Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 11(2), 92-98, 2023

Bioactive Compounds and Microbacteria of Peel-Off Gel Mask *Caulerpa racemosa*

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Received: February 2023
Received in revised: March 2023
Accepted: Juny 2023
Available online: August 2023

Abstract

Caulerpa racemosa is a type of seaweed that has the potential to be cultivated because it is known and favored by the public.*Caulerpa racemosa* is used not only for consumption as food but also for beauty cosmetics because of its ingredients. This study aims to determine the Bioactive content and total plate count (TPC) of the *Caulerpa racemosa* peel-off gel mask. The pour plate method was used to determine the total plate number of mycobacteria and phytochemical testing in the form of tests for flavonoid, alkaloid, phenolic, tannin, triterpenoids, and saponin. The results showed that the total colony total plate count until the 3rd repetition had an average of 31 col/g. The results of the phytochemical test of the *Caulerpa racemosa* peel-off gel mask contained bioactive compounds: flavonoid, alkaloid, phenolic, tannins, triterpenoid, and saponin. Conclusion: The Total plate count of *Caulerpa racemosa* peel off the gel mask complies with Indonesian National standards (INS) with the content of bioactive compounds, namely tannins dan saponins. Caulerpa racemose was used for a pell-off gel mask for the first time.

Keywords: Caulerpa racemosa, microbacteria, peel-off gel masks, phytochemical

INTRODUCTION

Seaweed is a leading commodity for coastal communities considering its positive contribution to employment absorption and increased income. The type of seaweed that has potential but has not been developed for cultivation is *Caulerpa racemose* (Supriadi et al., 2016). *Caulerpa racemosa* benefits beauty by containing vitamins and minerals the skin needs, namely vitamin B complex, vitamin C, magnesium, and other minerals that help skin cell metabolism.

Researchers have studied that *Caulerpa racemosa* has medicinal and beauty properties because it contains high levels of vitamin A, C, iron, iodine, and calcium (Ridhowati & Asnani, 2016). *Caulerpa racemosa* has photosynthetic pigments, namely chlorophyll a and b, and accessory pigments, namely carotenoids. The main carotenoids in green algae include ß carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin. Helpful in reducing the adverse effects of UV rays on the skin.

The potential of *Caulerpa racemosa* in the non-food sector can help increase the availability of natural cosmetic ingredients, which is often used and makes it easier to care for a peel-off gel mask. Using gel masks made from natural ingredients is better than synthetic materials because they cause side effects and can even damage the natural shape of the skin (Grace et al., 2015).

Peel-off gel masks are a type of mask made from natural active ingredients with the addition of gelling agents, humectants, pH regulators, and solvents. Peel-off gel masks have the advantage of not having a dependency effect on the product, in the form of cool gels or pastes, being able to relax and cleanse the face more quickly. They can be easily removed or removed like an elastic membrane (Rahmawanty et al., 2015). The novelty of the research is identifying secondary metabolites in Peel-off gel masks Caulerpa racemose as a Bioactive compound.

METHODOLOGY

Materials and Instrument

The materials used are *Caulerpa racemosa*, Polyvinyl Alcohol, Carbomer 940, Glycerin, Triethanolamine (TEA), Aquadest, and Perfume extract. The tools used are an Analytical balance, Water bath, Thermometer, Spatula, Tube, Erlenmeyer, Autoclave, Test tube, Petri dish, volume pipette, incubator, colony counter, pH meter, viscometer, beaker, glass plate.

Production of *Caulerpa racemosa* peel-off gel mask

Polyvinyl alcohol (PVA) is crushed until finer, then expanded by dissolving it in hot distilled water over a bath at 70-80 ^{o}C. Carbomer 940 was developed with cold distilled water until dissolved, then mixed into the polyvinyl alcohol base while stirring to form a

homogeneous mixture. Glycerin and TEA were homogenized into polyvinyl alcohol bases and added preservatives with a concentration of 1%. Add perfume extract to the gel base while stirring until it is estimated to be homogeneous. Then add distilled water until it reaches 100 mL to form a homogeneous gel base mixture. Store in a tightly closed container opaque. Leave it for 24 hours to avoid air bubbles forming on the gel preparation before testing.

Mycobacterial Test (Total Plate Count)

Calculate the number of colonies total plate number of microorganisms selected from a petri dish with the number of colonies between 30-300 with a dilution of 10^{-3}. In determining the total plate number, the pour plate method was used, and the number of bacterial colonies growing on agar media was counted after incubation at 37 ^{o}C for 24 hours. After 4 x 24 hours of incubation, colony counts were performed. It can be assumed that each colony comes from a cell, so the number of colonies can represent the number of counted cells (Sundari & Fadhliani, 2019).

Phytochemical test

Alkaloid Test

A solution of 1% alkaline ammonia and chloroform in a test tube was shaken, then the (lower layer) was pipetted, and 2N HCl was added and then shaken. The solution obtained was divided into three as a blank, and the remainder reacted with Mayer's and Dragendorf's reagents, respectively. Positive results is a mixture with Mayer's reagent gives rise to a white precipitate, and a mix of Dragendorf's reagent causes turbidity and an orange deposition (Dali et al., 2022).

Flavonoid Test

Ethanol extract 2 mL was added with a little magnesium powder and 2 mL of 2N HCl. The positive result is that the solution changes color to orange or yellowish red (Dali et al., 2022).

Phenolic Test

The ethanol extract was dripped onto the drop plate, and the $FeCl_3$ reagent solution was added. A change in the color of the solution to blackish green indicates a positive result.

Triterpenoid Test

The ethanol extract was added with the addition of Lieberman-Burchard reagent. The appearance of brownish red color indicates positive results for Triterpenoid compounds.

Saponin Test

Pipetted two drops of ethanol extract, put into a test tube, then added 10 mL of hot water. After that, it was cooled and shaken vigorously for 10 minutes until a steady froth was formed for not less than 10 minutes, as high as 1 cm to 10 cm. On adding one drop of 2 N hydrochloric acid, the foam does not disappear. It is saponins.

Taninns Test

The extract in the test tube was dissolved with distilled water, heated over a water bath, and then dripped with 1% (1:1) gelatin solution. The formation of a white precipitate indicates a positive result.

RESULTS AND DISCUSSION

Mycobacterial (Total Plate Count)

Contamination of microorganisms in a product is avoided because it is easy to damage the quality of the product. Based on INSINS 01-2897-1992, the total plate number calculation is only on Petri dishes containing 25-250 bacterial colonies. The total plate count test uses solid media with the final result in the form of colonies that can be observed visually, and the interpretation of the results is calculated in the form of colony numbers/ml.

Figure 1. Peel Off Gel Mask *Caulerpa racemos*

The results of calculating the number of colonies of *Caulerpa racemosa* peel-off gel mask seaweed can be seen in Table 1

Table 1. Total colonies of peel-off gel mask *Caulerpa racemosa*

Sample	(colony/ml)	Standard
A_1	32	Maximum 10^2 Kol/g. INS 2332.3:2015
A_2	28	
A_3	35	

Based on Table 2, the number of colonies of the peel-off gel mask *Caulerpa racemosa* preparation in the first replicate showed 32 colonies. In contrast, the second replicated 28 colonies. However, in the third

iteration, there were 35 colonies. The presence of antibacterial compounds in Caulerpa racemosa seaweed plays an active role in the preparation. *Caulerpa racemosa* seaweed contains alkaloid, terpenoid, phenolic, and flavonoid compounds, which act as antioxidants and antibacterials (Noor & Nursandi, 2014). Phenol is a secondary metabolite that acts as an antibacterial (Nurjanah et al., 2018). Antibacterial mechanisms of phenolic compounds are done by denaturing bacterial cell proteins. Hydrogen bonds between phenolic compounds and cell proteins cause the protein structure to break down. This protein binding affects the permeability of the bacterial cell wall and cytoplasmic membrane, which will cause an imbalance of macromolecules and ions present in the bacterial cell, so the cell becomes lysed. The decrease in bacterial colony growth during storage is thought to be due to the water content that has evaporated due to the influence of uncontrolled temperature and humidity. Microorganisms can grow if there is excessive water content, which can cause a decrease in the quality of a product. The higher the water content of the material, the greater the opportunity for microbes to grow (Adawyah, 2007).

Phytochemicals

Phytochemical tests were conducted to determine the bioactive compounds in the *Caulerpa racemosa* peel-off gel mask. Phytochemical analysis was carried out qualitatively to provide an overview of the active compounds contained in *Caulerpa racemosa* seaweed extract. However, it is the initial step to identify antibacterial chemical compounds capable of counteracting free radicals and ensure the presence of compounds with antioxidant activity (Ambari et al., 2021).

Table 2. Bioactive phytochemical compounds of the *Caulerpa racemosa* gel peel-off mask

Bioactive compounds	Sample		
	A 1	A2	A3
Flavonoids	++	+	++
Alkaloids	+++	++	+++
phenolics	++	++	+++
Tannins	+++	+++	+++
Triterpenoids	++	+	++
Saponins	+++	+++	+++

Information: (+++) = many ; (++) = moderate; (+) = less.

The results of the phytochemical test (Table 1) on the *Caulerpa racemosa* peel-off mask gel extract showed the presence of bioactive compounds, namely alkaloids, flavonoids, phenolics, tannins, triterpenoids and saponins (Mahardika et al., 2023).

Flavonoid

Flavonoids are good reducing agents, inhibiting many oxidation reactions, both enzymatic and non-enzymatic. Flavonoids act as hydroxyl and superoxide radicals, protecting membrane lipids against damaging reactions. The structure of flavonoids can be seen in Figure 2.

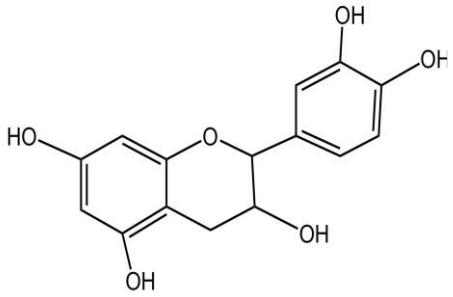


Figure 2. Structure of flavonoids

The function of flavonoids to maintain average skin growth and damage to the skin plays an essential role in absorbing and neutralizing free radicals. Flavonoids can be used as ultraviolet light filters, protecting cells from ultraviolet B radiation (280-320 nm) (Rahmi & Minerva, 2022). A positive result for samples containing flavonoids is the solution changes color to yellowish or orange (Figure 3).

Before

After

Figure 3. Flavonoids Test

Alkaloids

Alkaloids are compounds that contain nitrogen and are often present in heterocyclic rings. The mechanism of action of alkaloids is also antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death. The structure of alkaloids can be seen in Figure 4. Alkaloids contain basic nitrogen atoms and are part of a heterocyclic ring. Alkaloids have prominent

physiological activities and are often used extensively in medicine.

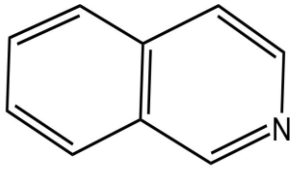
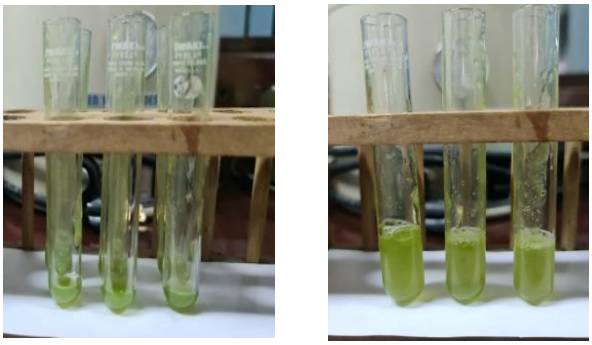


Figure 4. Structure of alkaloids

Alkaloids are compounds with one or more nitrogen atoms usually combined and part of the cyclic system and also function as antioxidants. After adding the reagent, positive results in the sample give rise to a white residue (Figure 5).



Before After

Figure 5. Alkaloids Test

Phenolic

Based on the results of the test measurements that phenolic compounds play a role in preventing oxidation events. The high content of phenolic compounds in the sample means that the antioxidant activity will be high. It is suspected that with an increase in total phenol, there is ongoing antioxidant activity. The entire phenol content can be produced from several simple molecules, namely phenolic compounds (Figure 6), to complex molecules. This condition indicates the presence of phenolic compounds in peel-off gel mask extracts (Dungir et al, 2012)

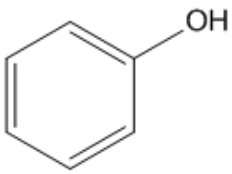


Figure 6. Structure of phenol

Antioxidants function to protect the body from free radicals, both internally and externally. The part of the body that is often exposed to free radicals externally is the skin, such as ultraviolet radiation and cigarette smoke. High exposure to free radicals on the skin can cause damage to skin cells or tissues such as autoimmune and premature aging (Putri et al., 2021). One of the most visible effects of antioxidants is their ability to stimulate collagen production, which is an important part of the structure and process of facial skin rejuvenation (Toripah et al., 2014) and can prevent scaly skin, is effective for rejuvenating the skin and inhibiting the premature aging process of facial skin (Rahmi & Minerva, 2022). A positive result for samples containing phenol is the solution changes color to dark green.

Before After

Figure 6. Phenolic Test

Tannins

Tannins are active secondary metabolites compounds with several properties, such as an antigen, anti-diarrhoea, antibacterial, and antioxidant. Tannins are generally defined as polyphenolic compounds that have a relatively high molecular weight (more than 1000) and can form complexes with proteins. The more tannin content, the greater the antioxidant activity because tannins comprise polyphenolic compounds with free radical scavenging activity (Malangngi et al., 2012). The structure of tannins can be seen in Figure 8.

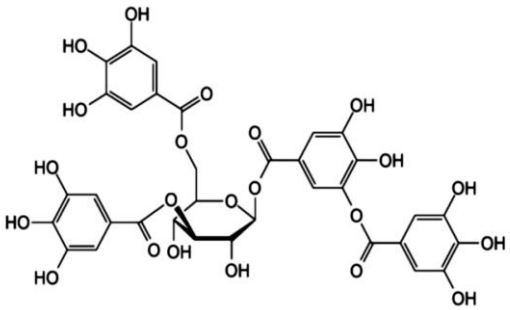


Figure 8. Structure of tannins (Sa'adah, 2010)

The results of the phytochemical test of peel-off gel mask extract with $FeCl_3$ produced a brownish-green color like Figure 8 because the reaction between

tannins and $FeCl_3$ formed a complex compound. The formation of complex compounds between tannins and $FeCl_3$ is due to the presence of Fe^{3+} ions as the central atom and tannins have an O atom that has a lone pair of electrons that can coordinate to the central atom as a ligand (Sa'adah, 2010) (Figure 9).

Before After

Figur 9. Tannins test

Antimicrobials can inhibit or kill microbial growth with relatively little toxicity to humans. Antimicrobials include antibacterial, antifungal, and disinfectant. Antibacterial substances can kill or inhibit the growth of bacteria, so they can be used to prevent and treat bacterial infections such as acne in teenagers. Acne occurs when the skin pores are filled with oil, dead skin cells, and bacteria. Bacteria that cause acne include Propionibacterium acne, and another bacteria that clog hair follicles is Staphylococcus aureus (Winahyu et al., 2021).

Triterpenoids

Triterpenoids are part of antioxidants that have a function to strengthen skin cells and improve skin repair. Triterpenoids can serve essential functions for skin health. Besides that, it can convert alanine and proline into collagen, which treats the skin. Another function is accelerating the healing of post-operative wounds, pimples and black spots on facial skin (Sulastri & Chaerunisaa, 2016). Terpenoid compounds, one of the active compounds, inhibit bacteria by damaging the membrane (Jumrah et al., 2023). The structure of triterpenoids can be seen in Figure 10.

The results of testing the triterpenoid compounds in each extract, namely in the *Caulerpa racemosa* peel-off gel mask, contained triterpenoid compounds characterized by a change in the color produced in each replicate. In this test, the sample was extracted with ethanol, then the filtrate was added with chloroform reagent and concentrated sulfuric acid. The appearance of a brownish color marks a positive result for samples containing triterpenoids (Figure 11).

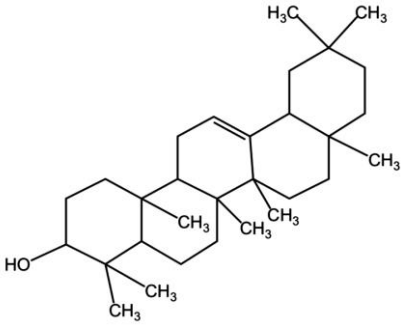


Figure 10. Structure of triterpenoids

Before After

Figure 11. Triterpenoids test

Saponins

Saponins are compounds in the form of widely distributed glycosides that form facial skin collagen tissue, maintain skin oil balance, and do not make facial skin dry (Rahmi & Minerva, 2022). Besides that, saponins are compounds that have anti-inflammatory, analgesic, anti-functional, and cytotoxic effects (Gunawan, 2018). The structure of saponins can be seen in Figure 12.

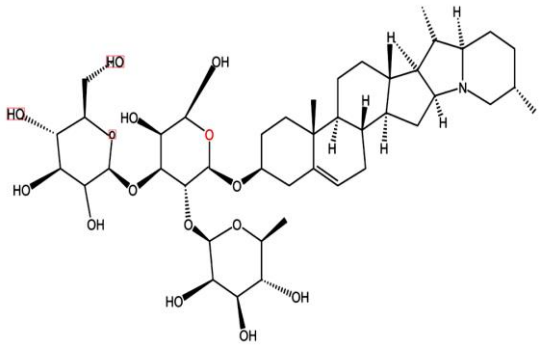


Figure 12.Structure of saponins

The extracted extract was shown two layers were formed. The formation of this layer depends on the density of each n-hexane and extract (saponins). The top layer is clear n-hexane and the bottom is orange

(Liem et al., 2018). This indicates the high antibacterial content in the peel off gel mask, which can form complex compounds with proteins through hydrogen bonds. If a hydrogen bond is formed between the tannins and the protein, the protein will be denatured, disrupting bacterial metabolism (Angelina et al., 2015). Gel mask extract 0,1 g is put into a test tube, then 10 ml of warm or hot water is added and shaken for 30 minutes. Look at the foam and measure how many cm of foam is formed. Leave it for 5 minutes, and if the foam does not disappear, add HCl 2 N. If there is constant foam, it shows a positive result (Figure 13).

Before After

Figure 13. Saponins test

Antibacterials are substances that function to eradicate bacteria and have selective toxicity. Antibacterials can stop or inhibit the growth of microorganisms (bacteria). The number of bacteria becomes stationary, unable to reproduce and multiply.

CONCLUSION

This study concludes that the value of the Total Plate Number (ALT) of the *Caulerpa racemosa* peel-off gel mask complies with the INS standards with bioactive compounds Flavonoids, alkaloids, phenolics, tannins, triterpenoids, and saponins. The highest content of bioactive compounds in the *Caulerpa racemosa* Seaweed Peel-Off Gel Mask are tannins and saponins.

ACKNOWLEDGMENT

The authors thank Organic Chemistry Laboratory, Hasanuddin University, and BPMPP for providing the research facilities.

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