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ผลของไบโอพลาสติกฟิล์มชนิดอะกาโรสที่เสริมด้วยสารสกัดหยาบจากเห็ดนางฟ้าต่อการหายของแผลในไฟโบรบลาสต์ตัวอ่อนไก่

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บทคัดย่อ: การศึกษาวิจัยครั้งนี้มีวัตถุประสงค์ในการตรวจวิเคราะห์อัตลักษณ์ทางเคมีของสารเบต้ากลูแคนที่พบในสารสกัดหยาบจากเห็ดนางฟ้าและการนำมาใช้เสริมในไบโอพลาสติกฟิล์มชนิดอะกาโรสเพื่อศึกษาผลต่อการหายของแผลที่เกิดขึ้นกับไฟโบรบลาสต์ตัวอ่อนไก่ โดยทำจำลองบาดแผลด้วยเทคนิค cell scratching assay ลงบนไฟโบรบลาสต์ตัวอ่อนไก่ที่มีเจริญเป็นเนื้อเยื่อชั้นเดียวเต็มพื้นที่ จากนั้นทำการทดลอง 3 รูปแบบ ประกอบด้วย การใช้ไบโอพลาสติกฟิล์มชนิดอะกาโรสที่ไม่เสริมสารทดสอบ การใช้ไบโอพลาสติกฟิล์มชนิดอะกาโรสที่เสริมด้วย yeast β-glucan ที่ความเข้มข้น 10-40 μg/ml และการใช้ไบโอพลาสติกฟิล์มชนิดอะกาโรสที่เสริมด้วยสารสกัดหยาบจากเห็ดนางฟ้า ที่ความเข้มข้น 10-40 μg/ml จากผลการทดลองพบว่ากลุ่มทดลองที่ใช้ผลดีที่สุดต่อการหายของแผล ได้แก่ กลุ่มทดลองที่ใช้ไบโอพลาสติกฟิล์มชนิดอะกาโรสเสริมด้วย yeast β-glucan ที่ความเข้มข้น 10 μg/ml, yeast β-glucan ที่ความเข้มข้น 20 μg/ml และสารสกัดหยาบจากเห็ดนางฟ้า ที่ความเข้มข้น 40 μg/ml นอกจากนี้ยังพบการเปลี่ยนแปลงรูปร่างของ fibroblast ในลักษณะ stellate-shaped with oval concentric nuclei การค้นพบในครั้งนี้จึงอาจบ่งชี้ได้ถึงประโยชน์ของไบโอพลาสติกฟิล์มชนิดอะกาโรสที่เสริมด้วยสารสกัดหยาบจากเห็ดนางฟ้าว่ามีผลช่วยสนับสนุนการหายของแผล

คำสำคัญ: ไฟโบรบลาสต์ตัวอ่อนไก่ เห็ดนางฟ้า เบต้ากลูแคน สารสกัดหยาบ ไบโอพลาสติกฟิล์มชนิดอะกาโรส วัสดุปิดแผล การหายของแผล

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Effect of Agarose Bioplastic Film Enriched with *Pleurotus sajor-cajor* Crude Extract on Wound Healing in Chicken Embryo Fibroblast

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Abstract: The present study aims to verify the chemical characteristic of β-glucan in *Pleurotus sajor-caju* crude extract and its effect as enriched agarose bioplastic film on wound healing of scratched chicken embryo fibroblast (CEF) cells. Performed cell scratching assay on confluent monolayer CEF then assigned to 3 different treatment conditions, including blank agarose bioplastic film (agarose bioplastic film control), agarose bioplastic film enriched with 10-40 µg/ml of yeast β-glucan, and agarose bioplastic film enriched with 10-40 µg/ml of *P. sajor-caju crude extract.* This experiment was triplicated to observe wound healing at 0, 24, and 48 hours. The result shows the peak effect on wound healing in experimental groups, including yeast β-glucan 10 µg/ml, yeast β-glucan 20 µg/ml, and crude extract 40 µg/ml. In addition, the morphology of CEF cells post-treated by enriched agarose film appeared stellate-shaped with oval concentric nuclei. This finding might suggest the benefits of agarose bioplastic film-enriched *Pleurotus sajor-caju* crude extract on effective wound healing.

Keywords: Chicken embryo fibroblast, *Pleurotus sajor-cajor*, β-glucan, Crude extract, Agarose bioplastic film, Wound dressing, Wound healing

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Introduction

A wound defines as an injury caused by damage to various kinds of biological tissue, such as organ tissues, skin, and mucous membranes. Furthermore, the consequences of a wound lead to conformational changes in the normal anatomic structure of injured organs and tissue even their physiological functions (Robson *et al.,* 2001). This evident results from pathological processes beginning internally or externally to the involved organ(s) (Robson *et al.,* 2001). Eventually, the orchestrated complex cascades of body healing against damaged tissue generally occur to inhibit further severity of the affected wound areas including coagulation and hemostasis, inflammation, proliferation, and wound remodeling, these phases are crucial for wound healing. (Velnar *et al.,* 2009). However,

the physiological wound healing cascades remain a challenging clinical problem without appropriate management, therefore, efficient wound management is indispensable (Velnar *et al.,* 2009) due to a variety of wound types and each category has its distinctive healing requirements (Mir *et al.,* 2018).

Wound dressing is an important step of clinical management during the healing process of wounds (Uzun, 2018). This realization has motivated the development of a myriad of wound dressings, each with specific characteristics (Mir *et al.,* 2018). Not any wound dressing is ideal for various types of injuries, nevertheless, rapid healing, the affordable cost to the patient, aesthetics, and prevention of infection, respectively, are the minimum characteristics required for wound management (Mir *et al.,* 2018). The commonly used wound dressing keeps the wound sealed, prevents infection, and is safe from loss of wound hydration and further tissue damage from exposure to the environment. In addition, the proper wound dressing should be ventilated, maintaining moisture and appropriate wound temperature that promotes tissue regeneration, also should be easy to apply and remove, causes no trauma on removal, leaves no debris in the wound bed, and induces no allergic reactions. (Mir *et al.,* 2018; Nuutila and Eriksson, 2020; Pai and Madan, 2013).

Currently, wound dressing design is rapidly growing in the field of the medical wound-care market worldwide (Gardikiotis *et al.,* 2022). Researchers are motivated to achieve the ideal wound dressing for the high demand for the global wound care market, which is predicted to increase from 18.35 billion US dollars in the year 2017 to 22.01 billion US dollars by the year 2022 (Ovais *et al.,* 2018). Thus, the present and future trends of studies attempt to create modern wound dressing by focusing on healing enhancement, pain control, antibacterial activity, and drug delivery system (Gardikiotis *et al.,* 2022; Delavari and Stiharu, 2022). Modern methods focus on the use of synthetic polymers and biopolymers, in the form of hydrogels, thin films, and nanofibrous scaffolds. Many studies have documented the use of polymers in dressings or as medical devices provides a potential improvement in controlling wound healing (Mir *et al.,* 2018). These findings indicate that modern wound dressing can prevent infections induced by microbes, stimulate the growth rate, and be non-toxic, non-antigenic, biocompatible, and biodegradable (Gardikiotis *et al.,* 2022; Delavari and Stiharu, 2022).

One distinctive notable of wound dressing made by bioplastic polymer is the biodegradable accordingly to the current environmental awareness and social concerns for the global ecological problems that have pressured various industries to develop and use environmentally friendly materials in their manufacture, including wound dressing (Delavari and Stiharu, 2022; Arikan and Ozsoy, 2015; Bano *et al.,* 2018). Therefore, wound dressing requirements also increase continuously

worldwide. However, the increase in medical production results in a high amount of pollution and waste (Napavichayanun *et al.,* 2021). Consequently, bioplastic is a suitable material for wound management that has attracted attention recently (Delavari and Stiharu, 2022; Arikan and Ozsoy, 2015; Bano *et al.,* 2018). Bioplastics are plastics made of biomass or renewable resources and produced by various microorganisms (Arikan and Ozsoy, 2015; Saharan *et al.,* 2012). Bioplastics are Eco-friendly materials that can be completely degradable and can be recycled (Bano *et al.,* 2018). These substances have been increasingly highlighted as means for saving fossil fuels, reducing CO_2 emission, and plastic wastes (Saharan *et al.,* 2012). Bioplastics contain no toxins and are a biocompatible benefit in biomedical applications (Arikan and Ozsoy, 2015; Bano *et al.,* 2018). However, due to the variety of bioplastics and plasticizers, using this material still requires more work to meet the application demand and fit the ideal wound dressing concept.

During the last few decades, various forms of the agarose-based systems have been developed and applied in pharmaceutical industries and medical researches (Bao *et al.,* 2010). Agarose is a linear polysaccharide extracted from marine red algae, and consists of α-1,3 linked D-galactose and α-1,4 linked 3,6-anhydro-αL-galactose residues (Normand *et al.,* 2000). Agarose is an abundant and biodegradable polymer with a strength comparable or higher than other commonly used natural polymers (Awadhiya *et al.,* 2016). Moreover, agarose also has it benefits for wound dressing material including biocompatibility, moisturizing capacity, water-soluble, transparent, neutral charge, and thermal tolerance (Napavichayanun *et al.,* 2021; Bao *et al.,* 2010). Overall benefits described above indicate that agarose is an excellent property as a material for wound dressing invention in the form of gel or film.

Besides of the applicable of agarose as a wound dressing material, furthermore, adding of certain kinds wound-healing accelerating agents into the wound dressing could enhance the effectiveness of wound healing and also would make the wound dressing more commercially valuable (Napavichayanun *et al.,* 2021). The development of alternative wound healing products has been a popular topic in recent years, not only chemical or synthetics wound healing agents are investigated, various herbal sources, in particular, now also increasing attention (Deng *et al.,* 2022). To obtain wound healing biological ingredients, conventional extraction methods, such as solvent extraction, maceration, cold pressing, and hydro-distillation, are commonly used to isolate several chemical compounds from natural matrices (Dias *et al.,* 2021). Nevertheless, there is still a lack of standardization methods for the evaluation of the composition of natural products, which makes it difficult to estimate the efficacy of these compounds in wound management (Deng *et al.,* 2022).

Pleurotus sajor-caju is an edible mushroom with high nutritional value and therapeutic properties. *P. sajor-caju* has been used widely in traditional medicine because of its various bioactive potential. β-glucan is one of the highlighted components for pharmacologic properties that is found in the mushroom cell wall. β-glucan varies in terms of structure and biological efficiency. A high level of biological efficiency has been found in β-glucans, especially β-1,3-D-glucans and β-1,6-D-glucans. One of the significant deal interests in the medical properties of β-glucan is the stimulation of wound healing activity. Studies have confirmed that β-glucan involves in the proliferation and migration of fibroblasts. (Finimundy *et al.,* 2018; Dalonso *et al.,* 2010; Konusova *et al.,* 2020; Son *et al.,* 2005) Therefore, this present work aims to study the effect of agarose bioplastic film enriched with *P. sajor-caju* crude extract on wound healing of chicken embryo fibroblast. To achieve the goal of creating a degradable wound dressing with a wound healing stimulation effect.

Materials and methods

Material preparation

Raw materials

For the experiment, oyster mushrooms (*Pleurotus sajor-caju*) were purchased from a local market in Thailand and yeast β-glucan, a positive control of the experiment, was acquired from Xi'an Hench Biotechnology Co., Ltd., China. A specific pathogen-free egg, which was later used for the preparation of chicken embryo fibroblast cells, obtained from CPF Co., Ltd.. For the preparation of a bioplastic film, agarose (Vivantis technologies Snd. Bhd., Malaysia) and glycerol (Univar, Thermo Fisher Scientific, Australia) were used as bioplastic and plasticizer, respectively.

Pleurotus sajor-caju crude extract preparation

The 2 kg of fresh mushrooms (*Pleurotus sajor-caju* (Fr.) Singer) were washed through the running tap water and then immersed in 10% Sodium bicarbonate w/v for 20 minutes (Andrade *et al.,* 2015) for detoxication. Thereafter, the mushroom was rinsed and placed in an aluminum strainer, then air-dried in a hot air oven at 60 °C for 72 hours. The dried mushroom was ground into powder using a blender and stored in a desiccator for later use. The crude extract was then isolated from ground mushrooms by using a hot water extraction technique, briefly, the ground mushroom was suspended in distilled water in a ratio of 1:20 w/v and boiled at 70 °C for 4 hours. After that, the boiled sample was cooled down to room temperature and subsequently filtered through a Nylon liquid filter bag pore size 60 micrometer (DE PERFECT Co.,Ltd., Thailand) and stored at 4°C overnight. The chilled crude extract was sent to Salaya Central Instrument Facility, Mahidol University, for lyophilization in a lyophilizer (FreeZone® Plus™ 6, Labconco, USA). Finally, the lyophilized crude extract was stored in a desiccator (Ahmed *et al.,* 2017; Jantaramanant, 2014).

Characterization of β-glucan

The chemical characteristics of the lyophilized *P. sajor-caju* crude extract and a control sample, yeast β-glucan, were sent to Salaya Central Instrument Facility, Mahidol University, to be determined by Fourier transform infra-red spectroscopy (FT-IR) analysis (Nicolet iS50, Thermo Fisher Scientific, USA) at the absorbance between 4000 and 400 cm^{-1} (Jantaramanant, 2014).

Chicken embryo fibroblast (CEF) cells preparation

To prepare chicken embryo fibroblast, firstly, on the outer bottom of the 6-well plate was marking to create reference points by lightly scratching with a razor blade (Liang *et al.,* 2007). The, chicken embryo fibroblast cells from the specific pathogen-free eggs at the age of 9-11 days were prepared following the protocol of Kunanusont (2019), then, adjusted cell concentration to $1.5x10^6$ cells/ml, and added into the well. Cells were incubated and allowed to grow at 37 °C with 5% CO_2 in the cell incubator for 24 hours (Kunanusont, 2019) or until cells become the confluent monolayer.

Agarose bioplastic film enriched with *P. sajor-caju* crude extract

To prepare enriched agarose bioplastic film, there were two main components including a bioplastic mixture and experimental agents. For the bioplastic mixture, 2 g of agarose powder and 1 ml of glycerol were added into the Erlenmeyer flask, then filled up with sterile PBS (pH 7.4) to a final volume of 50 ml., Next, the mixture was boiled in a microwave oven at 800 W for 4 min. During boiling, the mixture was stirred intermittently until the mixture becomes a homogenized viscous solution during the boiling process. After that, the bioplastic mixture was allowed to cool at approximately 60°C, then 2X growth medium was combined with the bioplastic mixture in a ratio of 1:1 and was then cooled down to 40°C. For the experimental agents, either yeast β-glucan (positive control) or *P. sajor-caju* crude extract was added with deionized water to the final concentration from 10-40 μg/ml and filtered through a syringe filter (Puradisc™, Whatman™, UK) for decontamination. Finally, each agent, yeast β-glucan or *P. sajor-caju* crude extract was mixed with the bioplastic mixture to obtain melt-enriched bioplastic film. Once the growth medium was removed from scratched cells, a melt-enriched bioplastic film was cast over the scratched cells in a 6-well plate. All experimental conditions were summarized in a table shown below (table 1).

Method

Evaluation of enriched agarose bioplastic film on scratch cells

To observe the effect of bioplastic film enriched with *P. sajor-caju* crude extract on wound healing *in vitro*, thus, the cell scratching assay was performed as described by Liang *et al.* (2007). Briefly, the CEF cells were scratched using a 200 μl sterile pipet tip to create a straight line on monolayer cells and removed cell debris. Then to smooth the edge of the scratch, the old

Table 1 The composition of bioplastic mixture

Experimental group	Enriched agarose bioplastic film
Agarose bioplastic film control	Blank bioplastic mixture
Yeast β-glucan 10 μg/ml	Bioplastic mixture enriched Yeast β-glucan 10 μg/ml
Yeast β-glucan 20 μg/ml	Bioplastic mixture enriched Yeast β-glucan 20 μg/ml
Yeast β-glucan 30 μg/ml	Bioplastic mixture enriched Yeast β-glucan 30 μg/ml
Yeast β-glucan 40 μg/ml	Bioplastic mixture enriched Yeast β-glucan 40 μg/ml
crude extract 10 μg/ml	Bioplastic mixture enriched *P. sajor-caju* crude extract 10 μg/ml
crude extract 20 μg/ml	Bioplastic mixture enriched *P. sajor-caju* crude extract 20 μg/ml
crude extract 30 μg/ml	Bioplastic mixture enriched *P. sajor-caju* crude extract 30 μg/ml
crude extract 40 μg/ml	Bioplastic mixture enriched *P. sajor-caju* crude extract 40 μg/ml

growth medium was removed and washed with 1 ml sterile PBS (pH 7.4); after that replaced with 3 ml of a fresh growth medium and later incubated at 37 °C with 5% CO_2 waiting for the bioplastic mixture adding. Finally, replace the growth medium, then pour the prepared bioplastic mixture on top of the scratched cells. The bioplastic film was allowed to completely solidify before incubating at 37 °C with 5% CO_2. The wound healing process of CEF cell treatment with enriched agarose bioplastic film was observed under an inverted light microscope at 0, 24, and 48 hours.

The scratched cells area treated by each enriched bioplastic film was observed at 0, 24, and 48 hours by an inverted light microscope (Olympus™, Japan) during the experiment and photographed using a mouse control HDMI camera (ToupCam™ XCAM0720PHB HDMI, China). The reduction of a scratched area at each time point by tracing the cell-free area of the scratch was measured using the Image-J software (National Institute of Mental Health, USA) then report as wound area.

To improve the visualization of the scratch area and observe the morphology of fibroblasts clearly on the culture plate. Enriched bioplastic film was removed, then 1% crystal violet in 10% formalin solution was used to fix and stain the cell, incubated at room temperature for 10 minutes, rinsed a few times gently with distilled water, and observed the scratch under an inverted light microscope and photographed by using a mouse control HDMI camera. (Punyadarsaniya *et al.,* 2011; Nilles *et al.*, 2022).

Data collection and statistical analysis

Performed the statistical analysis of the changing wound area spot in time 0, 24, and 48 hours by One-way ANOVA followed by multiple comparison tests using IBM SPSS Statistics 26 to detect the significant difference. Presented the data as mean and considered the significant value of $p<0.05$. Then calculated %wound

healing following the formulation of Treloar and Simpson (2013).

Result and discussion

The characteristics of β-glucan from *P. sajor-caju* crude extract

The chemical structural analysis of polysaccharides like glucan in crude extraction from *P. sajor-caju* crude extract was determined by FT-IR spectroscopy. FTIR is a suitable scientific instrument due to the sensitivity of the position and anomeric configuration of glycosidic linkages in glucans. As for the FT-IR spectra, the important characterization region of polysaccharides is the sugar region around 1,200-950 cm^{-1} and the anomeric region around 950-750 cm^{-1}. Also, the stretching vibrations of the C-O-C glycosidic bridge appear in two spectral ranges include 1160-1130 and 999-965 cm^{-1}. Furthermore, the FT-IR spectroscopy applied to polysaccharide analysis would show the characteristic peak of β-glucan around 1073-1077 cm^{-1} and 1044-1046 cm^{-1} represent β-(1⟶3) glucan, 1026–1028 cm^{-1} represent β-(1⟶4) glucan and 993-994 cm^{-1} represent β-(1⟶6) glucan (Sermwittayawon *et al.,* 2018; Synytsya and Novak, 2021; Nikonenko *et al.,* 2000; Satitmanwiwat *et al.,* 2012). As for the result, figure 1A shows FT-IR spectrum of yeast β-glucan as β-(1⟶3) glucan at 1149.50, 1075.72, 1015.72, and 996.31 cm^{-1}, whereas figure 1B shows FT-IR spectrum of *P. sajor-caju* crude extract as β-(1⟶3) glucan at 1145.93, 1075.32, 1030.66 and 987.99 cm^{-1}. Overall, the data of FTIR spectra are shown in figure 1.

The result of FTIR analysis indicates that the composition of both experiment agents, including yeast β-glucan and *P. sajor-caju* crude extract contain β-(1⟶3) glucan as an active ingredient. Several studies described the benefit

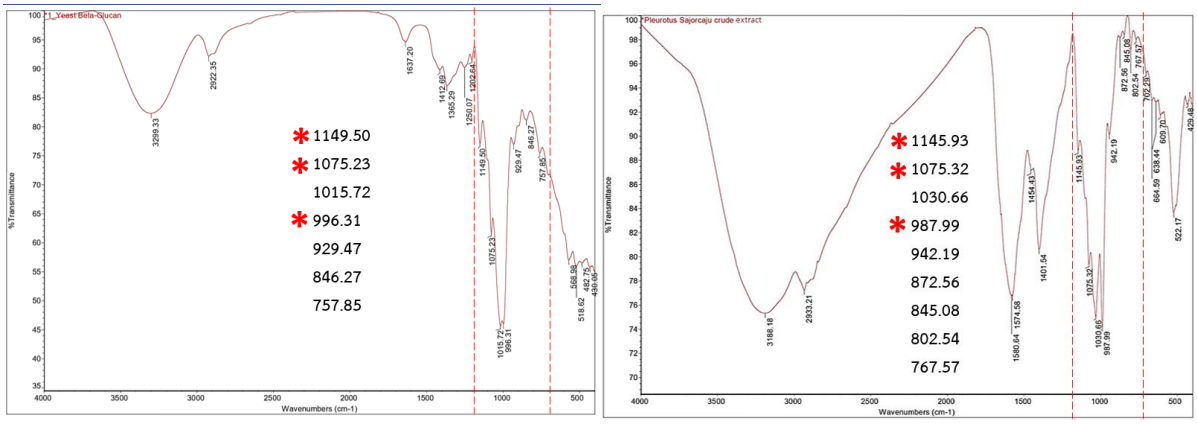
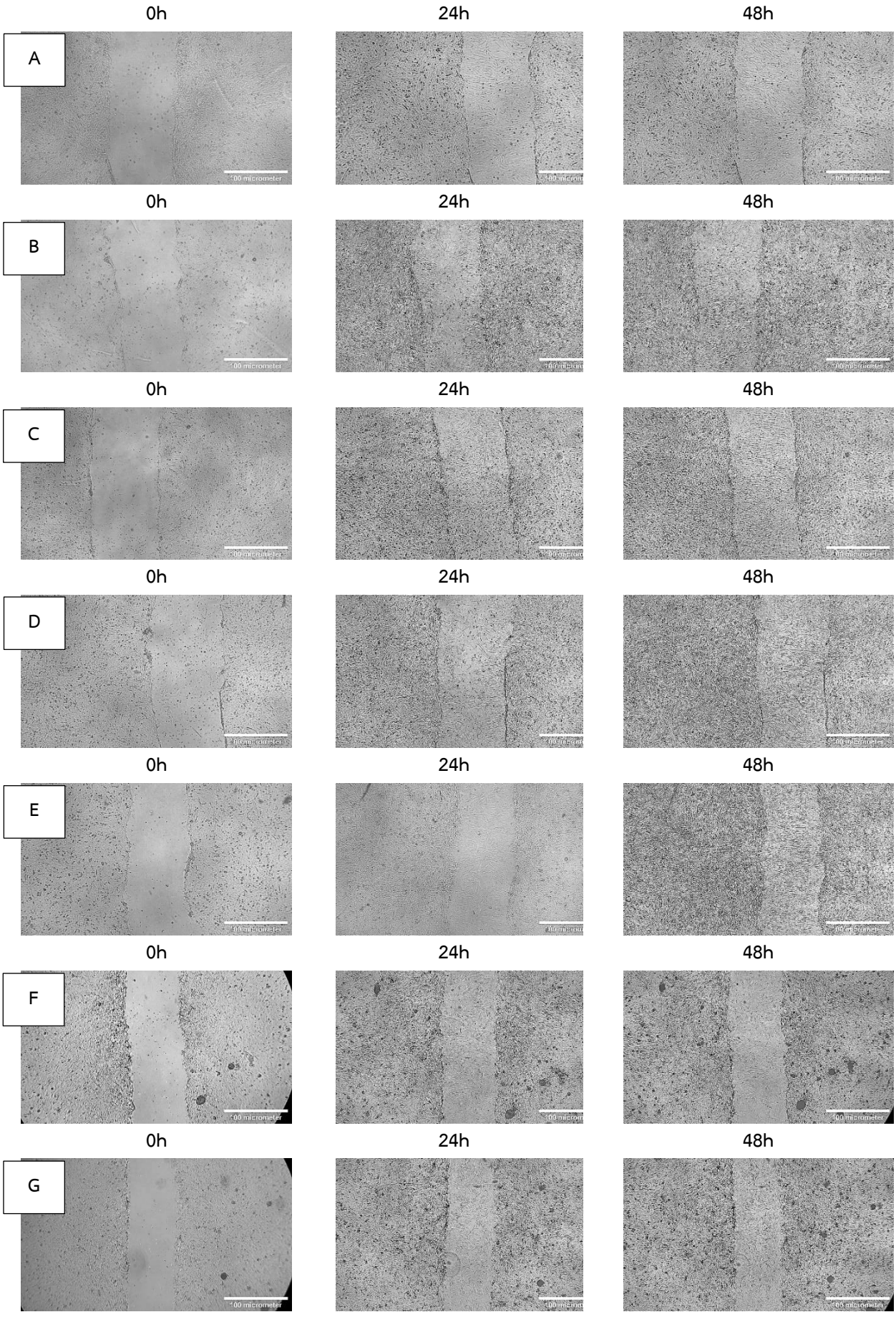


Figure 1: FT-IR spectrum of yeast β-glucan (A) and *P.sajor-caju* crude extract (B)

Asterisk (*) in figure 1A shows FT-IR spectrum of yeast β-glucan as β-(1⟶3) glucan at 1149.50, 1075.72, 1015.72, and 996.31 cm^{-1}, whereas Asterisk (*) in figure 1B shows FT-IR spectrum of *P. sajor-caju* crude extract as β-(1⟶3) glucan at 1145.93, 1075.32, 1030.66 and 987.99 cm^{-1}.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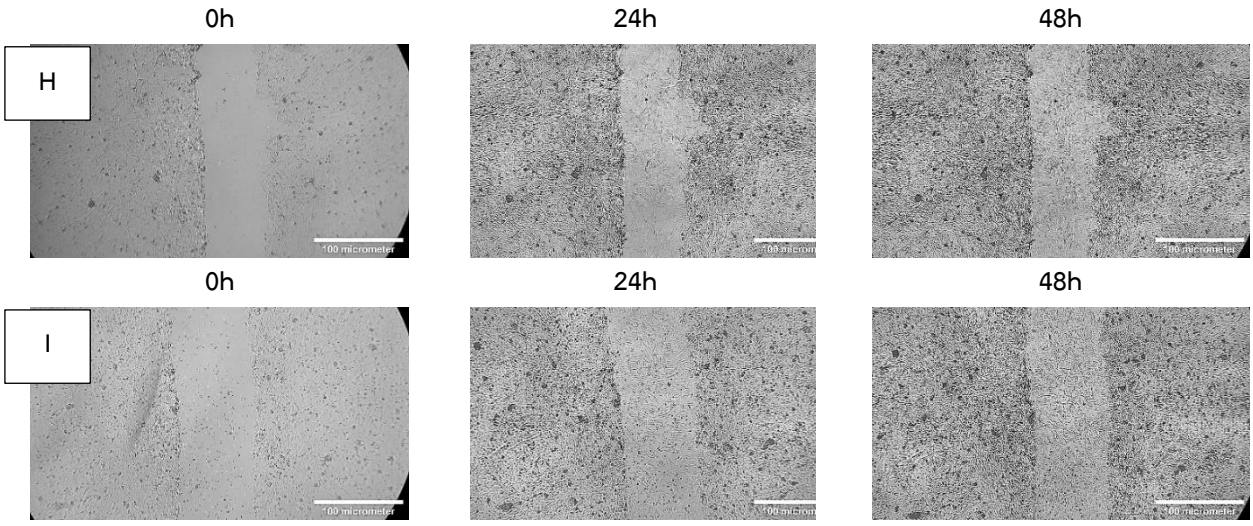

Figure 2 Scratch images of the experimental group (4X) and scale bar = 100 μm; A: Agarose bioplastic film control 2; B: Yeast β-glucan 10 μg/ml; C: Yeast β-glucan 20 μg/ml; D: Yeast β-glucan 30 μg/ml; E: Yeast β-glucan 40 μg/ml; F: Crude extract 10 μg/ml; G: Crude extract 20 μg/ml; H: Crude extract 30 μg/ml; I: Crude extract 40 μg/ml

of β-glucan involved in wound healing, such as the ability to modulate the wound healing process, reduce scar formation, induces the production of growth factors, stimulate fibroblast proliferation and migration, and promotes collagen biosynthesis. Indeed, support the idea of using the chosen experimental reagent as a bioactive substant in developing β-glucan-loaded wound dressing. (Seo *et al.,* 2019; Grip *et al.,* 2021; Choi *et al.,* 2016; Son *et al.,* 2007).

Effect of agarose bioplastic film enriched with *P. sajor-caju* crude extract on wound healing

The stimulatory effects of agarose bioplastic film enriched with *P. sajor-caju* crude extract on the wound healing process using cell scratching assay of CEF cells were determined in this study as a model *in vitro* at 0, 24, and 48 hours to observe the wound healing process that happens on the cells surrounding the scratch area into the cell-free area under the inverted light microscope. Then the cell-free area that reduced in time was used to calculate the wound area (figure 2).

The result of figure 2(A-I) focuses on the agarose bioplastic film used with or without adding an experimental reagent. The visualizing of wound healing showed the wound closure pattern of CEF cells appeared as the surrounding cell was still in contact and moved coordinately into the cell-free area. Eventually, the cell moving into the cell-free area created a new monolayer fibroblast tissue. This result was related to Seo *et al.* (2019), which investigated the molecular mechanism of 4 types purify β-glucan from different natural sources, including mushroom and yeast β-glucan and found that

the effect of β-glucan on wound healing by driven through the Integrin/FAK/Src pathway and its impact on cell migration by forming lamellipodia starting from the concentration of 20 μg/ml. During the lamellipodial protrusion, causing the change in cell morphology of fibroblast, this reason indicates the finding of stellate-shaped cells with oval concentric nuclei. These cells essential in maintaining wound healing and enhancing wound closure (Ravikanth *et al.,* 2011). However, only visualization could not quantify the effects of the enriched agarose bioplastic film on wound healing in different treatments, *P. sajor-caju* crude extract and yeast β-glucan. Therefore, the wound healing process was accessed by calculating the cell-free area and representing data as log Mean μm^2, summarized in table 2. Then the wound area was used to calculate the % wound healing (table 3) described above in data collection and statistical analysis.

From the result of table 2, among the experimental groups, the wound area decreases

significantly differed in the group using enriched agarose bioplastic film as follows; yeast β-glucan 10 μg/ml, yeast β-glucan 20 μg/ml, and crude extract 40 μg/ml; these experimental groups had wound areas (log Mean) as 0.62, 0.00, and 1.15 μm^2 respectively, at 24 hours and also had % wound healing as 90.07, 100, and 81.53

Table 2 Wound area spot in time at 0, 24 and 48 hours

Time	Wound area (log Mean μm^2)								
(hours)	AC	YB10	YB20	YB30	YB40	CE10	CE20	CE30	CE40
0	6.20^{a}	6.25^{a}	6.26^{a}	6.26^{a}	6.21^{a}	6.28^{a}	6.28^{a}	6.23^{a}	6.22^{a}
24	3.66^{A}	0.62^{B}	0.00^{B}	1.19^{A}	2.02^{A}	4.54^{A}	1.95^{A}	3.12^{A}	1.15^{B}
48	0.00^{I}	0.00^{I}	0.00^{I}	0.00^{I}	0.00^{I}	2.17^{II}	0.62^{I}	1.12^{I}	0.00^{I}

One-way ANOVA: different letters indicate significant differences ($p<0.05$) (Turkey's HSD)

AC = agarose bioplastic film control; YB10 = Yeast β-glucan 10 μg/ml; YB20 = Yeast β-glucan 20 μg/ml; YB30 = Yeast β-glucan 30 μg/ml; YB40 = Yeast β-glucan 40 μg/ml; CE10 = Crude extract 10 μg/ml; CE20 = Crude extract 20 μg/ml; CE30 = Crude extract 30 μg/ml; CE40 = Crude extract 40 μg/ml

Table 3 % wound healing spot in time at 24 and 48 hours

Time	% Wound healing								
(hours)	AC	YB10	YB20	YB30	YB40	CE10	CE20	CE30	CE40
24	40.92	90.07	100.00	80.92	67.37	27.77	68.99	49.94	81.53
48	100.00	100.00	100.00	100.00	100.00	56.87	90.16	82.03	100.00

AC = agarose bioplastic film control; YB10 = Yeast β-glucan 10 μg/ml; YB20 = Yeast β-glucan 20 μg/ml; YB30 = Yeast β-glucan 30 μg/ml; YB40 = Yeast β-glucan 40 μg/ml; CE10 = Crude extract 10 μg/ml; CE20 = Crude extract 20 μg/ml; CE30 = Crude extract 30 μg/ml; CE40 = Crude extract 40 μg/ml

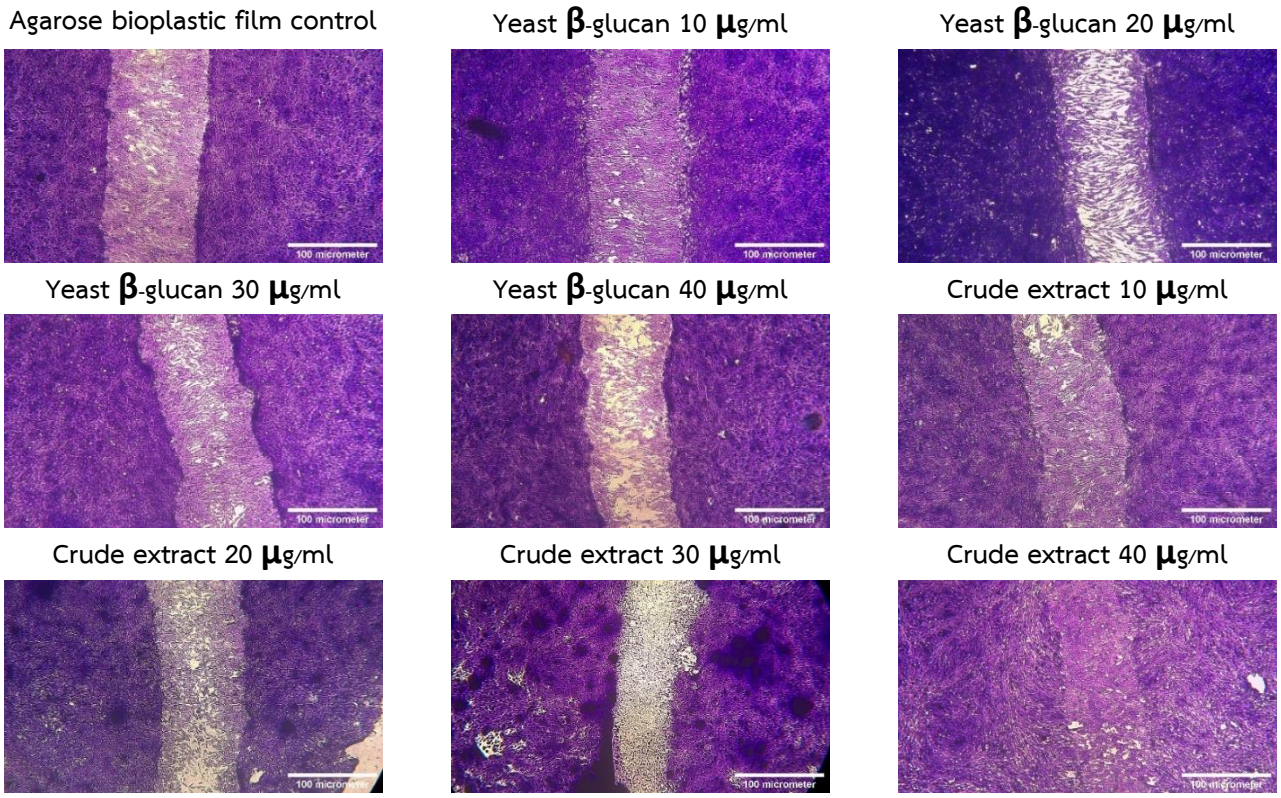

Figure 3 Crystal violet staining to improve the visual of the scratch image (4X) and scale bar = 100 μm

respectively, at 24 hours (table 3). These imply that *P. sajor-caju* crude extract and yeast β-glucan affect fibroblast migration and proliferation (Son *et. al.*, 2005), leading to an advantage of wound healing; however, the physicals and chemicals used for preparing *P. sajor-caju* crude extract were not observed for any alterations such as cytotoxicity due to chemical residues (Pijuan *et al.*, 2019). To elucidate cell viability of CEF during the experiment, the crystal violet staining was used on CEF post-treatment by different enriched agarose bioplastic films to improve the scratch area's visualization and cell morphology and confirm the cell viability of the new monolayer tissue that forms during wound healing (figure 3). From figure 3 indicates the direct contract between agarose bioplastic film and CEF cells did not cause an abnormality in cell morphology or stop wound healing. As a result, this implies that agarose bioplastic film is biocompatibility. The staining can improve the visualization of the scratch image, showing the difference between old and new tissue, and also improve the visualization of active fibroblast morphology (Ozcelikkale *et al.,* 2017; Zafar *et al.,* 2019).

Conclusion

This experiment implies the benefit of agarose bioplastic film enriched *P. sajor-caju* crude extract fits the ideal wound dressing concept, including inducing rapid healing, releasing bioactive ingredients when connecting with the wound surface, be biocompatible,

minimizing trauma or maceration to wound edges, provide a barrier to external contaminants, and easily remove at the end of treatment and be biodegradable. The agarose bioplastic film enriched *P. sajor- caju* crude extract is effective for wound healing of CEF cells at 40 μg/ml concentration, which promotes cell migration and proliferation and induces the morphology change to active fibroblast.

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