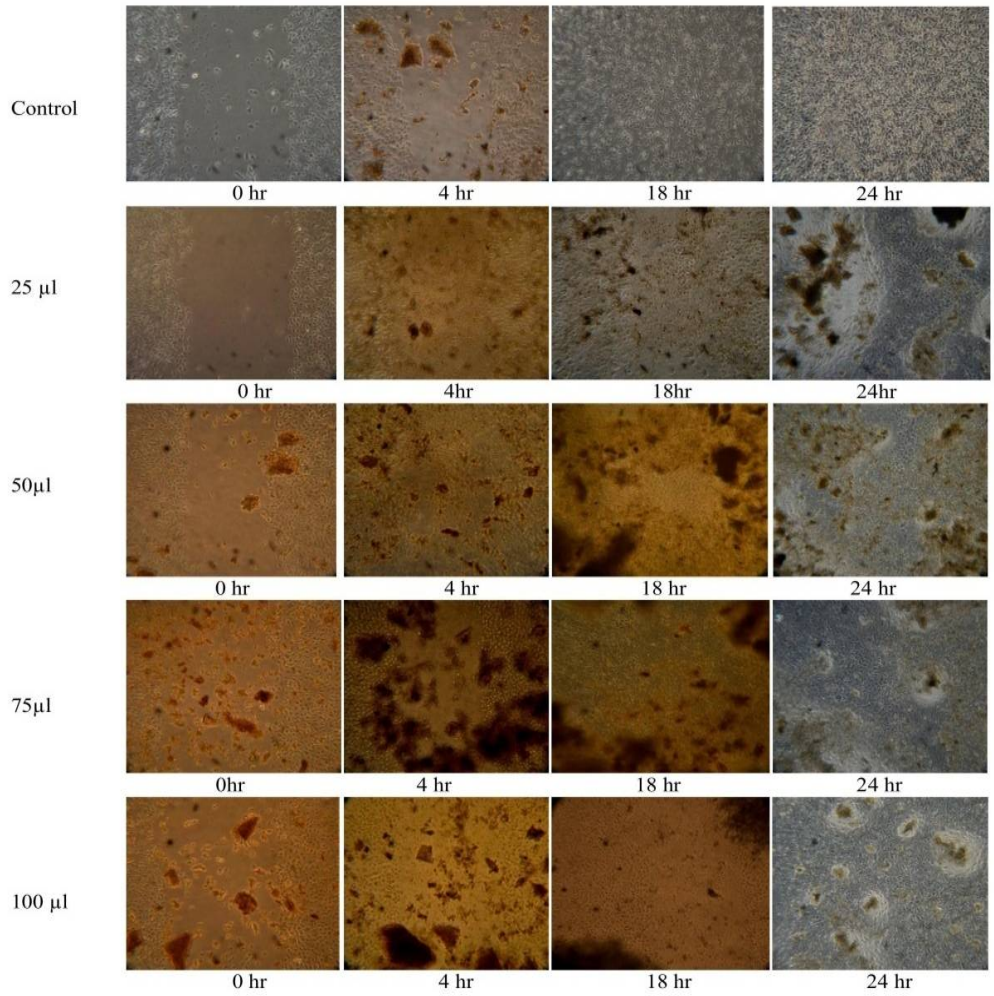

Figure 3: Microscopical images representing the *in vitro* wound healing properties of *Musa paradisiaca* latex

Microscopical pictures demonstrating the latex from *Musa paradisiaca's* capacity to heal wounds *in vitro*, emphasizing the migration, growth, and eventual closure of the wound region. Similar to this, Sundhani *et al*. reported that NIH3T3 fibroblast cells were not cytotoxically affected by the *Musa paradisiaca* var. *sapientum latex* or *Carica papaya* L., (which contains alkaloids and saponins), as demonstrated by IC_{50} (IC_{50}>1000 µg/mL) values showing increased migration and proliferation of these cells [12]. This demonstrates the possible contribution of latex derived from plants to the stimulation of cellular functions necessary for wound healing. To produce granulation tissue and close wounds, fibroblast

migration and proliferation are crucial [13]. In this investigation, the methanolic extract's capacity to promote wound healing of *Musa paradisiaca* using L929 fibroblast cells. The microscopical image demonstrated efficacy across all tested concentrations, with the highest wound closure observed at 100 µg/mL within 24 hours (Figure 3). As the concentration of *M. paradisiaca* methanolic extract increased, the wound healing assay showed a notable increase in fibroblast cell migration and wound closure. As shown in Table 2, the extract exhibited a dose-dependent effect, with wound closure improving from 44.1 ± 0.91% at 25 µg/ml to a maximum of 91.2 ± 0.64% at 100 µg/ml. The results of statistical analysis demonstrated the extract's effectiveness in encouraging cell migration, showing that the observed differences have been significant ($p < 0.05$). Wound closure was greatest at the highest concentration (100 µg/ml), indicating that the extract's bioactive ingredients, especially beta-sitosterol may be essential for hastening the healing process.

CONCLUSION

This study offers scientific proof of the wound-healing capabilities of *Musa paradisiaca* latex on tested fibroblasts. The methanolic extract demonstrated significant efficacy in promoting cell migration and wound closure, which can be credited to the bioactive substances that the GC-MS study revealed. These results demonstrate the possibility of *M. paradisiaca* latex as a valuable resource for developing therapeutic applications in wound management. Further studies are necessary to isolate and characterize the bioactive constituents and to evaluate their mechanisms of action through *in vivo* studies.

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