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EFEKTIVITAS ANTI-BAKTERI DARI EKSTRAK *Cymodocea rotundata* DAN MENILAI KOMPOSISI BIOAKTIF UTAMA

ANTI-BACTERIAL EFFECTIVENESS OF *Cymodocea rotundata* EXTRACT AND ASSAY FOR PRIMARY BIOACTIVE COMPOSITION

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Abstrak

Antibakteri merupakan suatu senyawa yang dapat digunakan untuk mengambat pertumbuhan bakteri. Adapun senyawa yang berperan dalam merusak dinding sel antara lain fenol, flavonoid, dan alkaloid. Senyawa fitokimia di atas berpotensi sebagai antibakteri alami pada bakteri patogen, contohnya terhadap *Escherichia coli*. *Escherichia coli* merupakan mikroba yang bersifat patogen pada manusia yang dapat menyebabkan gangguan pencernaan serta mengganggu sistem kerja dari organ lambung. Lamun *C. rotundata* mempunyai senyawa yang bersifat sebagai antibakteri yaitu, alkaloid, flavonoid, phenol, steroid dan tannin. *C. rotundata* dapat ditemui di perairan Indonesia. Namun, belum banyak dimanfaatkan. Penelitian ini bertujuan untuk mengetahui perbedaan konsentrasi dari ekstrak lamun *C. rotundata* terhadap aktifitas Antibakteri *E. coli*. Metode penelitian yang digunakan yaitu experimental laboratories dengan perbedaan konsentrasi ekstrak lamun (10%, 20%, 30%, dan 40%). Hasil penelitian menunjukkan bahwa Ekstrak lamun *C. rotundata* efektif sebagai antibakteri dengan kategori sedang yaitu zona hambat berkisar antara 5-10 mm. Berdasarkan penelitian yang dilakukan, masa inkubasi 72 jam pada konsentrasi 40% merupakan konsentrasi terbaik untuk mencegah *E. coli* pada zona hambat 8,5 mm Selanjutnya, senyawa bioaktif yang dihasilkan oleh *C. rotundata* yaitu senyawa flavonoid dengan menunjukkan perubahan warna larutan menjadi kuning jingga. Selain itu, juga menghasilkan senyawa bioaktif Fenol dengan menunjukkan perubahan warna larutan menjadi kehijauan, dan juga menghasilkan senyawa bioaktif Tanin dengan menunjukkan perubahan warna larutan menjadi hijau kehitaman. Hasil penelitian menunjukkan bahwa *C. rotundata* dapat dijadikan acuan untuk pengembangan obat antibakteri di masa depan.

Kata Kunci: Anti-bakteri, Lamun, Senyawa Bioaktif

Abstract

Anti-bacterial is a compound that can be used to inhibit bacterial growth. The compounds that play a role in damaging the cell membrane are phenols, flavonoids, and alkaloids. The phytochemical compounds above have the potential as natural Anti-bacterial on pathogenic bacteria, for example against *Escherichia coli*. *Escherichia coli* is a pathogenic microbe in humans that can cause digestive disorders and disrupt the work system of the stomach organs. Seagrass *C. rotundata* has compounds that are antibacterial, such as alkaloids, flavonoids, phenols, steroids, and tannins. *C. rotundata* can be seen in Indonesian waters. However, it has not been widely used. This study aims to determine the difference in concentration of seagrass extract of *C. rotundata* against *E. coli* anti-bacterial activity. The research method used was experimental laboratories with different concentrations of seagrass extract (10%, 20%, 30%, and 40%). The results showed that the seagrass extract of *C. rotundata* was effective as an antibacterial with a middle category, which is the inhibition zone ranging from 5-10 mm. Based on the studies conducted, 72 hours incubation period at 40% concentration was the best concentration to prevent E. coli at the 8.5 mm inhibition zone. Furthermore, bioactive compounds produced by *C. rotundata* are flavonoid compounds by showing changes in the color of the solution to yellow-orange. In addition, also produces phenol bioactive compounds by showing a change in the color of the solution to greenish, and also produces tannin bioactive compounds by showing a change in the color of the solution to blackish green. The results showed that *C. rotundata* can be used as a recommendation for the development of Anti-bacterial drugs in the future.

Keywords: Anti-bacterial, Seagrass, Bioactive component

INTRODUCTION

Seagrasses are plants which live and are submerged in the sea; they are vascular, leafy, rhizome, rooted, and spread reproductively (seeds) and vegetatively. The word seagrass appeared in America in the 1960s and in Europe in the 1970s when research using the word seagrass was published (Poli *et al*., 2022). In fact, tens or even hundreds of years ago, English names (common names) for seagrass species emerged that were adapted to certain animals' morphology or diet. In Indonesian waters, seagrasses usually grow in the intertidal zone and around coral islands (Unsworth *et al*., 2018). It grows on silt, silty sand, sand, and crushed coral substrats. Worldwide there are 60 seagrass species consisting of 2 families and 12 genera (Kuo and McComb 1989). In Indonesian waters, there are 15 species consisting of 2 families and 7 genera. There are 12 types of algae namely *Enhalus acoroides, Thalassia hemprichii, Cymodocea rotundata, Cymodocea serrulata, Haludole pinifolia, Halodule uninervis, Halophila decipiens, Halophila ovalis, Halophila minor, Halophila spinulosa, Syringodium iseotifolium,* and *Thalassodendron ciliatum* (Mariño *et al*., 2019).

Some seagrass species have different activities, such as antibacterial activity. Anti-bacterial is a compound that can be used to inhibit bacterial growth. The compounds that play a role in damaging cell walls include phenols, flavonoids, and alkaloids. The phytochemical compounds above have the potential as a natural anti-bacterial on pathogenic bacteria, for example against *Escherichia coli*. *Escherichia coli* is a pathogenic microbe in humans that can cause digestive disorders and disrupt the work system of the stomach organs (Kim *et al*., 2021).

One species of algae that has anti-bacterial activity is *C. rotundata*. *C. rotundata* was derived from the family of Cymodoceaceae. *C. rotundata* is widespread along the coasts of the Indian Ocean and western Pacific and stretches along the coast of Africa from the Red Sea south to Maputo and Madagascar. *C. rotundata* is also found along the southern coast of the Indian subcontinent, the Andaman and Nicobar Islands, and extends into the western Pacific (Bismarck, Caroline, New Caledonia, and Queensland Islands) and its marginal seas, including the Gulf of Thailand, Vietnam, and the Ryukyu Islands (Dilipan & Arulbalachandran., 2022). *C. rotundata* is widespread in Lampung, Riau Islands, Peninsular Malaysia, Java, Lesser Sunda Islands, Maluku, Philippines, Borneo, and New Guinea (Ambo-Rappe *et al*., 2019). *C. rotundata* seagrass has functions and uses for water and humans. This species acts as a substrate for many marine organisms, can stabilize bottom sediments to make the water clearer, and plays an important role in nutrient cycling in the marine environment. In addition, algae can be a source of antibacterial because they contain bioactive compounds such as alkaloids, flavonoids, phenols, steroids, and tannins. But so far it has not been widely used. Therefore, this study aimed to determine the different concentrations of seagrass extract *C. rotundata* on *E. coli* antibacterial activity. Thus, it can be used as a reference for the development of antibacterial drugs in the future (Kim *et al*., 2020).

MATERIAL AND METHODS

Materials

The materials used in this study were seagrass *C. rotundata*, ethanol 96%, aluminum foil, distilled water, plastic wrap, cotton, label paper, and a filter. The primary tools used in this study included glass jars, rotary evaporator (RV 10 Digital V), autoclave (Tomy ES-215), Laminar air flow (Thermos Fisher Scientific), and incubator (Thermos Fisher Scientific).

Method

Sample preparation

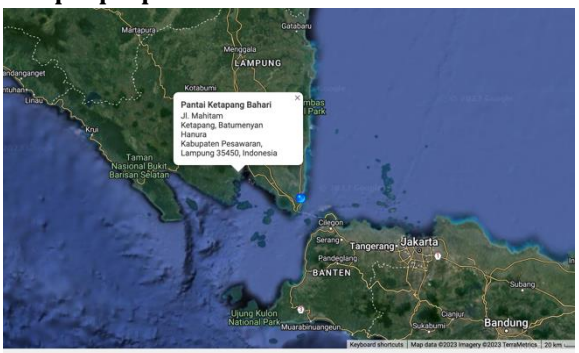

Figure 1. Location of study

Seagrasses were collected from Ketapang Sea, Lampung. We used Seagrass of as much as 500 g. The processing of seagrass samples was done by washing the samples using seawater to remove the embedded sand and then washing again using fresh water to remove the dirt that was still sticking to the samples. The samples were then dried by breezing at room temperature for 5 days. The dried samples were then chopped 2-3 cm, then were crushed using a blender to make it easier during the extraction process.

The bacterial cultivation of *E. coli*

The first step was to prepare the tools and materials. Measuring Nutrient Agar (Oxoid) as much as 5.6 grams with an analytical scale. Dissolved Nutrient Agar (Oxoid) into 200 ml of distilled water in an Erlenmeyer, mixed until homogeneous, and then sealed using aluminum foil. The next stage was to put the

Erlenmeyer containing the agar solution and petri dish at 121°C for 20 minutes in an autoclave. After the autoclave was completed, turn on the laminar airflow and place the Erlenmeyer that contained the agar solution into the LAF to be sterilized using UV for 15 minutes. Furthermore, pour the agar into the Petri dish in a volume of approximately 2 ml and let it solidify. After that, put the bacterial inoculant into the agar media for as much as 1 l using a micropipette and spread it on the media using a spreader in laminar airflow. Petri dishes that contained cultured bacteria were isolated with plastic wrap and labeled with a marker pen. Incubate the isolated in an incubator at 37°C for five days.

The next stage is to examine the petri dish that has been colonized with E. coli bacteria. We made a Nutrient Broth (Oxoid) with a ratio of 2.6 grams of agar and 200 ml of distilled water in an Erlenmeyer. Autoclave the Erlenmeyer and test tubes at 121°C for 20 minutes. After the autoclave was complete, turn on the laminar airflow and place the Erlenmeyer contained Nutrient Broth solution into the LAF to be sterilized by UV for 15 minutes. Furthermore, we poured approximately 10 ml of Nutrient Broth solution into a test tube. Subsequently, isolate the bacteria from the petri dish into a test tube with an ose syringe aseptically. Seal the test tube with cotton or aluminum foil and label it with a label or marker. Place the test tube into an incubator at 37°C for 5 days. *E. coli* bacteria samples are already used in this study.

Seagrass extraction

The extraction method that was used in this study is maceration. Maceration was carried out with 96% ethanol solvent. The principle of this method is done by using samples that are soaked using a solvent that is polar. During the maceration, the shaking was done every 10 minutes once a day for 5 days as referred to by Hernán *et al*., (2022). The first step, making a 70% ethanol solution by diluting 96% ethanol with distilled water with a ratio of 729 ml ethanol and 271 ml distilled water and insert into a glass jar. The mashed seagrass was weighed as much as 500 grams and then immersed with 70% ethanol into a glass jar. Glass jars are kept in a sterile room or place protected from direct daylight. After 5 days, seagrass was filtered and put into an evaporating flask. The products of maceration were then evaporated with a rotary evaporator at 40°C at 100 rpm. After the extract is obtained, the subsequent phytochemical test of the seagrass extract is carried out.

Analysis of the anti-bacterial activity of seagrass against *Escherichia coli*

This study used different concentrations of extracts. The extract concentrations used were 10%, 20%, 30%, and 40% with an incubation time of 72 hours.

Phytochemical measurements

1. Flavonoid measurement procedure

This study used four treatments. Samples of seagrass extract *C. rotundata* were weighed as much as 5 grams, then dissolved in 100 ml of distilled water. The solution was then homogenized with a centrifuge and then filtered to separate the sediment and filtrate. The filtrate was then taken as much as 1 mL, 2 mL, 3 mL, and 4 mL then each added 3 mL of 5% AlCl3 solution was and then added and distilled water to a volume of 10 mL Observe the color change in the solution. Positive results contain flavonoid compounds if the solution turns green and orange-yellow (Tian *et al*., 2019).

2. Tannin measurement procedure

This study used four treatments. Samples of seagrass extract *C. rotundata* weighed as much as 5 grams and were then dissolved in 100 mL of distilled water. The solution was then shaken until homogeneous then homogenized with a centrifuge and then filtered to separate the sediment and filtrate. The filtrate was then taken as much as 1 mL, 2 mL, 3 mL, and 4 mL then added 0.5 mL of Follin Denis (Follin 1: 1) and 1 mL of saturated NaCO3 solution. The solution was then added with distilled water up to a volume of 10 mL and homogenized with a vortex until homogeneous. Observe the color change in the solution. Positive results contain tannin compounds if the solution turns into a clear greenish and yellow color (Sepperer *et al*., 2019).

3. Phenol measurement procedure

This study used four treatments. Samples of seagrass extract *C. rotundata* weighed as much as 5 grams and were then dissolved in 100 mL of distilled water. The solution was then homogenized with a centrifuge and then filtered to separate the sediment and filtrate. The filtrate was then taken as much as 1 mL, 2 mL, 3 mL, and 4 mL added 0.5 mL Follin Denis (Follin 1:1) and 1 mL saturated Na2CO3 solution, then allowed to stand for 10 minutes. The solution was then added with distilled water up to a volume of 10 mL and centrifuged until homogeneous. Observe the color change in the solution. Positive results contain phenol compounds if the solution turns greenish, yellow, and brown (Ghomari *et al*., 2019).

Anti-bacterial activity

The antibacterial activity test was carried out by making agar media, namely nutrient agar (NA). The medium that has been prepared is sterilized by autoclave with a temperature of 121oC for 20 minutes. The next step is making test medium and making wells which are done by making wells using a dropping pipette as many as two wells and then naming them K- and K+. Furthermore, 1µl of *C. rotundata* seagrass extract was added to the K+ well with different concentrations of 10%, 20%, 30%, and 40%, then incubated at 37^{o}C. Observation and measurement of the inhibition zone were done at 72 hours of incubation time. The negative control test was carried out using the solvent used to make extract concentrations aqua dest.

RESULTS

Phytochemical composition of the seagrass of *C. rotundata*.

The results of phenol, tannin, and flavonoid tests on seagrass extracts of C. *rotundata* with ethanol solvent can be seen in Table 1. Based on Table 1. Seagrass C. *rotundata* is positive for flavonoid compounds. The results of phenol, tannin, and flavonoid tests on seagrass extracts of C. *rotundata* with ethanol solvent can be seen in Table 1. Based on Table 1. Seagrass C. *rotundata* positively contains flavonoid compounds. These qualitative test results show this. In 1 ml of seagrass extract, there is a color change from green to orange-yellow, while in the 2 ml sample of seagrass extract, there is a color change from green to bright green, whereas for the 3 ml sample of seagrass extract, there is a color change from green to deep green, and for the 4 ml sample of seagrass extract there is a color change from green to orange-yellow. The color change from green to orange-yellow and deep green is the key to identifying that the seagrass used as a sample of this study has flavonoid bioactive compounds. Based on Table 2. C. *rotundata* certainly contains tannins and phenol compounds. This is shown by the results of qualitative tests. In the phenol and tannin test in 1 ml of seagrass extract, there was a change in color from green to greenish, then in the phenol test in 2 ml of seagrass extract there was a color change from green to brown, but during the tannin test, the color changed from green to translucent yellow. Whereas for the phenol test and tannin sample of 3 ml of seagrass extract, there was a change in color from green to translucent yellow, and for the phenol test and tannin sample of 4 ml of seagrass extract there was a change in color from green to greenish. The change in color from green to greenish and translucent yellow indicates that the seagrass used as the sample for this study has phenol and tannin bioactive compounds.

Anti-bacterial activity test

The results showed that at 72 hours of isolation, the different diameters of seagrass extract in each treatment formed a bacteriostatic zone. The zone of inhibition was not seen at 24 and 48 hours of isolation. The maximum inhibition zone that occurred at 40% extraction dose was 8.5 mm. The minimum zone of inhibition formed at a dose of 10% was 6 mm.

Table 1. Test Results of Flavonoid of seagrass *C. Rotundata*

Treatment	Composition	Result
1	1 ml seagrass extract + 3 ml $AlCl_3$ + 6 ml aquadest	Shown in an orange-yellow color
2	2 ml seagrass extract + 3 ml $AlCl_3$ + 5 ml aquadest	Shown in bright green color
3	3 ml seagrass extract + 3 ml $AlCl_3$ + 4 ml aquadest	Shown in deep green color
4	4 ml seagrass extract + 3 ml $AlCl_3$ + 3 ml aquadest	Shown in an orange-yellow color

Table 2: Test Results of Tannins and Phenols of seagrass *C. Rotundata*

Treatment	Composition	Result
1	1 ml seagrass extract + 0,5 ml follin denis + 8,5 ml aquadest	Phenols: Shown in greenish color Tannin: Shown in greenish color
2	2 ml seagrass extract + 0,5 ml follin denis + 7, 5 ml aquadest	Phenols: Shown in brown color Tannin: Shown in translucent yellow color
3	3 ml seagrass extract + 0,5 ml follin denis + 6,5 ml aquadest	Phenols: Shown in translucent yellow color Tannin: Shown in translucent yellow color
4	4 ml seagrass extract + 0,5 ml follin denis + 5,5 ml aquadest	Phenols: Shown in greenish color Tannin: Shown in greenish color

Table 3. The result of Antibacterial Activity Test

Treatment	Comncentration of Extraction	Inhibition Diameter Results
1	10%	6 mm
2	20%	7,5 mm
3	30%	8 mm
4	40%	8,5 mm

DISCUSSION

Based on the results of the Phytochemical composition of the seagrass of C. *rotundata*. Extract of C. *rotundata* with methanol solvent showed a positive response to the qualitative test of flavonoids (Table 1) with a change in the color of the sample. This shows that all extracts contain reducing sugars and bioactive compounds belonging to the flavonoid class. Flavonoid compounds are also found in extracts of *Thalassia testudiunum* (Jensen *et al.,* 1998). Another researcher Qi *et al.* (2008) also succeeded in isolating four flavonoid compounds and five steroid compounds from Enhalus acoroides extract. Flavonoids are polar compounds, so generally soluble in ethanol, methanol, butanol, acetone, dimethylsulfoxide, dimethylformamide, and water (Tajik *et al*., 2019). This is confirmed by Guedes *et al*., (2020) that ethanol solvent is a universal solvent with a high polarity index.

Flavonoids are natural pigment compounds that are yellow to colorless, soluble in water, and resistant to heat (Sillero *et al*., 2021). Flavonoids are normallynormally synthesized by flora in response to microbial infection (Dzoyem *et al*., 2018). The mechanism of antibacterial action is the utilization of soluble cell extract proteins and microbial walls (Godoy-Gallardo *et al*., 2021). Another possibility is that flavonoids play a specific role by interfering with microbial cell function and inhibiting the microbial cell cycle (Li *et al*., 2022).

In addition to flavonoids, other bioactive compounds such as phenols and tannins were also found in the phytochemical content test in C *rotundata* extract as shown in table 2 with a change in the color of the sample.

According to study from Mani *et al.,* (2012) are *Syringodium isoetifolium* extract was reported to contain phenolic and alkaloid chemical compounds, and C. *rotundata* extract contained alkaloid chemical compounds.

C*. rotundata* extract is said to contain tannin and phenol compounds that can prevent bacterial growth and even inactivate bacteria (Otmani *et al*., 2021). According to research (O'Connor *et al*., 2022), 95% of seagrass ethanol extracts are known to contain tannins. The use of 96% ethanol as a solvent in the extraction of biologically active compounds because ethanol is beneficial for the extraction of tannin and phenol antibacterial compounds, as ethanol more easily penetrates cell membranes to extract intracellular substances. The seagrass phytochemical composition showed that ethanol solvent made the seagrass extract of *C. rotundata* contain tannin compounds. This secondary metabolite is characterized by antibacterial compounds.

Tannins are secondary metabolites in plants that have antibacterial properties, have the ability to treat skin diseases, and are also known as astringents. According to (Bading Taika *et al*., 2018), tannins can be used as anti-inflammatory agents, antidiarrheal, treatment of skin and oral infections, and treatment of burns, based on their antimicrobial properties. Therefore, tannins can be used in the field of medicine as antibacterials. The next compound that is also contained in *C. rotundata* seagrass extract is phenol compounds. Phenols, compounds produced by marine flora, algae, and invertebrates, are used to repel predators and act as highly effective antimicrobials because they cause protein denaturation and cell death. (Stamogiannou *et al*., 2021) indicates that phenols also work through protein coagulation and damage cell membranes. Depending on the concentration used, phenolic compounds can have both bactericidal and bacteriostatic characteristics. The presence of chemical compounds belonging to the flavonoid, phenol, and tannin classes in the crude extract of C. rotundata in this study indicates that this species of seagrass has potential as a natural antifouling, antibacterial, antifungal, and other pharmaceutical raw material (Bading Taika *et al.,* 2018; Cho *et al.,* 2019).

Based on the results of the Antibacterial activity test showed the existence inhibition of E. coli bacteria in all isolates of C. *rotundata* extract with inhibition diameters ranging from 6 mm to 8.5 mm. This can be seen in Table 3. In the second stage of the inhibition test ethyl acetate was used as a negative control, and showed no inhibition. This inhibition is thought to be due to the presence of antibacterial compounds secreted by C. *rotundata* on the media agar as a secondary metabolite product of C. *rotundata*. The more antibacterial compounds that are excreted, the greater the inhibition (Xu *et al*., 2022).

The formation of the inhibition zone indicates that C. *rotundata* extract has the potential as a source of antibiotics in natural materials. Bacteriostatic results are classified as medium-diameter bacteriostatic zones. This is in accordance with (Cho *et al.,* 2019) that the higher the concentration of C. *vulgaris* extract, the higher the inhibitory effect on pathogens. E. *coli* has a more complex cell wall structure than *Staphylococcus aureus.*

This is also confirmed by the statement (Hajdinjak *et al.,* 2019) E. *coli* is a Gram (-)-negative bacterium that is resistant to several antimicrobial agents. The cell wall of Gram (-) Gram-negative bacteria contains three polymer layers, outer lipoproteins, middle lipopolysaccharides, and inner peptidoglycans, and the outer membrane is a double-layer structure (good resistance to compounds entering or leaving the cell and causing toxins). Xu *et al.,* (2022) add that basically, the most

denatured cell walls are those composed of polysaccharides compared to those composed of phospholipids. Gram-positive cell walls contain peptidoglycan and teichoic and teichuronic acids. Therefore, the cell wall of Gram (+) bacteria is partially polysaccharide. Whereas in the cell wall of gram-negative bacteria, there is very little peptidoglycan and is between the outer membrane and the inner membrane of the cell wall. The outer cell wall of Gram (-) bacteria is a component consisting of phospholipids and several proteins that are often referred to as auto layers. It can be concluded that Gram (+) gram-positive bacteria experience the cell denaturation process first compared to Gram (-) bacteria.

CONCLUSIONS

Based on the results of phytochemical tests, seagrass extract contains flavonoids, tannins, and phenol compounds. Seagrass extract concentration and incubation time significantly affected the antibacterial activity of C. *rotundata* against E. coli bacteria. The antibacterial effect of C. *rotundata* was classified as the medium because the inhibition zone was 5-10 mm. Based on the studies conducted, 72 hours incubation period at 40% concentration was the best concentration to prevent E. coli at the 8.5 mm inhibition zone. These results showed that C. *rotundata* extract can be used as an antibacterial agent.

CONCLUSIONS

Based on the results of phytochemical tests, seagrass extract contains flavonoids, tannins, and phenol compounds. Seagrass extract concentration and incubation time significantly affected the antibacterial activity of *C. rotundata* against *E. coli* bacteria. The antibacterial effect of *C. rotundata* was classified as the medium because the inhibition zone was 5-10 mm. Based on the studies conducted, 72 hours incubation period at 40% concentration was the best concentration to prevent *E. coli* at the 8.5 mm inhibition zone. These results showed that *C. rotundata* extract can be used as an antibacterial agent.

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REFERENCES

Ambo-Rappe, R., & Moore, A. M. 2019. Sulawesi Seas, Indonesia. World Seas: An Environmental Evaluation (Second Edition), 559-581. https://doi.org/10.1016/B978-0-08-100853-9.00032-4

Bading Taika, B., Bouckandou, M., Souza, A., Bourobou Bourobou, H., MacKenzie, L., & Lione, L. 2018. An overview of anti-diabetic plants used in Gabon: Pharmacology and toxicology. Journal of Ethnopharmacology, 216, 203-228. https://doi.org/10.1016/j.jep.2017.12.036

Cho, C., Zhao, Y., & Yun, Y. 2019. QSAR modelling for predicting adsorption of neutral, cationic, and anionic pharmaceuticals and other neutral compounds to microalgae Chlorella vulgaris in aquatic environment. Water Research, 151, 288-295.https://doi.org/10.1016/j.watres.2018.12.033

Dilipan, E., & Arulbalachandran, D. 2022. Genetic diversity of seagrass Cymodocea species as an ecological indicator on the Palk Bay Coast, India. Ecological Genetics and Genomics, 23, 100119. https://doi.org/10.1016/j.egg.2022.100119

Dzoyem, J., Tchamgoue, J., Tchouankeu, J., Kouam, S., Choudhary, M., & Bakowsky, U. 2018. Antibacterial activity and cytotoxicity of flavonoids compounds isolated from Pseudarthria hookeri Wight & Arn. (Fabaceae). South African Journal of Botany, 114, 100-103. https://doi.org/10.1016/j.sajb.2017.11.001

Ghomari, O., Sounni, F., Massaoudi, Y., Ghanam, J., Drissi Kaitouni, L. B., Merzouki, M., & Benlemlih, M. 2019. Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnology Reports, 23, e00347. https://doi.org/10.1016/j.btre.2019.e00347

Godoy-Gallardo, M., Eckhard, U., Delgado, L. M., de Roo Puente, Y. J., Hoyos-Nogués, M., Gil, F. J., & Perez, R. A. 2021. Antibacterial approaches in tissue engineering using metal ions and nanoparticles: From mechanisms to applications. Bioactive Materials, 6(12), 4470-4490. https://doi.org/10.1016/j.bioactmat.2021.04.033

Guedes, A. R., de Souza, A. R. C., Turola Barbi, R. C., Nottar Escobar, E. L., Zanoello, É. F., & Corazza, M. L. 2020. Extraction of Synadenium grantii Hook f. using conventional solvents and supercritical CO2 + ethanol. The Journal of Supercritical Fluids, 160, 104796. https://doi.org/10.1016/j.supflu.2020.104796

Hajdinjak, T., Wergner, A., Prammer, W., Rigler-Hohenwarter, K., & Pelzer, A. 2019. Rectal swab cultures prior to transrectal prostate biopsy: Among Gram-negative isolates, in 42% of samples non-E.coli species are present. European Urology Supplements, 18(1), e55. https://doi.org/10.1016/S1569-9056(19)30040 -5

Jensen PR, Jenlins KM, Porter D, Fenical W. 1998. Evidence that a new antibiotic flavone glycoside chemically defends the seagrass Thalassia testudinum against zoosporic fungi. AEM, Vol 64(4); 1490 – 1496.

Kim, D. H., Mahomoodally, M. F., Sadeer, N. B., Seok, P. G., Zengin, G., Palaniveloo, K., Khalil, A. A., Rauf, A.,

& Rengasamy, K. R. 2021. Nutritional and bioactive potential of seagrasses: A review. South African Journal of Botany, 137, 216-227. https://doi.org/10.1016/j.sajb.2020.10.018

Li, A., He, Y., Zhang, S., & Shi, Y. 2022. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pesticide Biochemistry and Physiology, 188, 105221. https://doi.org/10.1016/j.pestbp.2022.105221

Mani AE, Bharathi V, Patterson J. 2012. Antibacterial activity and preliminary phytochemical analysis of seagrass *Cymodocea rotundata*. IJMR, Vol. 2(2): 99 – 103.

Mariño, M., Breckwoldt, A., Teichberg, M., Kase, A., & Reuter, H. 2019. Livelihood aspects of seaweed farming in Rote Island, Indonesia. Marine Policy, 107, 103600. https://doi.org/10.1016/j.marpol.2019.103600

O'Connor, M. I., Griffiths, G., Sanders-Smith, R., Hessing-Lewis, M., Davis, K. M., Forbes, C., Olson, A. M., Prentice, C., & Parfrey, L. W. 2022. A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota. Aquatic Botany, 179, 103511.https://doi.org/10.1016/j.aquabot.2022.103511

Otmani, A., Amessis-Ouchemoukh, N., Birinci, C., Yahiaoui, S., Kolayli, S., Rodríguez-Flores, M. S., Escuredo, O., Seijo, M. C., & Ouchemoukh, S. 2021. Phenolic compounds and antioxidant and antibacterial activities of Algerian honeys. Food Bioscience, 42, 101070. https://doi.org/10.1016/j.fbio.2021.101070

Poli, A., Varese, G. C., Garzoli, L., & Prigione, V. 2022. Seagrasses, seaweeds and plant debris: An extraordinary reservoir of fungal diversity in the Mediterranean Sea. Fungal Ecology, 60, 101156. https://doi.org/10.1016/j.funeco.2022.101156

Qi Shi-Hua, Si Zhang, Pei-Yuan Qian, Bin-Gui Wang. 2008. Antifeedant, antibacterial, and antilarval compounds from the South China Seagrass *Enhalus acoroides*. In Press. Botanica Marina, Vol 51.

Sepperer, T., Hernandez-Ramos, F., Labidi, J., Oostingh, G. J., Bogner, B., Petutschnigg, A., & Tondi, G. 2019. Purification of industrial tannin extract through simple solid-liquid extractions. Industrial Crops and Products, 139, 111502. https://doi.org/10.1016/j.indcrop.2019.111502

Sillero, L., Prado, R., Welton, T., & Labidi, J. 2021. Extraction of flavonoid compounds from bark using sustainable deep eutectic solvents. Sustainable Chemistry and Pharmacy, 24, 100544. https://doi.org/10.1016/j.scp.2021.100544

Stamogiannou, I., Van Camp, J., Smagghe, G., Van de Walle, D., Dewettinck, K., & Raes, K. 2021. Impact of phenolic compound as activators or inhibitors on the enzymatic hydrolysis of cellulose. International Journal of Biological Macromolecules, 186, 174-180. https://doi.org/10.1016/j.ijbiomac.2021.07.052

Tajik, S., Zarinkamar, F., Soltani, B. M., & Nazari, M. 2019. Induction of phenolic and flavonoid compounds in leaves of saffron (Crocus sativus L.) by salicylic acid. Scientia Horticulturae, 257, 108751.https://doi.org/10.1016/j.scienta.2019.108751

Tian, C., Chang, Y., Zhang, Z., Wang, H., Xiao, S., Cui, C., & Liu, M. 2019. Extraction technology, component analysis, antioxidant, antibacterial, analgesic and anti-inflammatory activities of flavonoids fraction from Tribulus terrestris L. leaves. Heliyon, 5(8), e02234. https://doi.org/10.1016/j.heliyon.2019.e02234

Unsworth, R. K., Ambo-Rappe, R., Jones, B. L., La Nafie, Y. A., Irawan, A., Hernawan, U. E., Moore, A. M., & Cullen-Unsworth, L. C. 2018. Indonesia's globally significant seagrass meadows are under widespread threat. Science of The Total Environment, 634, 279-286. https://doi.org/10.1016/j.scitotenv.2018.03.315

Xu, C., Chen, T., Zhang, S., Zhou, C., Liao, W., Fang, R., Chen, L., & Zhou, T. 2022. In vitro activity of imipenem-relebactam alone and in combination with fosfomycin against carbapenem-resistant gram-negative pathogens. Diagnostic Microbiology and Infectious Disease, 103(3), 115712. https://doi.org/10.1016/j.diagmicrobio.2022.115712