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BIOVIABILITY OF BIDURI LEAF EXTRACT (*Calotropis Gigantea* L.) ON FIBROBLAST CELL CULTURE

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Abstract

Background: Biduri is recognized for its medicinal properties, particularly in wound healing. The active compounds influence the number of fibroblast cells, which play a role in wound healing. Research on the bioviability of biduri leaf extract has never been conducted, so in vitro testing is necessary to determine the ability of biduri leaf extract to influence fibroblast cells.

Objective: To assess the effect of Biduri leaf extract on the viability of BHK-21 fibroblast cells in vitro.

Methods: This experiment used a posttest-only group Design with an MTT assay to assess the viability of BHK-21 fibroblast cells after exposure to different concentrations of Biduri leaf extract. The study employed four treatment groups with varying extract concentrations and used one-way ANOVA followed by an LSD test to analyze the proportion of living cells statistically.

Results: The viability percentages of fibroblast cells varied with the concentration of Biduri leaf extract. The highest viability was observed at 5% concentration (80.82%), while the lowest was at 20% concentration (60.25%). A one-way ANOVA test was used to analyze the data, and differences were considered statistically significant at $p < 0.05$..

Conclusions: The highest viability was observed at 5%, while the lowest was at 20% concentration. All treatment groups had cell viability above 60%, indicating that the Biduri extract is generally biocompatible with fibroblast cell culture.

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INTRODUCTION

Oral health significantly impacts overall quality of life, as dental and oral diseases often lead to pain, inflammation, and tissue damage. Wounds in the oral cavity can arise from various factors, including poorly fitting dentures, sharp edges of teeth, extremely hot food and beverages, or even vigorous brushing with a stiff toothbrush. These wounds can result in painful ulcers, leading to pain and discomfort, significantly impacting the patient's daily life (1). In response to such injuries, the body initiates a physiological wound-healing process. This involves complex events, including inflammation, tissue formation, and remodeling, which are essential for restoring oral integrity and function (2). Understanding this process is crucial for developing effective strategies to prevent and manage oral wounds, ultimately enhancing oral health and overall well-being.

The wound healing process consists of various complex processes to restore tissue integrity. Wound healing is a biocellular activity that occurs sequentially and is influenced by many intrinsic and extrinsic factors (3). One plant that shows promise as a medicinal and wound-healing agent is biduri (Calotropis gigantea L). This wild plant is particularly notable for its resilience, as it reproduces rapidly, making it difficult to eradicate (4).

Biduri is known to have analgesic, antimicrobial, antioxidant, anti-inflammatory, anti-carcinogenic, and procoagulant properties. The chemical content of biduri is spread throughout the plant, in the leaves, flowers, sap, and root bark. Phytochemical analyses have identified various compounds in biduri, such as cardiac glycosides, flavonoids, terpenoids, alkaloids, tannins, steroids, and resins (5,6).

A plant can have potential as a medicine because there is a secondary metabolite process. Plants produce secondary metabolites that vary in structure, function, and content (7). Secondary metabolites in plants function as antibacterials, antivirals, and anti-inflammatories (8).

Fibroblasts are the most common cell type in the body, making them easy to obtain from various tissues. Fibroblasts are often chosen for studies because they are readily available, easy to culture, and vital to tissue structure and repair. The MTT assay measures cell viability because it is a simple, colorimetric method that reflects metabolic activity in living cells.

This research is necessary to determine biocompatible Biduri leaf concentrations for wound healing. Materials in dentistry that are applied in the oral cavity must be biocompatible, which means the body can accept them, they are not irritating, are not cariogenic, do not cause allergic reactions, and are not toxic. The viability test in cell culture is one of the in vitro biocompatibility tests that can be used and meets standards according to ethical, practical, and economic factors.

MATERIALS AND METHODS

The research is an experimental laboratory with a post-test only control group design. Collecting biduri leaves was carried out at Watu Ulo Beach, Jember. The collection process was carried out in the morning, selecting leaves that were intact, good, and green in color.

Preparation of leaf extract; 2 kg of leaves were washed and cut into small pieces, dried for 2 days at room temperature, away from direct sunlight, then oven-dried at 40˚C for 24 hours. Dried leaves were blended and sieved through an 80-mesh sieve to obtain a fine powder. Fine powder was macerated with 70% ethanol for 3 days, stirring every 24 hours. Furthermore, concentrate the solution using a rotary evaporator at 50 ˚C and 90 rpm. Cell culture: Utilized the BHK-21 fibroblast cell line for further experimentation.

Four groups with four replications for the MTT (3- (4,5 4,5-dimethylthiazol) 2-yl)-2,5 2,5-diphenyltetrazolium bromide) Assay test technique for cell culture. The cell culture's optical density value is determined using an ELISA Reader, and the proportion of living cells is computed following a microscope examination.

Baby Hamster Kidney-21 (BHK-21) fibroblast cell culture was prepared with RPMI-1640 culture media with fetal bovine serum in a Roux culture bottle and incubated at 37 °C and 5% CO2 for 2 days. After forming a monolayer of fibroblast cells, the cells are transferred to the MTT Assay microplate. Add 50 µL of serum-free media and 50 µL of MTT solution into each well. Incubate the plate at 37°C for 3 hours. After incubation, add 150 µL of MTT solvent into each well. Elisa Reader readings use a wavelength of 620 nm. The research data in the form of OD (optical density) values are then entered into a formula to obtain the percentage of living cells.

From the microplate, the following divisions are carried out: (1) Media Group (culture media), (2) Control Group (culture media and fibrobalast Cells), (3) Group P1 (culture media, fibroblast cells, 5% v/v biduri leaf extract), (4) Group P2 (culture media, fibroblast cells, 10% v/v biduri leaf extract), (5) Group P3 (culture media, fibroblast cells, 15% v/v biduri extract), (6) Group P4 (culture media, fibroblast cells, 20% v/v biduri leaf extract).

The data were analyzed using SPSS with significance ($\alpha = 0.05$). The normality test used the Shapiro-Wilk and the homogeneity test with the Levene test. The parametric One-way ANOVA and Least Significant Difference (LSD) tests are used.

RESULTS AND DISCUSSIONS

Based on Table 1, the highest viability is at 5% concentration with 80.82%, and the lowest is at 20% concentration with 61.25%. The following table data is presented as a graph in Figure 1.

Table 1. Viability of Biduri leaf extract against BHK-21 fibroblast cell culture

Concentration	% Viability cell
Control	100
5%	80.82
10%	74.16
15%	65.83
20%	61.25

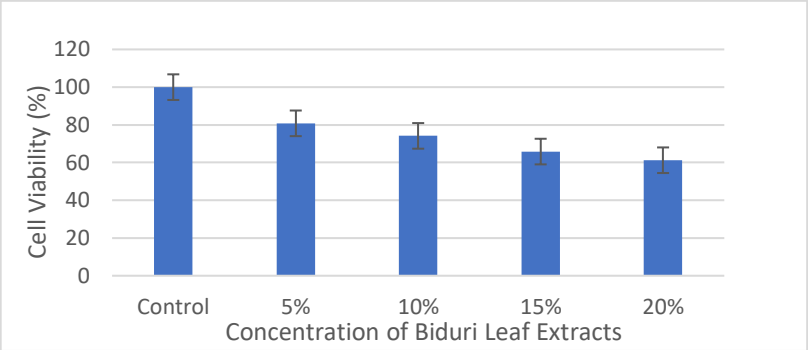

Figure 1. Graphic of viability cell in various concentrations of Biduri leaf extract

Observations were also made on fibroblast cell cultures. The fibroblasts have characteristic morphological features, including an elongated spindle shape and round to oval cell nuclei. Fibroblast cell culture has characteristics of branched cytoplasm surrounding an oval-shaped nucleus with one or two nucleoli.

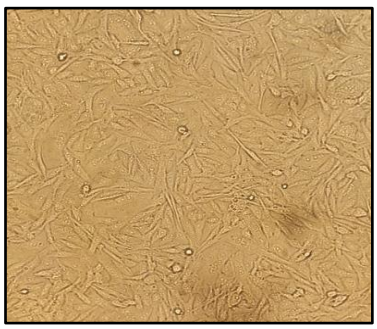

Figure 2. BHK-21 (Baby Hamster Kidney-21) fibroblast cell culture

Data analysis showed that the data was normally distributed and homogeneous with a significance value ($p > 0.05$). The data was continued to the ANOVA test with a significance value of 0.00 ($p < 0.05$). This shows differences between the concentration variations of the treatment groups.

This research used biduri leaves that grow abundantly in the coastal areas of Jember to raise the local potential of natural materials in the Jember area. Biduri plants are abundant in Watu Ulo Beach and its surroundings. Based on previous research, according to the results of phytochemical screening, Calotropis gigantea's leaves, flowers, sap, and root bark include phenol, tannin, and steroids. Whereas flavonoids are exclusively present in leaves and flowers, saponins are present in

both leaves and sap. Compared to flowers, leaves have a higher overall flavonoid concentration. As a result, selected leaves, which have the maximum flavonoid concentration, were investigated (9).

Biduri leaves are then made into simplicia and extracted with 70% ethanol. Based on a study by Suhaenah et al, which compared ethanol solvents of 50%, 70%, and 96% in biduri extraction, good results were obtained using 70% ethanol because it is polar, so the active compounds obtained are higher (10). This is the basis for maceration with 70% ethanol. After the maceration process with ethanol, the filtrate is filtered to obtain a liquid extract of Biduri leaves. The liquid extract was then concentrated using a rotary evaporator and evaporated to achieve a stable extract weight.

This research is an experimental laboratory study to determine the viability of fibroblast cell culture at specific concentrations. In this study, biduri leaf extract was tested with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay at concentrations of 5%, 10%, 15%, 20% against Baby Hamster Kidney-21 (BHK-21) fibroblast cells. BHK-21 cell culture is used because fibroblast cells are the most critical and significant components of the pulp, periodontal ligament, and gingiva (11,12).

Viability or toxicity testing is a formal requirement in drug development. Cell-based toxicity models have the best potential to reliably predict drug toxicity in humans, as they are developed using the cells of the target organism (13). Based on the study's results, the viability value decreased with increasing concentration of biduri leaf extract. The lowest cell viability was at a concentration of 20%, at 61.25%. All treatments were biocompatible with fibroblast cells because they met the requirements to be called biocompatible (living cells ≥ 60%). This shows that the content of active ingredients and nutrients in biduri leaf extract does not interfere with the biological function of fibroblast cells.

Proliferation of fibroblast cells is an effort to improve or accelerate wound healing. Fibroblasts have many roles in the wound healing process, one of which is as a cellular component that plays a role in the coagulation process and inflammation, such as producing fibril components. Fibroblasts also play a role in producing defensins and cathelicidins, which are amphipathic and antimicrobial peptides. Fibroblasts also produce growth factors such as Fibroblast growth factor (FGF), Transforming growth factor β (TGFβ ß), and Platelet-derived growth factor (PDGF). Fibroblasts are the primary source of dermal substitutes by producing many structural components of the extracellular matrix (ECM) such as collagen, elastin, laminin, and glycosaminoglycans (14,15). Biduri

leaf extract maintains cell viability due to the content of active ingredients such as flavonoids and alkaloids. Flavanoids are polyphenol compounds in plants that exhibit potent antioxidant and biological properties related to their chemical structure, confer an excellent radical scavenging ability (16).

Antioxidants can help cell viability by holding back reactive oxygen species (ROS) so that they do not interfere with the cell cycle, and antioxidants can promote cells to enter the S phase in the cell cycle. The generation of ROS and antioxidant defense systems must be balanced for cellular health (17). Oxidative stress can cause cycle arrest in the G1 phase and induce apoptosis or return to the next phase if the oxidant level is controlled (18,19)

Common flavonoids have antibacterial, antioxidant, and anti-inflammatory properties, which advance wound healing and have anti-scar properties by interfering with the transmission of key signaling pathways involved in scar formation (20). The utilization of cancer prevention agents, such as most flavonoids, is believed to hasten wound healing and recovery by decreasing oxidative stress within the wound (21).

The ability of flavonoids to maintain cell viability is supported by the theory that flavonoids can activate calcium in the mitochondria, which makes cells able to produce ATP so that they can survive (22). In addition to flavonoids, there are alkaloid compounds that have the potential to heal wounds by playing a role in the collagenase process, matrix formation, and fibroblast proliferation (23).

The decrease in cell viability in the treatment group can be caused by the formation of purple formazan by tetrazolium salts, which refers to metabolically active cells. The metabolic activity of each cell is different. Sometimes, living cells are found and show metabolic activity, but only a little or even no proliferation. Two main processes can lead to a decrease in the number of viable cells: either real cell death (cytotoxic impact) or suppression of cell metabolism and/or proliferation (cytostatic effect) (24).

CLINICAL IMPLICATION

Biduri leaf extract demonstrates biocompatibility across all tested concentrations, making it a promising candidate for further research in wound-healing applications.

LIMITATIONS

The limitation of this research is the lack of variation in concentrations that can increase fibroblast cell proliferation, so that the wound healing process becomes faster.

CONCLUSIONS

The highest viability percentage was at a biduri extract concentration of 5%, and the lowest was at a concentration of 20%. All treatment groups had cell viability above 60%, indicating that the Biduri extract is generally biocompatible with fibroblast cell culture.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

Conceptualization, S.S., P.A and ZM; methodology, S.S, A.W.S.D; software, A.W.S.D and S.S; formal analysis, Z.M and A.W.S.D; writing the original draft, S.S, writing the review and editing, P.A, Z.M, S.S.

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