# ประสิทธิภาพของสารสกัดสาหร่าย *Caulerpa racemosa* ต่อการยับยั้งแบคทีเรียก่อโรคบางชนิด

# The Performance of *Caulerpa racemosa* Extracts on Inhibition of Some Pathogenic Skin Bacteria

สุนันทา ข้องสาย [1*] อุทร เจริญเดช [1] และ ชาคริยา ฉลาด [1]

Sunanta Khongsai [1*], Uton Chareondat [1] and Chakriya Chalad [1]

## บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของสารสกัดสาหร่าย *Caulerpa racemosa* ต่อการยับยั้งแบคทีเรียก่อโรคบางชนิด โดยนำสารสกัดหยาบและน้ำสาหร่ายมาทดสอบการยับยั้งแบคทีเรียก่อโรคผิวหนัง ได้แก่ *Staphylococcus aureus* (TISTR 2326), *Staphylococcus epidermidis* (TISTR 518) และ *Pseudomonas aeruginosa* (TISTR 1287) ด้วยวิธี disc diffusion, broth microdilution และ microbicidal activity เทียบกับยาปฏิชีวนะ Streptomycin ผลการทดสอบพบว่าสารสกัดหยาบที่สกัดด้วยตัวทำละลายอินทรีย์ทุกชนิดมีฤทธิ์ยับยั้งการเจริญของแบคทีเรียทดสอบทุกชนิด สารสกัดเอทิลอะซิเตตมีฤทธิ์ยับยั้งการเจริญของ *S. aureus* ดีที่สุด มีขนาดเส้นผ่านศูนย์กลางวงใสของการยับยั้ง 10.12 ± 0.18 มิลลิเมตร มีค่า MIC และ MBC เท่ากับ 6.25 และ 25.00 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ สารสกัดเมทานอลและสารสกัดเฮกเซนมีฤทธิ์ยับยั้งการเจริญของ *S. epidermidis* ดีที่สุด มีขนาดเส้นผ่านศูนย์กลางวงใสของการยับยั้ง 13.03 ± 0.55 และ 13.01 ± 0.37 มิลลิเมตรตามลำดับ มีค่า MIC และ MBC เท่ากับ 0.39 และ 1.56 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ สารสกัดเอทิลอะซิเตตและสารสกัดเฮกเซนมีฤทธิ์ยับยั้งการเจริญของ *P. aeruginosa* ดีที่สุด มีขนาดเส้นผ่านศูนย์กลางยับยั้งวงใสของการ 12.38 ± 0.26 และ 12.07 ± 0.31 มิลลิเมตรตามลำดับ สารสกัดเอทิลอะซิเตตมีค่า MIC และ MBC เท่ากับ 0.78 และ 12.50 มิลลิกรัมต่อมิลลิลิตรตามลำดับ สารสกัดเอทิลอะซิเตตมีค่า MIC และ MBC เท่ากับ 0.78 และ 25.00 มิลลิกรัมต่อมิลลิลิตรตามลำดับ ซึ่งแสดงให้เห็นว่าสาหร่าย *Caulerpa racemosa* เป็นแหล่งทรัพยากรธรรมชาติหนึ่งที่มีศักยภาพในการต้านเชื้อแบคทีเรียการเลือกตัวทำละลายในการสกัดจึงเป็นอีกหนึ่งปัจจัยสำคัญที่ส่งผลต่อการนำสาหร่ายไปพัฒนาเป็นผลิตภัณฑ์ที่มีคุณสมบัติในการยับยั้งเชื้อจุลินทรีย์ก่อโรคผิวหนังได้ในอนาคต

**คำสำคัญ:** *Caulerpa racemosa*, แบคทีเรียก่อโรคผิวหนัง, ความเข้มข้นต่ำสุดในการยับยั้งเชื้อแบคทีเรีย, ความเข้มข้นต่ำสุดในการฆ่าเชื้อแบคทีเรีย

[1] คณะวิทยาศาสตร์และเทคโนโลยีการประมง มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย เลขที่ 179 หมู่ที่ 3 ตำบลไม้ฝาด อำเภอสิเกา จังหวัดตรัง 92150

[1] Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, 179 Moo 3 Maifad, Sikao, Trang 92150, Thailand.

[*] Corresponding author, e-mail: Ta047@hotmail.com

## ABSTRACT

The purpose of this study was to investigate the effectiveness of *Caulerpa racemosa* extracts on the inhibition of some pathogenic skin bacteria. Crude extracts and juice of *Caulerpa racemosa* were tested for their antibacterial activity against pathogenic bacteria (which were *Staphylococcus aureus* (TISTR 2326), *Staphylococcus epidermidis* (TISTR 518), and *Pseudomonas aeruginosa* (TISTR 1287)) by using disc diffusion assay, broth microdilution test and microbicidal activity test compared with Streptomycin. The results revealed that all organic solvent crude extracts of *Caulerpa racemosa* exhibited significant antibacterial activity against all of bacterial strains tested. The ethyl acetate extract showed the highest antibacterial activity against *S. aureus* with the average inhibition zone 10.12 ± 0.18 mm. MIC and MBC values were 6.25 and 25.00 mg/ml, respectively. The methanol extract and hexane extract showed the highest antibacterial activity against *S. epidermidis* with the average inhibition zone 13.03 ± 0.55 and 13.01 ± 0.37 mm., respectively. MIC and MBC values were 0.39 and 1.56 mg/ml, respectively. The ethyl acetate extract and hexane extract showed the highest antibacterial activity against *P. aeruginosa* with the average inhibition zone 12.38 ± 0.26 and 12.07 ± 0.31 mm., respectively. MIC and MBC values were 0.78 (12.50) for ethyl acetate extract and 0.78 (25.00) for hexane extract, respectively. It clearly showed that *Caulerpa racemosa* is a potential source of various antibacterial active marine organisms. Hence, the selection of solvent medium used for extraction is one of the important factor that should be investigated more in future with greater attention in pathogenic skin antibacterial.


**Key words:** *Caulerpa racemosa*, pathogenic skin bacteria, minimum inhibitory concentration, minimal bactericidal concentration

## INTRODUCTION

Marine macroalgae, known as seaweeds, are usually classified into three groups based on their pigmentation, that is, brown (Phaeophyceae), red (Rhodophyceae) and green (Chlorophyceae) algae (Elena, 2013). Seaweeds have valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations (Rajasulochana, 2009). As such, edible seaweeds may be the only relevant dietary source of some of these benefit. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is growing given the number of new diseases and resistant strains of microorganisms (Janarthanan and Kumar 2013). In recent years, seaweeds have served as important sources of bioactive natural substances. Moreover, many metabolites isolated from seaweeds have been shown biological active and potential health benefits (Ratih and Se-Kwon, 2011). Several workers have reported that the seaweed extracts obtained from the brown algae found along the coast of Japan exhibited inhibitory activity against

a number of gram positive and gram negative bacterial pathogens (Mansuya *et al*., 2010). Kandhasamy and Arunachalam (2008) were studied in *vitro* antibacterial property of seaweeds such as *Caulerpa racemosa*, *Ulva lactua*, *Gracillaria folifera*, *Hypneme muciformis*, *Sargassum tenneerinum*, *Sargassum myriocystem* and *Padinatetra stromatica* that collected from Koodankullam village, Tirunelveli district of Tamilnadu, India which exhibited antibacterial activity against gram negative and gram positive pathogenic bacteria. Different active molecules from seaweeds showed antimicrobial activity against pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which commonly cause infection in the human (Chandrasekaran *et al*., 2014).

*Caulerpa racemosa* is a green seaweed that is spread widely throughout tropical regions. In particular, we can be found along in the Andaman coastal area such as Trang, Satun, Krabi and Pang-nga province. Local people was usually used *Caulerpa racemosa* as food. In South East Asian countries, it is usually served raw as salad or eaten cooked. In addition, it is used as animal feed and in folk medicine to reduce blood pressure and to treat rheumatism (Chew *et al*., 2008). More recently, it has been growing interest in marine algae and their ingredients as functional foods and nutraceuticals with potential health benefit effects. Therefore, marine algae are considered as an important source of bioactive ingredients which can be applied to functional foods for antiallergic treatment (Vo *et al*., 2012). Hence, the present study undertaken to investigate antibacterial activity of different organic solvent extracts and juice of *Caulerpa racemosa* were examined against multi-drug resistant standard and clinical bacteria strains to search for the discovery of new antibacterial agents.

## MATERIALS AND METHODS

### Plant Materials

*Caulerpa racemosa* (Forsskål) J. Agardh var. corynephora (Montagne) Weber-van Bosse, 1898 was collected from Palean district, Trang province, Thailand. This seaweed in the form of fresh sample was cleaned well with seawater to remove all the extraneous matter such as epiphytes and other contaminated materials. Samples were then thoroughly washed with tap water followed by distilled water and kept under sunshade until nearly dry followed by oven drying at 50 °C for 24 hours. After drying the sample, it was cut to small pieces.

### Preparation of the crude extract

500 g of small pieces sample were soaked thrice with different solvents like hexane, dichloromethane, ethyl acetate, ethanol and methanol for 5 days. The extracts were filtrated by

**Figure 1** *Caulerpa racemosa*

Whatman No.1 filter paper and the solvent were evaporated under vacuum by ratory evaporator (Heidolph, Germany) at 40 °C and the crude extracts were stored at 4 °C in refrigerator for antibacterial assay.

500 g of small pieces sample were boiled in distilled water for 1 hr. The extract was filtrated by Whatman No.1 filter paper and the solvent was evaporated under vacuum by ratory evaporator (Heidolph, Germany) at 60 °C and freeze-dried. The crude extracts were stored at 4 °C in refrigerator for antibacterial assay.

**Preparation of *Caulerpa racemosa* juice**

1 kg of fresh *Caulerpa racemosa* was squashed and the residues were discarded. The juice was boiled for 1 hour and stored at 4 °C in refrigerator for antibacterial assay.

**Antibacterial Activities**

**Inoculums preparation**

The stocks of pathogenic bacteria, *Staphylococcus aureus* (TISTR 2326), *Staphylococcus epidermidis* (TISTR 518) and *Pseudomonas aeruginosa* (TISTR 1287), obtained from Thailand Institute of Scientific and Technological Research, were used in this trial. All tested inoculums had been produced as $1\times10^6$ CFU/ml through the modified method of Sritunyalucksana *et al*. (2005).

**Disc diffusion assay**

The antibacterial activities of *Caulerpa racemosa* crude extracts and juice were determined by disc diffusion method according to Raheela (2012) with modifications against pathogenic skin bacteria such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Standardized inoculums were introduced on the surface of the plates containing Muller Hinton agar (MHA), which was spread evenly with swab sticks. The aseptic paper discs (6 mm. in diameter; Whatman No.1 filter paper) were impregnated with 20 µl of each extract that concentration was equal to 100 mg/ml in DMSO and DMSO was used as a negative control. The discs were incubated at 37 °C for 18-24 hours. The antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). Three replicate trials were conducted against each organism.

**Broth microdilution test**

All of samplesand streptomycin, the comparative antibacterial agent, were prepared as stock solutions with initial concentration of 100 mg/ml, and diluted with Mueller Hinton broth as 2-fold serial dilution in sterilized 96-well microtiter plate to produce 50 µl of final concentration per well in quadruplicate. Afterwards, 50 µl of inoculums ($1\times10^6$ CFU/ml) were filled in each well and gently mixed with multichannel auto-pipette in order to produce the final concentration of $5\times10^5$ CFU/ml in each well. After mixing, the 96-well microtiter plate were covered with lid and incubated at 35 °C for 24 hr. Then, the turbidity of solutions was checked, and *p*-Iodonitrotetrazolium chloride (INT) was filled in each well to confirm the bacterial growth from discoloration of the mixture. The lowest concentration of the extracts appearing clearness

and no discoloration of the mixture was the minimal inhibitory concentration (MIC) value. In addition, the effect of solvent (DMSO) on bacterial growth was also tested in quadruplicate following aforementioned method, and four wells of inoculums were set up as negative control (Eloff, 1998 modified method).

**Microbicidal activity test**

This test was performed after broth microdilution test in order to evaluate minimal bactericidal concentration (MBC) value through streak plate technique. In this case, one loopful of the clear solution presenting in broth microdilution test was streaked onto agar plates, Mueller Hinton agar (MHB). Afterwards, the agar plates were incubated at 35 °C for 24 hr. The MBC value had been estimated from the appearance of bacterial colony on the agar plates, on which antibacterial agent concentrations were specified. The lowest concentration presenting no bacterial colony was the MBC value (Clinical and Laboratory Standards Institute, 2000).

**Statistical Analysis**

The results are expressed as the mean ± SD. All statistical analyses were performed using statistical software. Comparison of means for antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. P value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

The six different organic solvents of hexane, dichloromethane, ethyl acetate, ethanol, methanol and aqueous extracts including the juice of *Caulerpa racemosa* were tested against skin pathogenic bacterial such as *S. aureus*, *S. epidermidis*, and *P. aeruginosa*. All of *Caulerpa racemosa* extracts and juice possessed significant antibacterial activity against all the bacterial strains tested. The mean values of inhibition zone are presented in Table 1.

**Table 1** Inhibition zone of *Caulerpa racemosa* extracts and juice.

| Sample | | Inhibition Zone (mm) | | |
|---|---|---|---|---|
| | | ***S. aureus*** | ***S. epidermidis*** | ***P. aeruginosa*** |
| Extract | Hexane | $8.96 \pm 0.15^{b}$ | $13.01 \pm 0.37^{a}$ | $12.07 \pm 0.31^{a}$ |
| | Dichloromethane | $8.93 \pm 0.35^{b}$ | $10.62 \pm 0.37^{b}$ | $10.42 \pm 0.36^{b}$ |
| | Ethyl Acetate | $10.12 \pm 0.18^{a}$ | $10.41 \pm 0.33^{b}$ | $12.36 \pm 0.26^{a}$ |
| | Ethanol | $8.91 \pm 0.15^{b}$ | $8.28 \pm 0.19^{d}$ | $7.18 \pm 0.45^{c}$ |
| | Methanol | $8.90 \pm 0.33^{b}$ | $13.03 \pm 0.55^{a}$ | $8.93 \pm 0.27^{c}$ |
| | Water | $8.87 \pm 0.22^{b}$ | $8.95 \pm 0.09^{c}$ | $7.18 \pm 0.33^{c}$ |
| *C. racemosa* juice | | $6.14 \pm 0.12^{c}$ | $6.20 \pm 0.17^{e}$ | $6.28 \pm 0.09^{d}$ |
| DMSO | | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{f}$ | $0.00 \pm 0.00^{e}$ |

*Note*: - The values are represented by mean ± SD, n = 3.

Values within a column by the same letter are not significantly different (p>0.05).

When the different *Caulerpa racemosa* extracts and juice were assayed against pathogenic bacteria by disc diffusion method, the mean zone of inhibition obtained were between 6.14 ± 0.12 to 13.03 ± 0.55 mm. The ethyl acetate extract showed the highest antibacterial activity against *S. aureus* with the average inhibition zone 10.12 ± 0.18 mm., MIC and MBC values of 6.25 and 25.00mg/ml respectively. The methanol extract and hexane ex tract showed the highest antibacterial activity against *S. epidermidis* with the everage inhibition zone 13.03 ± 0.55 and 13.01 ± 0.37 mm., respectively, MIC and MBC values of 0.39 and 1.56 mg/ml respectively. Finally, the ethyl acetateex tract and hexane extract showed the highest antibacterial activity against *P. aeruginosa* with the everage inhibition zone 12.36 ± 0.26 and 12.07 ± 0.31 mm., respectively, MIC and MBC values of 0.78 (12.50) for ethyl acetateex tract and 0.78 (25.00) for hexane extract, respectively. as shown in Table 2.

**Table 2** Minimum inhibitory concentration of *Caulerpa racemosa* extracts and juice.

| Sample | | Minimum inhibitory concentration (mg/ml) | | |
|---|---|---|---|---|
| | | ***S. aureus*** | ***S. epidermidis*** | ***P. aeruginosa*** |
| Extract | Hexane | 12.50 | 0.39 | 0.78 |
| | Dichloromethane | 12.50 | 1.56 | 1.56 |
| | Ethyl Acetate | 6.25 | 1.56 | 0.78 |
| | Ethanol | 12.50 | 25.00 | 25.00 |
| | Methanol | 12.50 | 0.39 | 25.00 |
| | Water | 12.50 | 12.50 | 25.00 |
| *C. racemosa* juice | | > 50.00 | > 50.00 | > 50.00 |
| Streptomycin | | 0.19 | 0.43 | 3.12 |

**Table 3** Minimal bactericidal concentration of *Caulerpa racemosa* extracts and juice.

| Sample | | Minimal bactericidal concentration (mg/ml) | | |
|---|---|---|---|---|
| | | ***S. aureus*** | ***S. epidermidis*** | ***P. aeruginosa*** |
| Extract | Hexane | 25.00 | 1.56 | 25.00 |
| | Dichloromethane | 50.00 | 6.25 | 25.00 |
| | Ethyl Acetate | 25.00 | 12.50 | 12.50 |
| | Ethanol | > 50.00 | 50.00 | > 50.00 |
| | Methanol | > 50.00 | 1.56 | 50.00 |
| | Water | > 50.00 | 25.00 | > 50.00 |
| *C. racemosa* juice | | > 50.00 | > 50.00 | > 50.00 |
| Streptomycin | | 0.39 | 0.39 | 25.00 |

Many studies were reported about the biological activities of algal extracts from different coastal regions around the world (Chandrasekaran *et al*., 2014). In the present study, the different organic solvent such as hexane, dichloromethane, ethyl acetate, ethanol, methanol and aqueous extracts of *Caulerpa racemosa* possessed antibacterial activity against all of pathogenic skin bacteria strains tested. The hexane and methanol extract of *Caulerpa racemosa* showed the highest antibacterial activity than other extracts against *S. epidermidis* exhibited more inhibitory activity indicating the effective extraction than the other extracts. The absence of antibacterial activity in some extracts and juice indicated the insolubility of the active substances in these solvents (Shankar *et al*., 2010).

The variation in antibacterial activity may be due to the method of extraction, solvents used in extraction and season at which samples were collected (Chandrasekaran *et al*., 2014) and the different drying techniques was including (Angelina, *et al*. 2015). The extraction method is also an important factor that can affect the efficacy of the produced extracts and many of the prior researchs, the organic solvents were used for the extraction rather than water (Ioannis and Celine, 2015). Since the different extract of *Caulerpa racemosa* showed the different potential antibacterial activity against all of pathogenic skin bacteria strains tested. Moreover, it was indicated the potential source of a variety of biologically active marine organisms and it is hope that the present results will provide a starting point for investigations aimed at exploiting new natural antibacterial substances present in *Caulerpa racemosa*.

## CONCLUSION

The present investigation brings out adequate data on the antibacterial potential of different extract of *Caulerpa racemosa* according different pathogenic skin bacteria. Further research studies are being carried out on the other bioactive properties of *Caulerpa racemosa* in order to provide complete data. As such, the use of *Caulerpa racemosa* as natural antibacterial sources appears promising and should be investigated further.

## ACKNOWLEDGEMENTS

Funding for this research was provided by Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang, Thailand.

## REFERENCE

Angelina, L.M.L., Suhaimi, Y., Patricia, M. and Mohd, F.A.B. 2015. Effect of different drying techniques on the phytochemical contentand antioxidant activity of *Kappaphycus alvarezii*. **Journal of Applied Phycology** 27: 1717-1723.

Chandrasekaran, M., Venkatesalu, V., Raj, G.A. and Krishnamoorthy, S. 2014. Antibacterial properties of various extracts of *Sargassum wightii* against multidrug resistant bacterial strains. **Phykos** 44(2): 17-28.

Chew, Y.L., Lima, Y.Y, Omara, M. and Khoo, K.S. 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. **Food Science and Technology** 41: 1067-1072.

Clinical and Laboratory Standards Institute. 2000. **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Growth Aerobically; Approved Stand 5th Edition.** Clinical and Laboratory Standards Institute

Elena, M., Conde, B.E., Moure, A., Falqué, E. and Domínguez, H. 2013. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. **Food Chemistry** 138: 1764-1785.

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. **Planta Medica** 64: 711-713.

Ioannis, N.V. and Celine, R. 2015. Seaweed extracts as antimicrobial agents in aquaculture. **Journal of Applied Phycology** 27: 2017-2035.

Janarthanan, M. and Kumar, S.M. 2013. Qualitative and quantitative analysis of phytochemical studies on selected seaweeds acanthopora spicifera and sargassum wightii. **International Journal of Engineering Research and Development** 7(3): 11-15

Kandhasamy, M. and Arunachalam, K.D. 2008. Evaluation of in vitro antibacterial property of seaweeds of Southeast coast of India. African Journal of Biotechnology 7(12): 1958-1961.

Mansuya, P., Aruna, P., Sridhar, S., Kumar, J.S. and Babu, S. 2010. Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from gulf of Mannar region. **Journal of Experimental Sciences** 2(8): 23-26.

Rajasulochana, P., Dhamotharan, R. and Krishnamoorthy, P. 2009. Primary phytochemical analysis of *Kappaphycus* sp. **Journal of American Science** 5(2): 91-96.

Raheela, K., Asia, N., Erum, N., Huma, S. and Ghazala, H.R. 2012. Antibacterial, antimycelial and phytochemical analysis of *Ricinus communis* Linn, *Trigonella-foenumgrecum* Linn and *Delonix regia* (Bojer ex Hook.) Raf of Pakistan. **Romanian Biotechnological Letters** 17(3): 7237-7244.

Ratih, P. and Se-Kwon, K. 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. **Journal of Functional Foods** 3(3): 255-266.

Shankar, S.R., Rangarajan, R., Sarada, D.V.L. and Kumar, C.S. 2010. Evaluation of antibacterial activity and phytochemical screening of *Wrightia tinctoria* L. **Pharmacognosy Journal** 2(14): 19-22.

Sritunyalucksana, K., Gangnonnigw, W., Archakunakorn, S., Fegan, D. and Flegel, T.W. 2005. Bacterial clearance rate and a new differential hemocyte staining method to assess immunostimulant activity in shrimp. **Diseases of Aquatic Organisms** 63: 89-94.

Vo, T.S., Ngo, D.H. and Kim, S.K. 2012. Marine algae as a potential pharmaceutical source for anti-allergic therapeutics. **Process Biochemistry** 47: 386-394.