Effectivity of Patch Herbal Mixture Composed of Mangosteen Peel Extract and Bacterial Cellulose for Wound Healing

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ABSTRACT

Indonesia has a very abundant production of mangosteen, which places at the 14th largest mangosteen production in the world. High mangosteen production affects the amount of mangosteen peel waste. On the other hand, mangosteen peel has been identified as a rich source in health benefits and has proven to be antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, and anti-carcinogenic. Traditionally, one of the conventional medicines used in wound dressings or infection medicine. It can be a possibility for patch wound healing development, especially for diabetic ulcers with an open wound, wetness, chronic infections, and high inflammation. This research aims to innovate wound dressings (patch) that contain active compounds from mangosteen peel as herbs with many active compounds to enhance the wound healing process with low toxic side effects. The patch developed from organic material from bacterial cellulose. The research was carried out with the initial process of making patches from bacterial cellulose (BC), then adding mangosteen peel extract as patch dressing with different concentrations (0%, 1%, 2%, 5%, and 10% v/v). Furthermore, the patch was applied to the injured Wistar rat, for 15 days. The wound area was measured using Image-J. The results of measuring the wound area showed significant wound improvement every day at each dose of the mangosteen patch compared with the control group. The optimum dose that appears to be different from the control is 10% v/v mangosteen peel extract which has been able to leave 3% of the wound area on the 15th day.

Key words: Bacterial cellulose, Mangosteen peel extract, Herbal compound, Patch dressing, Wound treatment

INTRODUCTION

Indonesia is known as the second one of the biggest biodiversity countries centers, especially in tropical fruit.1 This biodiversity as biological resources are included in agriculture, plantation, forestry, livestock, and fisheries sectors. The plantation sector relates to a wide range of plants that could be advantageous as raw materials in the traditional medicine industry. One of the native Indonesian plantations is mangosteen (Garcinia mangostana Linn.).2 Based on Indonesia Central Statistics Agency in 2019, mangosteen production in 2018 increased by 41.05% from 2017 and ranked 14th highest production at 228,155 tons.3 The high mangosteen production affects the amount of mangosteen peel waste, while it has been identified as rich beneficial values for the heath. Mangosteen peels have been proven in antibacterial, antifungal, anti-inflammatory, antioxidant, anticarcinogenic, and other effects involved in traditional medicine utilization around Southeast Asia region.^{4,5} One of the conventional medicines used in applications in wound dressings or infectious drugs.6

The mangosteen peel power is related to its bioactive compounds such as xanthones, flavonoids, triterpenoids, and benzophenones. Xanthones being the main mangosteen's secondary metabolites have been found in as many as 68 types in a different part of mangosteen fruit, indeed 50 types being present in the mangosteen peels as the higher composition than the edible part portion.^{6,7} This compound plays an essential role

in the wound healing process due to inhibiting the enzyme cyclooxygenase (COX) production which is responsible for the inflammation process.^{8,9}

In addition, Wound healing is a natural physiological process to recover the wound which is related to the contribution of various cells around the wound site. It consists of several stages including hemostasis, inflammation, proliferation, and maturation.^{9,10} Those stages could overlap over time which depends on the wound condition.11 In brief, upon tissue damage, platelets are activated and released growth factors to promote the fibroblasts to produce a fibrin matrix. This fibrin contributed to supporting blood clotting.12 Furthermore, wounds proceed to the inflammation stage by recruiting macrophages and neutrophils to degrade the pathogens. Then, within the proliferation stage, several processes, including fibroblast migration, angiogenesis, and granulation tissue become the main process.13 Moreover, at the maturation stage, the process focuses on matrix remodeling and collagen deposition. Finally, wounds are healed which is followed by scar formation.14

However, the wound-healing process could be impaired and prolonged in elderly people. Recent research showed that chronic wounds in the elderly remained in the inflammatory stage for a long time and progress for the proliferation and remodeling stage was impaired. It was considered an imbalance of cytokines and growth factors for the healing process.¹⁵ Moreover, age and multimorbidity in older people are also known for having an influence on the effectiveness of the wound-healing process.¹⁶ However, the treatment approaches for chronic wounds in the elderly remain unknown. In addition,



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the healing process will rapidly through all the stages with the additional antioxidant and antimicrobial agent which is necessary to prevent the severity of wounds, especially in open and chronic injury,¹⁷ while additional synthetic drugs such as antiseptics possess toxicity effects such as hypersensitivity to the skin and hyperthyroidism.¹⁸

Therefore, this study aims to make innovations in wound dressing (patch) containing active compounds from mangosteen peels as herbal with plenty of active compounds possessed to increase the wound healing process engaged with low toxic side effects. ^{19,20} This innovation can certainly be a choice of wound dressing that is affordable and environmentally friendly since the utilization of mangosteen peel waste as well as natural polymers of bacterial cellulose.

MATERIAL AND METHODS

Preparation of Mangosteen extract

Mangosteen peels with maturity level 6 were stored and chopped into smaller parts before drying under sunlight then ground to powder and screened with 65 mesh. The mangosteen powder was macerated using 96% ethanol with 1%, 2%, 5%, and 10% w/v in 100 mL volume and was agitated using a magnetic stirrer for 1 hour then placed for 1 day before being filtered. The solution was allowed to evaporated until 50 mL volume on water bath with temperature of 55°C. The product was stored in the refrigerator for further use.

Animals

Twelve-weeks-old male Wistar rats were housed under standard condition in an animal facility with free access to food and water. All experiment was approved by the guideline of the Ethics Committee of the University of Surabaya. The rats divide into 5 groups; one group for control treatment and wound healing treatment by mangosteen peel extract for the rest of group within 4 different doses (1%, 2%, 5%, and 10%).

Bacterial culture

A strain of *Acetobacter xylinum* was provided by BIOTECHNO, Banten, West java, Indonesia. Coconut water was used as a fermentation media in this research. Fresh coconut water was obtained from Sopoyono Traditional Market, Surabaya, East Java, Indonesia. To minimize contamination, at first coconut water was sterilized using autoclave at 121°C for 15 minutes and filtered before being used to eliminate dirt. All glass bottles for inoculum culture and fermentation process were sterilized with autoclave at 121°C for 15 minutes before being used. As much as 900 mL of substrate, were added to 100 mL of starter *Acetobacter xylinum* to make it 1L working volume solution, 25 g of sugar and 4-5 g ZA fertilizer were added to increase fermentation nutrient and at the end 10 mL of acetic acid 30% was added into the solution to adjust the pH. For bacterial cellulose (BC) production, static incubations were performed in flasks for 7 days before harvest.

Synthesis of Bacterial Cellulose (BC)

To purify BC production, BC pellicles were rinsed with hot water to remove the culture medium, and then boiled in a 1N sodium hydroxide (NaOH) solution at 90°C for 10 minutes in order to eliminate bacteria cells from the cellulose matrix. Repeated these procedures three times and then washed BC pellicles with distilled water until the pH was neutral. This BC pellicles is fabricated for a wound healing patch that will be applied to rats with a wound model. Before usage, BC pellicles were cut into rectangular pieces 3 x 3 cm with a thickness around 2-3 mm and then dipped overnight into ethanol extract of mangosteen peel with 1%, 2%, 5% and 10%w/v variations. Finally, the BC pellicles with mangosteen peel extract werw dried overnight at room temperate.

Wound model and treatment

Rats were anesthetized with chloroform and a punch wound was generated in the dorsal skin. Punch wounds excision was manually made with average 1.5 cm in diameter, that was shaved, by using scissors cleaned with 70% ethanol before. After wounding, wounds were covered with a cellulose patch (3 x 3 cm²) which was enriched with various dosages of mangosteen peel extract. Patches were changed every two days and wounds were observed for 15 days. All the treated rats were separated to be feed with sufficient water and feed. The area of wounds was measured by using Image-J.

Statistical analysis

All data of the wound area was collected and analyzed by using ANOVA. Data is presented as mean \pm standard deviation (SD) for all comparison. The P-value <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Mangosteen peel extraction

The extraction used in this study is by maceration. Maceration is a sample-soaking process using 96% ethanol as an organic solvent at room temperature. During the soaking process, the cell wall and membrane will break down due to the pressure difference between the inside and outside of the cell. Therefore, the secondary metabolites present in the cytoplasm of the plant will dissolve in organic solvents. The extraction result is shown in Figure 1. The higher concentration of mangosteen peel, the browner of liquid color.

However, the solvent of the extraction process can affect the amount of yield and the type of phytochemical extracted, depending on the polarity of the substance.^{21,22} The 96% ethanol solvent is a polar solvent that can attract more plant secondary metabolites during extraction because ethanol can break down plant cell walls more effectively.^{23,24} In addition, ethanol has a boiling point of 79 degrees C which requires less heat than other organic solvents. Actually, water solvents have the greatest dielectric constant, yet they can extract other cell components that may mask or interfere with potential active substances.²⁵ Accordingly, water solvents are less frequently used due to the low purity of the active substance extraction, easy contamination (extracts break down quickly due to overgrowth of fungi), and excessive cell turgidity.

Fabrication of patch

Bacterial cellulose is a heterofermentative product produced by fermentation of coconut water using the starter *Acetobacter xylinum*. The physical appearance of the wet pellicle of bacterial cellulose is



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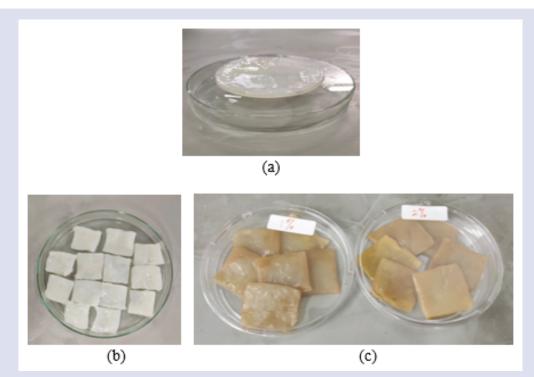


Figure 2: Patch fabrication from bacterial cellulose (a) Bacterial cellulose pellicle: white and almost thin, (b) Patch from BC, (c) Patch with mangosteen peel extract that brownish changed color

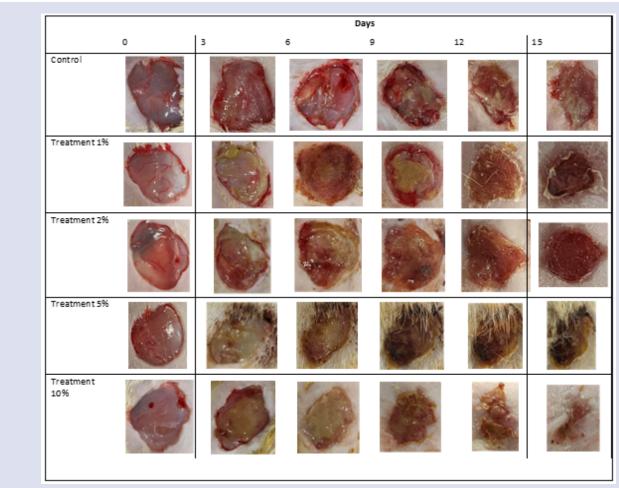
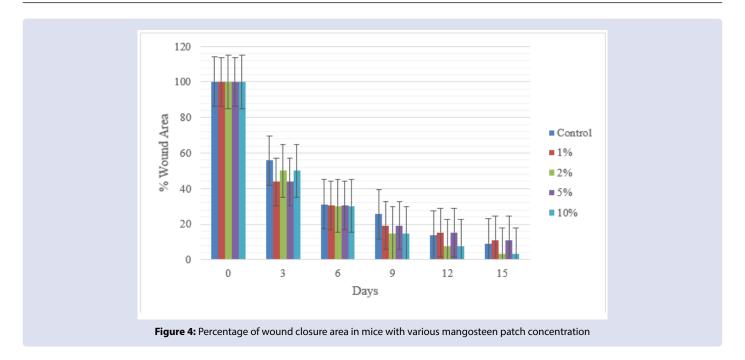


Figure 3: Observation of wound closure in mice with various concentration of mangosteen patch



shown in Figure 2. Bacterial cellulose pellicle has a white color and a smooth surface of 10 cm in diameter and 1cm thickness, respectively. BC has been widely used in biomedical applications such as wound healing patches, and skin or tissue artificial.

BC, like other cellulose polymers, possesses numerous hydroxyl bonds that form individual cellulose chains. These chains then interconnect to create microfibrils and ribbon fibrils. BC is synthesized through a liquid culture process, wherein a pellicle is formed during a network of fibrils combines to form a mat at the interface of the liquid and air. The hydroxyl group serves as the active site for both physical and chemical reactions, which contribute to the exceptional strength of BC materials when they are wet. Bacterial cellulose has better nanostructure cellulose than plant cellulose product due to it has better physicochemical and mechanical properties, outstanding biocompatibility, and biodegradability. Possible product due to it has better physicochemical and mechanical properties, outstanding biocompatibility, and biodegradability.

Hence, BC is employed as a wound healing material due to its distinct attributes, encompassing remarkable tensile strength, excellent flexibility, high water retention capacity, notable gas and liquid permeability, and strong compatibility with living tissues, especially human skin tissues. In terms of biocompatibility, BC exhibits non-cytotoxicity both in its natural state and throughout the production process, thereby avoiding the introduction of any toxic substances. Furthermore, BC does not elicit any adverse reactions when in contact with the human body, whether in the short or long term. These exceptional properties of BC are intricately linked to its origin, structure, and functional characteristics, and they play a significant role in promoting wound healing. ³³

To improve the biological properties, especially in an antibacterial activity that would be beneficial for medical treatment in the wound healing process, bacterial cellulose pellicle was immersed with ethanol extract of mangosteen peel at various concentrations (1%, 2%, 5%, and 10% w/v). After 24 hours, the bacterial cellulose pellicle exhibited a brownish color due to the mangosteen extract. Increasing mangosteen extract concentrations were added to bacterial cellulose pellicle, resulting in an increase in brownish color as shown in Figure 2.

The effectivity of patch

Observation of the wound healing process was conducted every three days for 15 days. In brief, observations conducted from day 0, 3, 6, 9,

12, and 15 demonstrated a repairing process within 0 days as an open wound then epitalization occurred, especially in 10% mangosteen peel extract at day 15th the wound almost close and heal (Figure 3).

Moreover, the results showed that compared to the control, both wounds treated with patch 5% mangosteen peel extract and patch 10% mangosteen peel extract promoted wound healing in 15 days. Of note, the comparison percentage of the wound area, patch 5% mangosteen peel extract: 10.93% and patch 10% mangosteen peel extract 3.12% (Figure 4) The effectivity within all treatments was not differe significantly (p>0.05), while 10% extract showed more improvement in wound area healing and epilatization process. According to those data, it suggested that patches with mangosteen peel extract possess the healing function which could be considered as a new approach for wound healing treatment, then 10% is the suit concentration applied.

The improvement of wound showed that patch from bacterial cellulose is able not only bind with active compound from mangosteen peel extract, but also adequate for transform the active compound into skin. It relates with study by Lin, *et.al.*, (2013) that fabricated wound dressing from bacterial cellulose with chitosan. The research revealed that bacterial cellulose with chitosan affect the high of epitalization and regenerate skin wound on rat compared with bacterial cellulose and commercial patch.³¹ Moreover, physical structure of bacterial cellulose has improve within the strength and humadity followed with additional dressing compound which more suitable for wound dressing material.³⁴ Beside that, this material confirmed for its ability on high loading drug within 90% at 24 hours.³⁵

In addition, the wound healing process consists of the hemostasis, inflammation, proliferation and remodeling stages which last for three weeks. In our previous research, we discovered that all stages of maturing mangosteen fruit contain various compounds such as γ -mangostin, garcinone-E, gartanin, and smeathxanthone-A. Among these compounds, maturity level 6 exhibited the highest concentration of xanthones, particularly in mangosteen peel extract. Therefore, our recent computational study investigating wound healing using mangosteen peel for burn injuries, we identified the most potent compounds for promoting burn wound healing, namely smeaxanthone A, Garcinone E, γ -mangosteen, and Gartanin, which all belong to the xanthone family. Computational predictions indicated that these four compounds have potential targets associated with interleukin 6, epidermal growth factor, and transforming growth factor beta 1, which

are involved in regulating epithelial cell proliferation, a crucial process in skin wound healing. $^{17,38}\,$

It is plausible that mangosteen peel extracts affect the expression of IL6 through TGFB2, as IL6 levels were higher compared to fibroblast growth factor 2 (FGF2). The primary mechanisms that impacted the body involve two areas of regulation: epithelial cell proliferation (FGF2, TGFB1, IL6, TGFB2) and leukocyte homeostasis (TGFB1, IL6). These pathways are strongly associated with wound healing, including epithelial cell proliferation and the elimination of antigens by leukocytes at the wound site. ^{39,40}

The previous studies by Cui *et al.* (2010) demonstrated that α -mangostin and γ -mangostin, compounds isolated from mangosteen, exhibit potent scavenging abilities against reactive oxygen species (ROS) in a dose-dependent manner. Additionally, α -mangostin has been shown to neutralize superoxide anion (O2-), singlet oxygen (1O2), and peroxynitrite anion (ONOO-) in a concentration-dependent manner, as observed in Pedraza-chaverri *et al.*'s (2009) study. These antioxidant properties of mangosteen peel extract likely contribute to the control of inflammation on the wounded skin, leading to accelerated reepithelization of the epidermis. Moreover, Sivaranjani *et al.* (2017) demonstrated the effective antimicrobial properties of α -mangostin against *S. epidermidis in vitro*. These antimicrobial effects of mangosteen extracts are crucial in preventing microbial infections in the wound area.

However, the wound healing process could be impaired by the age of patients. Elderly patients are considered to have an impaired wound healing process so the healing progress has failed to progress. It relates not only time wound healing needed,⁴⁴ but also the chronic wound might be advance.^{16,45} The patch from bacterial cellulose with mangosteen peel extract which has been evaluate in this research could be promising for wound healing treatment, especially for elderly patients. Indeed, the patch also potential for diabetic ulcer treatment which known chronic inflammation and infection.⁴⁶

CONCLUSION

Patches of mangosteen peel extract have been made from bacterial cellulose with flexibility and thickness qualified of being used as wound dressings. In addition, patches of bacterial cellulose can bind to active substances from mangosteen peel extract, which can be used as additives to accelerate wound healing. Mangosteen peel extract in the patch is proven to increase the wound healing process after 15 days that showed in improving significantly in wound healing by days. Moreover, the optimal concentration of mangosteen peel extract as patch dressing is 10%. This could be the basis for the development of patches for the treatment of diabetic ulcers.

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GRAPHICAL ABSTRACT



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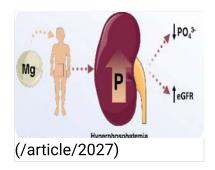
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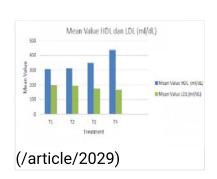
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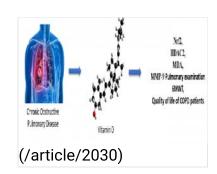
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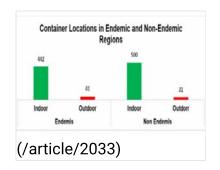
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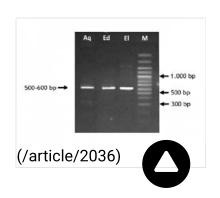
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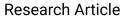
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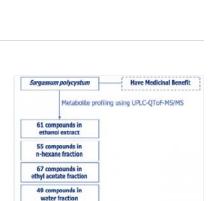
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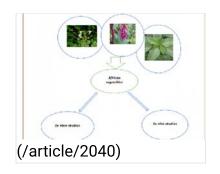
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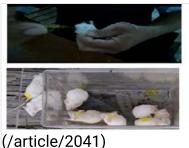
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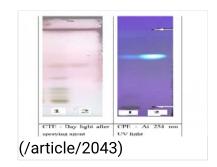
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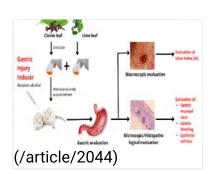
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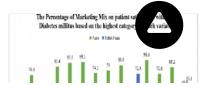
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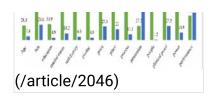
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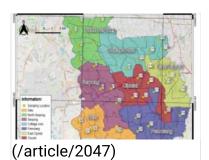
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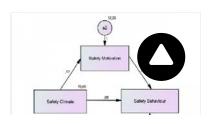
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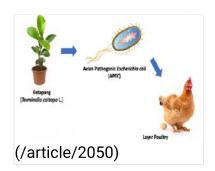
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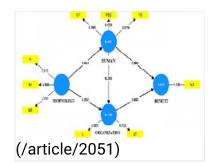
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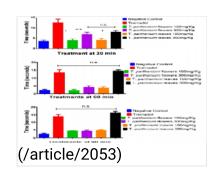
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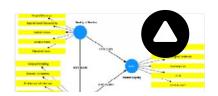
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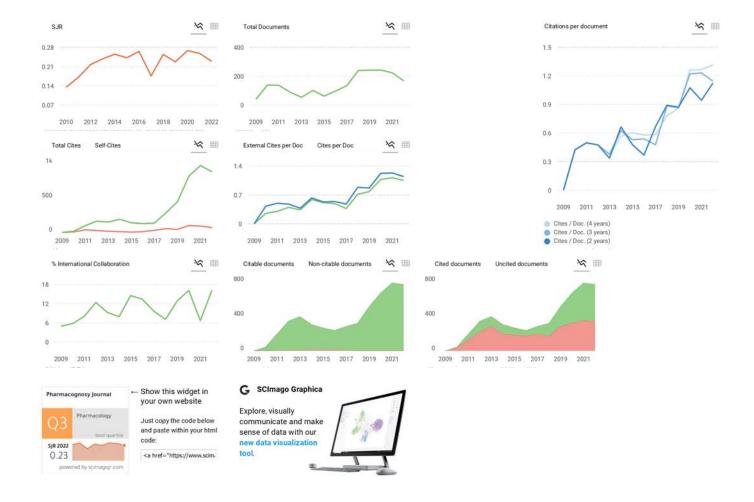
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T Tushar 4 years ago

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I just want to know that is these journals in the UGC care list because I didn't get the updated list of UGC.

kindly do needful

reply



SCImago Team

Melanie Ortiz 4 years ago

Dear Tushar,

Thank you for contacting us. SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus. Unfortunately, we cannot help you with your request referring

the index status. We suggest you to consult Scopus database (see the current status of the journal) or other databases (like WoS) for further information. You can also check that information in the journal's website or contact directly with the editorial staff. Best Regards, SCImago Team

A	aditi	5 years ago	
	Respe	ected Sir,	
		to know if while v	writing manuscript got Pharmacognosy journal, Can the results and d?
	re	ply	
			SCImago Tean
		Elena Corera	5 years ago
	109	Dear Aditi,	
		thank you very r	nuch for your comment, unfortunately we cannot help you with your
			gest you check author's instructions in journal website. You can find that
		information in S	JR website https://www.scimagojr.com
		Best Regards,	
		SCImago Team	
eave a comr	ment		
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mail vill not be publish	terfi		
van trot de poditis	ino)		
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Pharmacognosy Journal

Scopus coverage years: from 2009 to 2023

Publisher: EManuscript Technologies

ISSN: 0975-3575

Subject area: (Pharmacology, Toxicology and Pharmaceutics: Pharmacology)

Pharmacology, Toxicology and Pharmaceutics: Drug Discovery

Source type: Journal

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