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Research Article

EFFECT OF LATEX FROM *MUSA PARADISIACA* VAR. *SAPIENTUM* AND *CARICA PAPAYA* L. ON PROLIFERATION AND MIGRATION FIBROBLAST CELL NIH3T3

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ABSTRACT

Objective: The objective of this research was to screen phytochemical constituents and determine the activity of latex from *Musa paradisiaca* var. *sapientum* and *Carica papaya* L. to the process of wound healing in NIH3T3 fibroblasts cells through observations of the proliferation and migration of cells.

Materials and Methods: Screening phytochemical compounds of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. used chemical reagent. Cytotoxic activity using 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) method. The proliferation test used the doubling time method at a susceptible incubation time of 0, 24, 48, and 72 h with a concentration of 25 µg/mL–250 µg/mL. The migration test was carried out using the scratch wound healing method with a concentration of 25 µg/mL–250 µg/mL in the time range of 0, 12, 24, and 48 h.

Results: Phytochemical compounds contained in the latex from *M. paradisiaca* var. *sapientum* (saponin and tannin) and *C. papaya* L. (saponin and alkaloid). The cytotoxic assay results showed that no toxic effect for NIH3T3 fibroblasts cells (IC_{50} >1000 µg/mL). Cell proliferation and migration test results showed an increase in NIH3T3 fibroblast cell proliferation and migration process compared to controls. The concentration of 250 µg/mL of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. is the best to increase of proliferation and migration process of NIH3T3 fibroblast cells.

Conclusion: This study concludes that the latex of *M. paradisiaca* var. *sapientum* and *C. papaya* L. has the potential to increase proliferation and migration activity of NIH3T3 cells.

Keywords: *Musa paradisiaca* var. *sapientum*, *Carica papaya* L., NIH3T3, Cell migration, Proliferation.

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INTRODUCTION

Wounds are a condition of injury to the body caused by contact with physical, chemical, or high-temperature agents. One type of injury that often occurs is the wound caused by sharp objects. Naturally, the body will make repairs after incisions that disrupt the integrity of the skin with stages of hemostasis and inflammation, neovascularization, granulation, reepithelialization, and renovation. However, this requires a long time so that a chemical agent is needed that can help and speed up the healing process of the wound.

One alternative to wound healing agents is to use plants around the environment that are easy to obtain, safe, low cost, and better tolerated by patients [2]. Some active compounds from plants that are proven to help wound healing include tannins, triterpenoids, and alkaloids. Plants containing these compounds and thought to have wound healing activities were *Musa paradisiaca* var. *sapientum* and *Carica papaya* L. *M. paradisiaca* var. *sapientum* contains compounds such as tannins, flavonoids, saponins as antibiotics and Vitamin A, Vitamin C, fat, and protein while *C. papaya* L. also contains saponins, anthraquinones, reducing sugars, flavonoids, alkaloids, tannins, papain, steroids, glycosides, and Vitamin C which are thought to synergistically be able to stimulate the growth of new cells so that they can help in the healing process of wounds [3,4]. Based on previous study, tannins have the ability to precipitate proteins that form clots in wounds, and flavonoids can accelerating tissue regeneration during the process of wound healing [5,6].

Wound healing is defined by the Wound Healing Society as a complex and dynamic as a result of returning the continuity and anatomical function. Wound healing is a tissue regeneration and a complex process in human physiology. The wound healing phase is divided into three phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase. Fibroblasts play an essential role in the process of fibroplasia which is the stage of new blood vessel formation, migration, and proliferation of fibroblasts, extracellular matrix deposition, and maturation and organization of fibrous tissue (remodeling) [7]. At present, there are many wound healing products developed by the pharmaceutical industry but are still unsatisfactory due to the long process, high costs, and various adverse side effects [8]. This study was designed to see the ability of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. to induce proliferation and migration of N1H3T3 fibroblast cells which is a mechanism for wound healing and to identify the compounds contained in the latex of the two plants that play a role in the wound healing process.

MATERIALS AND METHODS

Materials

The tools used in this study include glassware, analytical balance, autoclave, Labconco Purifier Class II biosafety cabinet, CO_2 incubator, liquid nitrogen, inverted microscope, hemocytometer (Neubauer improved 0.100 mm Tiefe Depth Profondeur 0.0025 mm^2), and enzyme-linked immunosorbent assay (ELISA) reader. The main ingredients used in this study were latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. Material analysis of chemical compounds includes HCl,

Mg powder, 1% FeCl3, sulfuric acid, H2SO4, Mayer, Dragendorff, and Wagner reagent.

Cell culture materials used are cell growth media, Dulbecco's Modified Eagle's Medium (DMEM) containing fetal bovine serum 10% v/v, penicillin-streptomycin 1% v/v, and fungizone 0.5% v/v. Harvesting cells from tissue culture dish using trypsin-ethylenediaminetetraacetic acid (EDTA) were 0.25%. The material used in cell proliferation and migration testing is 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) reagent concentration of 5 mg/ml dissolved in phosphate-buffered saline (PBS) 1× pH 7.4. Work reagents are made by diluting 10 times the stock reactant using DMEM. The stopper used is sodium dodecyl sulfate (SDS) 10% in HCl 0.1 N. PBS 1× pH 7.4 is made by dissolving 8 g NaCl, 0.2 g KCl, 0.2 g KH2PO4, and 1.15 g Na2HPO4 into 1 L of aquabides.

Methods

Plant identification

The identification of *M. paradisiaca* var. *sapientum* and *C. papaya* L. was carried out at the Plant Taxonomy Laboratory, Faculty of Biology, Jenderal Soedirman University, Purwokerto.

Material collection

Preparation and collection of materials, the latex of *M. paradisiaca* var. *sapientum* was obtained from Panerusan Village, Wonosobo, Central Java, Indonesia. The latex is taken by cutting the midrib from *M. paradisiaca* var. *sapientum* trees with a height of 1.5 m close to the parent tree and then accommodated in a clean glass place. While the latex of *C. papaya* L. was taken from the village of Bulupayung, Kesugihan District, Cilacap Regency, Central Java, Indonesia. Taking latex of *C. papaya* L. is done every week to get new latex because the time to survive latex is about 3–5 days. Taking latex of *C. papaya* L. is done by cutting the papaya leaf stems. The latex of *M. paradisiaca* var. *sapientum* and *C. papaya* L. is taken using a knife and a container that has been cleaned using 70% ethanol to maintain sterilization from the knife and container used. The best time to take latex is in the morning; this is because, in the morning, the resulting latex is more and better [1].

Sample preparation

The latex of *M. paradisiaca* var. *sapientum* and *C. papaya* L. is dissolved in 1% dimethyl sulfoxide. From the mother liquor then diluted with DMEM culture media.

Screening phytochemical compounds

Identification of phytochemicals compound is using reagent specific for saponins, tannins, flavonoids, and alkaloids. Identification is made by observing the change in color of the sample added to the reagent.

N1H3T3 fibroblast cell culture

All tools that will be used are sterilized first. DMEM culture media made 3 ml aliquots in a conical tube. Cells are taken from the liquid nitrogen tank, then immediately thawed in a water bath at 37°C. The cell suspension was centrifuged at a speed of 650 rpm for 3 min and then the supernatant was removed. In the cell deposits, new media are added and suspended slowly until homogeneous. Then, the cells were grown in several tissue culture flasks or tissue culture dishes; each of which was 2 ml and added with 5 ml of culture medium and homogenized. The cell condition was observed under a microscope and then incubated in a CO_2 incubator at 37°C with 5% CO_2 flow. Twenty-four hours later, a culture media were replaced, then cells were grown to confluent, and the amount was sufficient for research.

After confluent cells, the media were removed, and the cells were washed PBS twice. Cells were added 500 µl trypsin-EDTA 0.25% to release cells and then incubated for 3–5 min in a CO_2 incubator. The DMEM media are added to the tissue culture dish or flask and cells resuspended until all of them are removed from the wall of tissue culture dish or flask. The cell suspension is then transferred into a new sterile conical tube. Cells are calculated by a hemocytometer and cell counter.

MTT assay

MTT assay used to know the half maximal inhibitory concentration (IC_{50}) from the latex of *M. paradisiaca* var. *sapientum* and *C. papaya* L. The cell concentration with NIH3T3 fibroblasts 5×10^3 cells distributed into the 96-well plate and incubation for 24 h at 37°C and 5% CO_2; then, the sample was added as much as 100 µl for each concentration variation from the sample. At the end of incubation (24 h), the culture medium containing the sample was removed, then washed with 100 ml PBS, and then into each well was added 100 ml of DMEM culture media containing MTT 0.5 mg/ml, incubated again for 4–6 h at 37°C with CO_2 flow. Living cells will react with MTT to form purple formazan crystals. After 4–6 h, the media containing MTT is removed; then, 10% SDS solution is added to dissolve formazan crystals. Cells were incubated overnight at room temperature and protected from light. At the end of incubation, the plate is shaken horizontally (with a shaker) for 3 min then read by ELISA reader at λ 595 nm.

Cell proliferation test

The proliferation of N1H3T3 fibroblast cells was detected by MTT assay. Briefly, 180 µl of 3×10^5 cells/ml is inserted into 24 wells. Incubated overnight at 37°C and 5% CO_2, then the sample was added as much as 20 µl for each concentration variation from the sample, incubated for 0, 24, 48, and 72 h and media control and cell control at 37°C and 5% CO_2. Percent viability cell is calculated based on the sample absorbance of each well at ELISA reader at 595 nm λ.

Cell migration test N1H3T3

Fibroblast cells were cultured and cultured in 24 wells. Then, it was injured by being scratched using the help of 10 µl micropipette tip by moving back and forth over the medium; the wound made 0.5 mm long. The well is then washed with PBS for 3 h to remove cell impurities. Then, the wound is seen with the help of a reverse phase microscope equipped with a digital camera. Add 20 µl of the variation in sample concentration into the culture medium and control with PBS. The migration of fibroblast cells was photographed 24 h after being treated with wounds. Photo taking is done at 0–24 h. The distance of cell migration in cells treated by the addition of samples is compared to the distance of migration of control cells; then, migration is calculated (Manoj *et al*., 2009).

Table 1: Phytochemical compounds

Chemical compound	Latex *Musa paradisiaca* var. *sapientum*	Latex *Carica papaya* L.	Latex *Musa paradisiaca* var. *sapientum*	Latex *Carica papaya* L.
Saponin	Steady foam	Steady foam	Positive	Positive
Tannin	Dark blue	Light brown	Positive	Negative
Alkaloid				
Dragendorff	-	Not formed deposits	Negative	Negative
Mayer	Not formed deposits	White deposits	Negative	Positive
Wagner	Not formed deposits	Deposits of brownish red	Negative	Positive
Flavonoid	Not color	Not color	Negative	Negative

RESULTS AND DISCUSSION

Identification of phytochemical compounds of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. shown in Table 1.

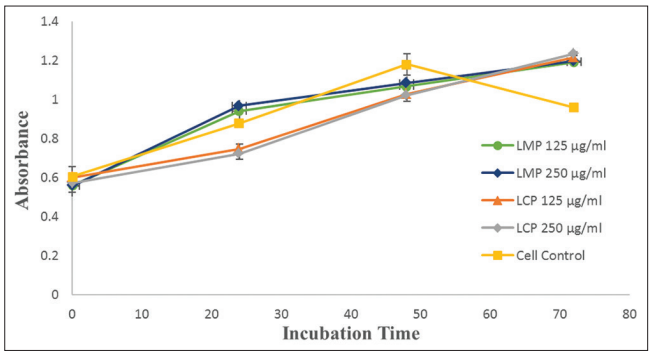

Fig. 1: NIH3T3 fibroblast cell proliferation test graph grown on 96-well plate and treated with a latex of *Musa paradisiaca* var. *sapientum* (LMP) and *Carica papaya* L. (LCP) with a series of concentrations of 50 µg/ml, 125 µg/ml, and 250 µg/ml

Latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. contains saponins, indicated with the formation of foam. Saponin is one of the compounds that trigger the formation of collagen that plays a role in wound healing [9]. Besides, saponins also have the ability as a cleanser is useful for healing a wound [10]. Saponins are active compounds that can cause the foam if beaten with water [11]. Tannin is a compound that has a therapeutic effect as an antiseptic wound tissue – latex from *M. paradisiaca* var. *sapientum* gave positive results with a dark blue color formation, while the latex from *C. papaya* L. showed a negative result due to light brown color formed. The color change occurs when the addition of $FeCl_3$ due to reaction with one hydroxyl group on tannins [12]. Tannins serve as an astringent which can cause small skin pores, stop the exudate and light bleeding [2]. The alkaloid is an organic base containing nitrogen (N) is generally derived from the plant, which has a powerful physiological effect against human beings. The benefits of an alkaloid in the field of pharmacology are to fight microbial infections (Pasaribu, 2009). Alkaloids have the ability as an antibacterial, achieved using disrupting the peptidoglycan constituent components in bacterial cells, making cell walls do not wholly form and cause cell death [10]; on the test, latex alkaloids from *M. paradisiaca* var. *sapientum* give negative results, and latex from *C. papaya* L. shows positive results.

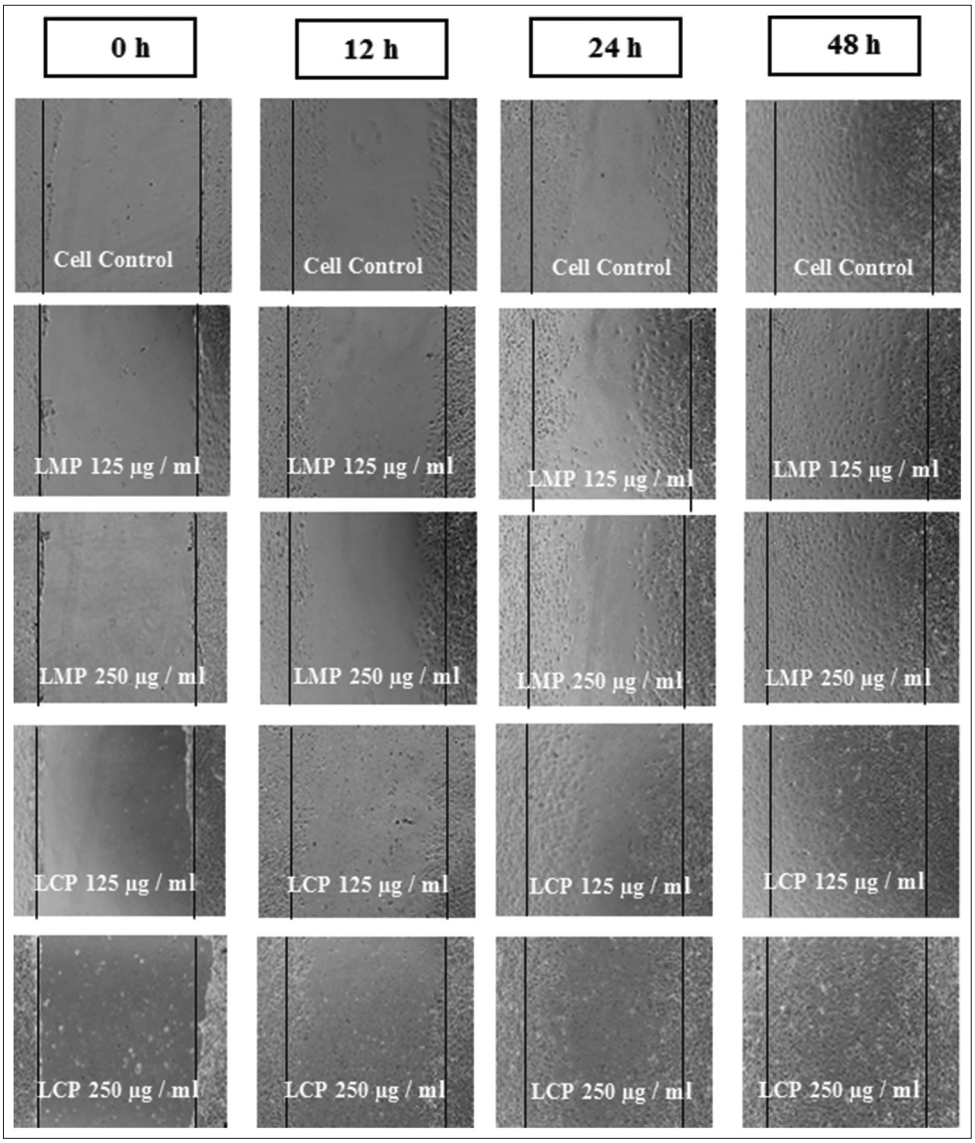

Fig. 2: NIH3T3 fibroblast cell migration test latex of *Musa paradisiaca* var. *sapientum* (LMP) and *Carica papaya* L. (LCP)

The cytotoxic test was carried out aimed to determine the cytotoxic potential of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. against cell NIH3T3. The method used in the cytotoxic test is using the MTT assay. MTT assay is a method based on the ability to live cells to reduce MTT salts. The principle of the MTT method is the reduction of MTT tetrazolium yellow salt (MTT) by the reductase system. Tetrazolium succinate which is included in the respiratory chain in the mitochondria of living cells forms purple formazan crystals and is not water soluble [13]. The results obtained in the cytotoxic test are in the form of IC_{50} values. The IC_{50} value indicates the concentration of the test compounds which can provide a cell viability barrier of 50% of the total cell number – the IC_{50} value of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. was analyzed using Microsoft Excel program, which was 2.801 µg/mL and 6.280 µg/mL. From the IC_{50} value obtained in the cytotoxic test, it can be seen that the latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. does not have a toxic effect because it has an IC_{50} value of more than 1000 µg/mL [14] so that it has the potential for NIH3T3 fibroblast cell.

In the cell proliferation test is performed by doubling time. Doubling time is a period needed by cells to be able to make the amount double the original number [15]. In this proliferation test, the lower the concentration of latex used, the better the activity in healing wounds. The longer the incubation period, the more fibroblast cells proliferate well. This is proven by the concentration of 50 µg/mL which has a more proliferative nature compared with concentrations of 125 µg/mL and 250 µg/mL. The following is the result of the proliferation of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. (Fig. 1).

Determination of the ability of NIH3T3 fibroblast cell migration is done by quantifying the width of the scratch at the beginning of the scratching until the cells migrate to cover the scratches. From the migration of fibroblast cells treated with latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. with concentrations of 50 µg/ml, 125 µg/ml, and 250 µg/ml so that the higher the concentration of latex and the longer the incubation period of the latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L. can help the NIH3T3 fibroblast cell migration process. Proliferation and migration of fibroblasts play an essential role in the formation of granulation tissue and wound closure [16].

At 0 h, the fibroblast cells grown on the 24-well plate were scratched using yellow, sterile tips. At 12 h, the scratched fibroblast cells begin to fuse and begin to stick to the plate again due to the response between cells with each other. At the 24th h, the scratches begin to shrink, and it is clear that the process of fibroblast cell migration is caused by the growth factor that will trigger the cell to approach. At 48 h, the scratches began to close, and at a concentration of 250 µg/ml, the scratches were closed, but there were not many cells. When compared with concentrations of 50 µg/ml and 125 µg/ml, the scratch distance at a concentration of 250 µg/ml was smaller, as well as at a concentration of 125 µg/ml, the scratch distance of cells was smaller than the concentration of 50 µg/ml. This shows that the higher the concentration of latex from *M. paradisiaca* var. *sapientum* and *C. papaya* L., the more it will be able to help the migration process of NIH3T3 fibroblast cells. The migration results can be shown in Fig. 2.

Cell proliferation has an essential role in the process of wound healing; this is because cell proliferation is a phase where the formation of new tissue that connects cells originating from the mesenchyme the latex from *M. paradisiaca* var. *sapientum* was applied topically in the form of fresh latex, in the process of wound healing using mice test animals. The latex accelerates the process of recapitalizing epidermal tissue, forming new blood vessels (neocapillarization), forming connective tissue (fibroblasts), and infiltrating inflammatory cells in the wound area [17]. This indicates that the latex from *M. paradisiaca* var. *sapientum* affects the proliferation of fibroblast cells where the more cells proliferate, the faster the wound healing process. This is influenced by the compounds contained tannins, saponins, and flavonoids. Tannin, saponins, and flavonoids function as antibiotics and stimulate the growth of new cells in the wound [18]. Gurung and Basnet conducted wound healing tests in rats using latex of *C. papaya* L. can be used to help heal wounds in mice [19]. Papain and chymopapain are known to be useful as antimicrobials and antioxidants. Ajani and Ogunbiyi also researched papaya latex used to help heal wounds in diabetic rats. The result is that papaya latex can help wound healing more than gentamicin, normal saline, and propylene glycol [1].

CONCLUSION

This study concludes that the latex of *M. paradisiaca* var. *sapientum* and *C. papaya* L. has the potential effect to help the wound healing process by induction of proliferation and migration on NIH3T3 fibroblast cell.

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CONFLICTS OF INTEREST

All authors have none to declare.

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