Curr. Biochem. 2023. 10(2): 46-51

CURRENT BIOCHEMISTRY
ISSN: 2355-7877 | e-ISSN: 2355-7931
Journal homepage: http://journal.ipb.ac.id/index.php/cbj

A Novel Plaster Dressing: A Combination of Chicken Eggshell Hydrogel Plus Button-Fungus Extract Enhances Wound Healing in Diabetic Patients

Akbar Rafiqi[1], Alivianisa Nabilatusyifa[1], Oyi Candra Santoso Putri[1], Ikromatul Hasanah[2], Sabrina Firdayani Rahmah[1], Dimas Andrianto[1,3*]

[1]*Department of Biochemistry, Faculty of Mathematic and Natural Science, IPB University Bogor, West Java, Indonesia, 16880*

[2]*Department of Chemistry, Faculty of Mathematic and Natural Science, IPB University Bogor, West Java, Indonesia, 16880*

[3]*Halal Science Center, IPB University Bogor, West Java, Indonesia, 16127*

Received: *19 September 2023 ;* Revised: *12 October 2023 ;* Accepted: *26 October2023*

*Corresponding author, email: dimasandrianto@apps.ipb.ac.id

ABSTRACT

Diabetic patients are susceptible to severe wound conditions due to high blood sugar levels and bacterial infections. This study aims to determine the formulation and effectiveness of the hydrogel plaster combination of laying hen eggshells with button mushrooms in curing diabetic wounds. The research methods consisted of making the hydrogel, coagulation, and antibacterial. Hydrogels were made with 5%, 10%, and 20% eggshell concentrations. The coagulation activity test used rabbit blood, which was with added glucose. Antibacterial was performed using Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). This research shows that the coagulation activity of F2 and F3 formulations significantly differed from the control, with an average blood clotting time of 64.67 minutes and 63.00 minutes. The antibacterial test results produced a clear zone for S. aureus and E. coli with diameters of 10.27 mm and 9.18 mm for F2, 6.90 mm, and 11.66 mm for F3. This research concludes that hydrogel formulation 3, with 20% eggshell and 6.4% button mushroom, had the best blood clotting effect and antibacterial activity.

Keywords: *agaricus bisporus***,** *diabetic wounds, eggshell, hydrogel wound dressing*

1. INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels caused by a lack of insulin secretion, impaired insulin activity, or both (Bulu *et al.* 2019). Based on data from the International Diabetes Federation (2021), Indonesia is ranked as the 5th highest diabetic population in the world, with as many as 19.5 million people in 2021. Diabetic foot ulceration (DFU) is one complication experienced by people with diabetes. DFU is a condition of severe wounding due to high sugar levels in diabetics, resulting in bacteria that are growing rapidly on damage tissue making is difficult to heal (Liu *et al.* 2021).

Liu *et al.* (2021) state that bacteria can aggravate diabetic wounds. Bacteria which are found in diabetic wounds, are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae* (Ogba *et al.* 2019). Diabetic wound covering must be antiseptic. One organism having antibacterial properties is the button mushroom. Research results of Risan *et al.* (2017) showed that a button mushroom 75% ethanol extract with a inhibited the growth of *Staphylococcus aureus*

by 29.9% and *Escherichia coli* by 27.7%. In addition, wound plaster for people with diabetes must absorb excessive wound exudate and provide sufficient moisture to facilitate the autolysis process, granulation, angiogenesis, and migration of epidermal cells in wounds (Everett & Mathioudakis 2018). Hydrogel has a highwater content for tissue granulation and epidermal cell migration (Liu *et al.* 2021).

According to the Badan Pusat Statistika (2021), the egg production in Indonesia from 2019 to 2021 increased 402,615.77 tons. Increasingly sluggish production leads to an increase in eggshell waste product. Therefore, coping with a large amount of eggshell waste is necessary. Research on the potential of eggshells in wound healing has been widely carried out. Research results from Guarderas *et al.* (2016) also showed that wounds covered with eggshell 21% faster than those in the control group. This is because eggshells contain calcium in the form of calcium carbonate, as high as 95%. Calcium helps prothrombin form fibrin threads when the wound secretes the enzyme thrombokinase (Sariyana *et al.* 2018).

The main causes of diabetic wound healing are difficulty coagulating blood and bacterial infections. The shell of chicken eggs contains calcium in the form of calcium carbonate, one of the substances needed in blood clotting. Button mushrooms contain flavonoids, alkaloids, terpenoids, and saponins that have antibacterial properties (Ekowati *et al*. 2018). Therefore, the potential of eggshells and button mushrooms as additives for diabetic wounds healing plasters needs to be studied further.

2. METHODOLOGY

Materials

The main materials of this study are laying hen egg shells obtained from Bogor Superkue by-products and button mushrooms identified by Dr. Gayuh Rahayu (Mycologist, Department of Biology, Bogor Agricultural University). Other materials used are ethanol, distilled water, polyvinyl pyrrolidone K30 (PVP-K30), polyvinyl alcohol (PVA), polyethylene glycol (PEG 400), DMDM Hydantoin, glucose, Mueller Hinton Agar (MHA), nutrient agar (NA), nutrient broth (NB), Bioplacenton, rabbit blood, *Staphylococcus aureus*, and *Escherichia coli*.

Methods

Preparation of Eggshell on Egg Membranes

The eggshell is selected clean, unstained, and eggshells were useda. Then, the shell is washed thoroughly. Next, the eggshells are dried in the oven for 30 minutes at 105°C. Then, the eggshell is mashed with a blender and sifted to a size of 250 mesh (Sariyana *et al*. 2018).

Preparation of Button Mushroom Ethanol Extract

The button mushrooms were washed under running water and dried in a 60°C oven for 24 hours. The sample is mashed and then sifted until a 50 mesh simplisia is obtained. 660 g of button mushroom simplicia was macerated with 6.6 L of 96% (v/v) ethanol for three days while stirring. After that, it is filtered with a vacuum pump and re-macerated the residue a used with 1.32 L of 96% (v/v) ethanol for 24 hours. Maserate is concentrated with a rotary vacuum evaporator at a temperature of 45°C (Nuryanti & Fitriana 2018).

Manufacture of Laying Hen Eggshell Hydrogel Plaster Containing Button Mushroom Ethanol Extract

Plaster hydrogel is made as three formulations, with the composition of each formulation following Table 1. Eggshell powder and button mushroom ethanol extract are placed into a goblet glass filled with distilled water. The solution is heated to 80°C and stirred until homogeneous. After that, the solution was combined with to PVP-K30, PVA, PEG 400, and DMDM Hydantoin, and then the remaining water was added to make 100 mL. The solution is stirred in a water bath for 15 minutes. After that, the mixture is poured into a mold and heated in an oven at 50 °C for 18 hours (Biu *et al.* 2018).

Table 1. Plaster hydrogel formulations (Biu *et al.* 2018)

Material	F1 (%)	F2 (%)	**F3 (%)**
Eggshell Powder (w/v)	5	10	20
Button Mushroom Ethanol Extract (w/v)	6.4	6.4	6.4
PVP-K30 (w/v)	7	7	7
PVA (b/v)	5	5	5
PEG 400 (w/v)	10	10	10
DMDM *Hydantoin* (v/v)	0.5	0.5	0.5
Distilled Water (v/v)	AD 100	AD 100	AD 100

Coagulation Test

This study used blood derived from rabbits. Blood is taken using a syringe from the part of the auricular vein located in the rabbit's ear. The coagulation test method used is the Lee-White method with modifications. To the rabbit's blood that was obtained added glucose until the blood glucose level became 400 mg/dL and the rabbit became 250 mg/dL. A total of 3 hydrogel formulation plasters were dripped with 0.2 mL of rabbit blood with added glucose. Then, a glass object is dripped with 0.2 mL of rabbit blood that has been given glucose as a negative control. After that, the rabbit's blood is examined for blood clotting. Observation of blood clots were repeated three times in each formulation. The best formulation for the blood clotting effect is used for antibacterial activity tests (Rohim 2018).

Antibacterial Activity Test

S. aureus and *E. coli* bacteria were rejuvenated on NA media and incubated for 24 hours at 35°C. Next, cultures of *S. aureus* and *E. coli* bacteria are taken with ose into NB. Mc. Farland's 0.5 solution was tested using UV-Vis absorption range 0.08–0.1 at a wavelength of 625 nm. The absorbance of Mc. Farland's standard 0.5 solution and NB media containing bacteria can't be measured with a UV-Vis spectrophotometer. The test of antibacterial activity is carried out by the pouring-plate method. A total of 15 mL of MHA media was mixed with 1.5 mL of *S. aureus* or *E. coli* bacterial inoculum in a test tube. Then, the mixture is put in a Petri dish and left until it solidifies. Next, four wells are made in one disc with sterile pipettes. Each well included a positive control (Bioplacenton), negative control (distilled water), and F2 and F3 as the best formulations of the coagulation test. Calipers were used to measure the diameter of the formed clear zone. Each test was repeated thrice (Putri *et al.* 2019; Rahmah *et al*. 2022; Septiani *et al.* 2017).

Data Analysis

The eggshell powder that has been filtered with a sieve of 250 mesh provides a yield of 36.00%. This result is lower than the amendment of the results of a previous study conducted, which uses a 100 mesh sieve and yielded 83.56%. This is because the larger the size of the mesh, the more material retained will be (Rahmah *et al*. 2022).

3. RESULTS

The length of control rabbit blood clotting coagulation time is 71.00 minutes, while the F1, F2, and F3 hydrogels have a faster blood clotting time than controls of 68.33, 64.67, and 63.00 minutes (Table 2). This is because the hydrogels have a high concentration of eggshells, so they have a higher calcium content.

Table 2. Rabbit blood clotting time

Treatment	Blood clotting time (minutes)
Control	71.00 ± 0.00^{a}
Formulation 1	68.33 ± 2.08^{ab}
Formulation 2	64.67 ± 2.08^{bc}
Formulation 3	63.00 ± 2.00^{c}

Description: The same letter indicates the sample does not differ significantly ($p<0.05$)

Testing of antibacterial activity was using the well diffusion method. The best hydrogels from the coagulation test, namely F2 and F3, were used in this study with positive control in the form of bioplacenton and negative control in the form of water. The clear zone produced by F2 in *S. aureus* bacteria is classified as medium with a clear zone diameter of 11.27 mm. In comparison, F3 is included in the medium category with a clear zone diameter of 6.90 mm. Antibacterial testing using *E. coli* bacteria produces a clear zone of F2, which is included in the medium category of 9.18 mm, and F3 is also included in the strong category with a clear zone of 11.66 mm (Table 3).

4. DISCUSSION

The eggshell powder that has been filtered with a sieve of 250 mesh yields 36.00%. This result is lower than the amendment of a study conducted by Hidayat *et al.* (2021), which uses a 100 mesh sieve with a yield of 83.56%. This is because the more dense the mesh size, the more material is retained (Makky *et al.* 2017).

Ethanol extract from button mushrooms is obtained by the method of maceration. The maceration process is an extraction method carried out through immersion of the material in the solvent corresponding to the active compound without any heating process. The immersion process causes the cell wall and cell membrane to rupture due to pressure differences outside and inside the cell so that the secondary metabolites contained in the cytoplasm dissolved in the solvent used (Chairunnisa *et al.* 2019). The button mushroom ethanol extract was 198.7294 g, so the yield was 8.97%. These results are below the standard of good amendment results. The yield is relatively good if it is more than 10% (Sunnah *et al.* 2021). The larger the yield indicates, a better the extraction treatment and the more bioactive compounds contained in the extract (Dewatisari *et al.* 2018).

According to Zimmerman *et al.* (1971), normal rabbit blood coagulation time is 80 minutes, so F2 and F3 hydrogels have a faster blood clotting time than controls of 64.67 and 63.00 minutes. This is because the F2 and F3 hydrogels have a high concentration of eggshells and then store a higher calcium content. The calcium content in the shell of chicken eggs plays a role in accelerating blood clotting, which helps prothrombin in forming fibrin threads when the wound secretes the enzyme thrombokinase (Sariyana *et al.* 2018). Blood clotting is one of the main processes that occur in wound healing. When the need occurs, the blood coagulation forms a blood clot that covers the wound. The blood clot is then converted into collagen scar tissue, permanently covering the wound (Rausch *et al*. 2021). Therefore, hydrogels F2 and F3 have the potential to be used as wound plasters for diabetic wound sufferers.

The clear zone produced by F2 and F3 in *S. aureus* bacteria is classified as medium with a clear zone diameter of 10.27 and 6.90 mm. Antibacterial testing using *E. coli* bacteria produces a clear zone of F2 the medium category of 9.18 mm, and F3 the strong category with a clear zone of 11.66 mm (Table 3).

The compounds in the hydrogel formulation can be used for antibiotic activity. Flavonoids in button mushrooms function as antibacterials because these compounds can damage the permeability of bacterial have organelles walls, microsomes, and lysosomes. Flavonoids can release transduction energy due to the interaction of flavonoids with bacterial DNA. In addition, flavonoids can change organic components in bacteria, which causes toxic effects. This is due to hydroxyl groups in flavonoid compounds (Egra *et al.* 2019). Another compound in button mushrooms is alkaloids that function as antibacterials with a mechanism to disrupt the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell lysis (Chairani & Harfiani 2018).

In concussion, formulation 3, with a content of 20% eggshell and 6.4% button mushroom ethanol extract from hydrogel plaster, is the formulation that produces the fastest blood clotting being 8 minutes faster than the control. Formulation 3 of the hydrogel

Table 3. Antibacterial activity of well diffusion method

Bacteria	Sample	Clear zone diameter (mm)	Inhibitory power[19]
S. aureus	Control (+)	14.10 ± 0.00	Strong
	Control (-)	0.00 ± 0.00	Weak
	Formulation 2	10.27 ± 0.09	Medium
	Formulation 3	6.90 ± 0.74	Medium
E. coli	Control (+)	23.25 ± 0.00	Very powerful
	Control (-)	0.00 ± 0.00	Weak
	Formulation 2	9.18 ± 1.39	Medium
	Formulation 3	11.66 ± 1.12	Strong

is the highest in the antibacterial activity test in *E. coli* and formulation 2 of the hydrogel is the highest in the antibacterial activity test in *S. aureus* with moderate inhibitory power.

5. ACKNOWLEDGMENT

Research was carried out with the help of various parties involved. The team would like to thank the Ministry of Research, Technology, and Higher Education for funding this research through the Student Creativity Program for Exact Research (PKM-RE) 2022. Thanks are also conveyed to the Bogor Agricultural Institute for providing moral and material support for the research so be carried out. The team also thanked Superkue for providing the main ingredients in the form of eggshells and Dr. Ir. Gayuh Rahayu, who assisted in the identification of button mushrooms.

6. REFERENCES

Badan Pusat Statistik. Egg Production of Laying Hens by Province (Ton), 2019–2021. Jakarta, 2021.

Biu MTS, Thomas NA, Mustapa MA. 2018. The formulation of hidrogel form of wound dressing from carrageenan red algae extract (*Eucheuma spinosum*) uses honey in healing burns [Thesis]. Gorontalo: Gorontalo State University.

Bulu A, Wahyuni TD, Sutriningsih A. 2019. The relationship between the level of adherence to taking medications and blood gula levels in patients with type ii diabetes mellitus. *Nursing News: Scientific Journal of Nursing*. 49(1):181–189.

Chairani A, Harfiani E. 2018. The effectiveness of castor sap as an antiseptic against the growth of *Staphylococcus aureus, Escherichia coli* and *Candida* sp. in vitro. *Unila Journal of Medicine*. 2(2): 84–92.

Chairunnisa S, Wartini NM, Suhendra L. 2019. Effect of temperature and maceration time on the characteristics of bidara leaf extract (*Ziziphus mauritiana* L.) as a source of saponins. *Journal of Engineering and Manajemen Agroindustry*. 7(4): 551–560.

Dewatisari WF, Rumiyanti L, Rakhmawati I. 2018. Amendment and phytochemical screening on *Sanseviera* sp. leaf extract. *Journal of Applied Agricultural Research*. 17(3): 197–202.

Egra S, Mardhiana, Rofin M, Adiwena M, Jannah N, Kuspradini H, Mitsunaga T. 2019. Antimicrobial activity of mangrove extract (*Rhizophora mucronata*) in inhibiting the growth of Ralstonia solanacearum causing wound disease. *Agrovigor*. 12(1): 26–31.

Ekowati N, Yuniati NI, Hernayanti, Ratnaningtyas NI. 2018. Antidiabetic ptentials of button mushroom (*Agaricus bisporus*) on alloxan-induced diabetic rats. *Biosaintifika*. 10: 655-662.

Everett E, Mathioudakis N. 2018. Update on management diabeteic foot ulcers. *Ann n y acad sci.* 1411(1): 153–165.

Guarderas F, Leavell Y, Sengupta T, Zhukova M, Megraw TL. 2016. Assessment of chicken-egg membrane as a dressing for wound healing. *Adv Skin Wound Care*. 29:131-134.

Hidayat KN, Nurbaeti SN, Kurniawan H. 2021. The effect of the ash content of the egg shells of laying hens on the organ index of female white rats of the wistar strain. *Journal of Pharmacy Students, Faculty of Medicine, UNTAN*. 5(1): 1–13.

International Diabetes Federation. IDF Diabetes Atlas Tenth Edition, 2021.

Liu H, Li Z, Zhao Y, Feng Y, Zvyagin AV, Wang J, Yang X, Yang B, Lin Q. 2021. Novel diabetic foot wound dressing based on multifunctional hydrogels with extensive temperature-tolerant, durable, adhesive, and intrinsic antibacterial properties of ice. *ACS Appl. Mater. Interfaces.* 13: 26770–26781.

Makky M, Napitu LS, Fahmy K. 2017. Development of sawdust waste grading tools for their use as composite mixture materials. *Journal of Agricultural Engineering Hue*. 10(1): 65–79.

Nuryanti S, Fitriana F. 2018. Antibacterial potential of button fungus ethanol

extract (*Agaricus bisporus*) as antibacterial. *Indonesian Journal of Phytopharmaceuticals*. 5(1): 266–270. ISSN: 2356-0398.

Ogba OM, Nsan E, Syam ES. 2019. Aerobic bacteria associated with diabetic foot ulcers and their susceptibility pattern. *Biomedical Dermatology.* 3(1): 1–6.

Putri RM, Diana VA, Fitri K. 2019. Comparison of antibacterial activity tests of ethanol extracts of flowers, leaves and roots of rosella plants (*Hibiscus sabdariffa* L.) against *Staphylococcus aureus* bacteria. *Journal of the Pharmaceutical World*. 3(3): 131–143.

Rahman IW, Fadlilah RN, Kaaba, Kristina HN, Dirga A. 2022. The potential of guava leaf extract (*Psidium guajava*) in inhibiting the growth of Serratia marcescens. *Journal of Natural Sciences and Environmentn.* 13(1):14–22.

Rausch MK, Parekh SH, Dortdivanlioglu B, Rosales AM. 2021. Synthetic hydrogels as blood clot mimicking wound healing materials. *Prog Biomed Eng (Bristol).* 3(4):1-23. doi: 10.1088/2516-1091/ac23a4.

Risan MH, Taemor SH, Muhsin AH, Hussan S. 2017. Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* extract against some gram negative and positive bacteria. *EJBPS*. 4(12): 9–15.

Rohim A. 2018. Test of anticoagulant activity of ethanol extract l pirdot leaves [Thesis]. Medan:University of North Sumatra.

Sariyana, Nofriani A, Sabarwati H, Sahidin. 2018. Potential waste of eggshells and tembelekan leaves (*Lantana camara* L.) as a natural antiseptic ointment. *Journal of Health Science Pharmacy.* 4(1): 22–25.

Septiani V, Choirunnisa A, Syam AK. 2017. Antimicrobial activity test ethanol extract of karuk leaves (*Piper sarmentosum* Roxb.) against *Streptococcus mutans* and *Candida albicans*. *Scientific Journal of Pharmacy*. 5(1): 7–14.

Sunnah I, Erwiyani AR, Aprilliani MS, Maryanti, Pramana, GA. 2021. Antihyperuricemia activity and evaluation of physical preparations of yellow pumpkin extract syrup (*Cucurbita maxima*). *Indonesian Journal of Pharmacy and Natural Product*. 1(4): 27–36.

Susanto D, Sudrajat Ruga R. 2012. Study of the content of the active ingredient of the red meranti plant (*Shorea leprosula* Miq) as a source of antibacterial compounds.

Zimmerman TS, Arroyave CM, MullerEberhard HJ. 1971. A blood coagulation abnormality in rabbits deficient in the sixth component of complement (C6) and its correction by purified C6. The Journal of Experimental Medicine. 134: 1591-1600.