Jurnal Pena Sains
Jurnal Pendidikan Sains dan Sains Murni
Journal homepage: https://journal.trunojoyo.ac.id/penasains

METHANOL EXTRACT PROFILE OF *Eucheuma cottonii* AS AN ANTIMALARIAL CANDIDATE

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ABSTRACT

Malaria is a tropical disease that endangers public health which is caused by the plasmodium parasite, and transmitted through the bite of a female *Anopheles* sp. mosquito. Malaria is a major health problem in Indonesia due to its high risk of transmission and drug resistance. This encourages a study to look for other antimalarial alternatives by utilizing natural ingredients. The purpose of this study is to identify the functional groups found in *Eucheuma cottonii* secondary metabolites using methanol 70% as the solvent. This study is included in the experimental research. The functional group of methanol extract of *Eucheuma cottonii* was evaluated using Fourier Transform Infrared (FT-IR) Nicolet iS 10 spectrometers. The FT-IR analysis identified the O-H functional group at 3347.1 cm-1, C≡C at 2094.8 cm-1, C=C at 1632.6 cm-1, phenol or O-H bend at 1401.5 cm-1, aromatic C-O at 1192.7 cm-1, and a C-C group at 1043.7 cm-1. The results of phytochemical analysis on Eucheuma cottoni revealed the presence of saponins. The functional groups formed in the 70% methanol extract of *Eucheuma cottonii* seaweed are the O-H, C≡C, C=C, phenol or O-H bend, aromatic C-O, and C-C groups which are flavonoid compounds in the flavanone group which have the potential to be antimalarial. Furthermore, the presence of the O-H, C=O, C-O, C=C, or C-H functional groups is a characteristic of a flavonoid compound. Therefore, it can be concluded that the identified functional groups indicate the existence of flavonoid molecules from the flavanone group, which potential as antimalarial properties.

Article Info	
Article history:	***Keywords:***
Received: March 19, 2024	Disesase;
Accepted: October 2, 2024	Flavonoid;
Published: October 31, 2024	Malaria;
	Metabolite.

Introduction

Malaria is a disease that endangers public health. Malaria is caused by the plasmodium parasite, which is transmitted via the bite of a female *Anopheles* sp mosquito. According to the WHO data, Indonesia ranks second in Southeast Asia for the largest number of malaria cases. One of the reasons for the increased number of malaria infections is the *Plasmodium* parasite's resistance to existing antimalarials. Furthermore, because there is currently no malaria vaccine available, control efforts are being carried out supplemented by research into pharmaceutical ingredients and antimalarial formulations (Phillips et al., 2017; Wykes, 2013).

When a mosquito carrying microscopic parasites bites human, it can transmit the deadly disease malaria. The mosquito injects malaria parasites into your bloodstream when it bites. Neither a virus nor a particular kind of bacteria may cause malaria; parasites can. Malaria can lead to serious health issues like convulsions, brain damage, breathing difficulties, organ failure, and even death if left untreated. A mosquito contracts malaria when it bites a person who has the disease. A parasite enters the other person's bloodstream when the mosquito bites them. The parasites proliferate there. Humans can contract malaria from five different kinds of malaria parasites. Rarely, pregnant women who have malaria may pass the illness on to their unborn child either before or during delivery. Although it is unusual, blood transfusions, organ donations, and hypodermic needles can all spread malaria.

However, repeated drug resistance can undermine the efficacy of both traditional and new antimalarial medications. The elimination of malaria is heavily dependent on the discovery of innovative effective drugs that are inexpensive and simple to administer. In this setting, plant metabolites impede malaria infection progression and could potentially be used as an alternative treatment for malaria, such as artemisinin (Habibi, Shi, Fatima Grossi-de-Sa, & Khan, 2022). Several plants such as *Lepidium sativum*, *Carica papaya*, *Allium sativum*, *Vernonia amygdalina*, *Croton macrostachyus* were identified as plant species based on an ethnomedicinal survey, while *C. papaya* and *A. sativum* were identified as traditional healers for malaria treatment. Plant leaves are the most commonly used plant portion, with alkaloids and terpenoids being the chemical families most frequently discovered for antiplasmodial efficacy (Suleman et al., 2018). While, the addition of flavonoid compounds to therapy with artemisinin, a well-known antimalarial medication, has been shown to have synergistic activity (Czechowski et al., 2019).

Plants contain secondary metabolites. Drugs, fragrances, flavors, dyes, pigments, pesticides, and food additives for use in industry, pharmaceuticals, and agriculture are examples of secondary metabolites compounds produced by an organism that are not necessary for primary metabolic processes but can have significant ecological and other uses. Any intermediate or byproduct of metabolism, the whole set of physical and chemical processes involved in the upkeep and reproduction of life where nutrients are broken down to produce energy and simpler molecules (catabolism), which can then be used to create more complex molecules (anabolism) is referred to as a metabolite.

Furthermore, metabolomics can be applied to a wide variety of species because it takes less time to reoptimize methods for a new species (Schauer and Fernie, 2006). Therefore, metabolomics offers a functional picture of a biological system's cellular phenotype at a certain moment in time. In general, metabolomics can be divided into three methods. In order to identify distinguishing metabolites, nontargeted (untargeted) metabolic profiling aims to identify and (relatively) quantify as many compounds as possible that alter or are synthesized in response to a stressor, environmental change, or genetic perturbation.

Flavonoid biosynthesis is not just found in plants; macroalgae have also been shown to be able to generate these molecules. The results of phytochemical analysis on *Eucheuma cottoni* revealed the presence of saponins (Purbosari, Warsiki, Syamsu, & Santoso, 2022). Information on the presence of flavonoids in these algae would be useful if obtained, because these algae have affordable economic value and are abundantly available in Indonesia. *Eucheuma cottonii*, commonly referred to as seabird's nest, is a red algae species that reduces tumor cell development, controls cancer cell apoptosis, promotes wound healing, and possesses anticoagulant, antioxidant, anticancer, and anti-inflammatory qualities.

Sea moss is low in calories and high in iron and iodine. It is a fantastic source of protein, fiber, carbs, and polyunsaturated fatty acids. Vitamins A, B, E, and C are among the many vitamins that are

10.21107/jps.v11i2.25437 Setyaningrum et al. E-ISSN: 2527-7634

abundant in it. Additionally, the antioxidants included in seaweeds of all kinds provide defense against the majority of cellular oxidative stressors. Because the plant contains antioxidants that support the immune system, it also helps to reduce inflammation throughout the body.

Eucheuma cottonii, the widely grown edible red seaweed, can be collected for human consumption every 45 days and grows extremely quickly in Southeast Asia's pristine waters. It has therapeutic applications and is rich in proteins, polyphenols, phytochemicals, minerals, vitamins, antioxidants, dietary fibers, and polyunsaturated fatty acids. Its use to overcome the threat of malaria provides an alternative and sustainability in its development. Therefore, this research aims to screen the profile of secondary metabolite compounds from extracts of the algae *Eucheuma cottoni* and its potential as an antimalarial candidate.

Research Methods

This research is included in an experimental study. The research tools that are used were containers, scissors, blender, analytical balance, filter paper, plastic wrap, polybag, label paper, measuring cup, Erlenmeyer, funnel, stirring rod, spatula, vacuum rotary evaporator, and oven. The required materials are *Eucheuma cottonii* seaweed powder and 70% methanol.

Eucheuma cottonii seaweed samples were obtained from Ruguk Village, Ketapang District, South Lampung Regency. Fresh seaweed is selected, then washed using distilled water until clean, and dried by hanging at room temperature until the water content is reduced. Then, seaweed is dried using an oven at a temperature of 30-35 ˚C. The dried seaweed is then blended until it forms a fine powder.

A total of 500 g of *Eucheuma cottonii* seaweed powder was macerated with 2 L of methanol for 72 hours. The maceration results were then filtered through filter paper, and the resulting residue was macerated again with 2 L methanol and left for 24 hours. Later, the macerate is filtered using filter paper. After filtering, the filtrate is evaporated using a vacuum rotary evaporator set at 50-60 ˚C. The extract is then heated in an oven at the same temperature until a thick seaweed extract forms.

The functional groups of the methanol extract of *Eucheuma cottonii* seaweed were analyzed using Fourier Transform Infrared (FT-IR) with the Nicolet iS 10 spectrophometer at a wavelength of 400-4000 cm-1. The results of FT-IR characterization of the methanol extract of seaweed are presented in graphical form using Origin software. The spectrum presented in this graph is then analyzed by comparing it with the literature to determine the functional groups of the methanol extract.

Results and Discussion

The Fourier Transform Infrared (FT-IR) test was carried out to determine the functional groups of active compounds contained in secondary metabolite extracts. This test was carried out with a wavelength of 400-4000 cm^{-1}, the test results were presented in the form of function graphs and transmittance percentages. Graphs with different wave absorptions were obtained. The results of the FT-IR test of the 70% methanol extract of *Eucheuma cottonii* are presented in Figure 1.

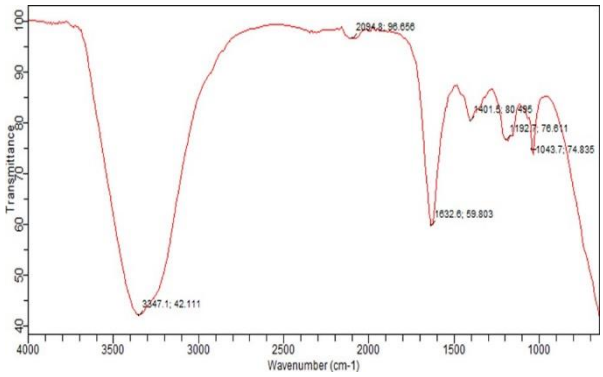

Figure 1. The sepctogram of extract *Eucheuma cottonii* using 70% methanol solvent

The results of IR analysis data (see Figure 1) show the presence of an O-H group at an absorption wave number of 3347.1 cm^{-1} with a wide band shape and strong intensity. The wave number 2094.8 cm^{-1} indicates the presence of the C≡C group because it is in the alkyne absorption area. Then the wave number 1632.6 cm^{-1} indicates the presence of an aromatic C=C carbonyl group. The wave number 1401.5 cm^{-1} shows the presence of a phenol group or O-H bend which strengthens the hydroxyl bonds produced. The wave number 1192.7 cm^{-1} indicates the presence of a C-C group and the wave number 1043.7 cm^{-1} indicates the presence of an aromatic C-O bond. Infrared spectrum analysis of the 70% methanol extract of *Eucheuma cottonii* seaweed is informed in Table 1.

Based on the results of IR analysis data for compounds contained in the 70% methanol extract

10.21107/jps.v11i2.25437 E-ISSN: 2527-7634

of *Eucheuma cottonii* seaweed (see Figure 1.), it shows that there is an absorption band at the wave number 3347.1 cm^{-1} which indicates the presence of an O-H group or hydroxyl bond because it is in the wave number area between 3650-3250 cm^{-1} and has strong intensity with a wide band shape. The formation of a widened absorption band is caused by intermolecular vibrations of hydrogen (Kopon, Baunsele, & Boelan, 2020). Then the wave number 2094.8 cm^{-1} shows a weak absorption intensity because the absorption band is not sharp and the presence of this area is in the alkyne absorption area, namely between 2000-2500 cm^{-1}, thus indicating the presence of an alkyne or triple C group (C≡C) (Nandiyanto, Oktiani, & Ragadhita, 2019). Furthermore, the wave number 1632.6 cm^{-1} indicates the presence of an aromatic C=C group because it is in the range 1650-1400 cm^{-1} (Darmawati, Bawa, & Suirta, 2015).

The wave number 1401.5 cm^{-1} indicates the presence of phenol or O-H bend because it is in the wave number area, namely between 1410-1310 cm^{-1}, the presence of the phenol group strengthens the hydroxyl bonds produced (Nandiyanto et al., 2019). The wave number 1192.7 cm^{-1} indicates the presence of a C-C group and the wave number 1043.7 cm^{-1} indicates the presence of an aromatic C-O bond (Kopon et al., 2020).

Based on the results of the IR spectrum interpretation of the 70% methanol extract of *Eucheuma cottonii* seaweed, it produces the functional groups O-H, C≡C, C=C, phenol or O-H bend, aromatic C-O, and C-C groups. The detected functional groups indicate the presence of flavonoid compounds, this is in accordance with the results of research by (Gafur, Isa, Bialangi, & Fakultas, 2011) in the methanol extract of jamblang leaves (*Syzygium cumini*), the presence of O-H, C=C, C=O, C-H and C-O groups was identified as indicating compounds. flavonoids. According to (Akbar, 2010) the presence of the O-H, C=O, C-O, C=C, or C-H functional groups is a characteristic of a flavonoid compound. Based on research by (Nugraha, Prasetya, & Mursiti, 2017) in the ethanol extract of mango leaves there were isolates of flavonoid compounds which were characterized by the presence of the functional groups O-H, C=O, C=C, C-H, C-OH and C-O. Flavonoid compounds have several groups, namely flavones, flavonols, flavanones, flavanols, anthocyanidins, and chalcones (Alfaridz, Amalia, Kunci, Flavonoid, & Klasifikasi, 2018).

Table 1. Infrared spectrum analysis of the 70% methanol extract of Eucheuma cottonii seaweed compared with a number of references

No	Wavenumber (cm^{-1})		Functional groups	References
	Spectra	References		
1.	1043,7	1059,40	Aromatic C-O bond	(Kopon et al., 2020)
2.	1192,7	1165,00	C-C	(Kopon et al., 2020)
3.	1401,5	1410-1310	Phenol or O-H bend	(Nandiyanto et al., 2019)
4.	1632,6	1650-1400	Aromatic C=C group	(Darmawati et al., 2015)
5.	2094,8	2000-2500	C≡C triple bond	(Nandiyanto et al., 2019)
6.	3347,1	3650-3250	OH hydroxyl bond	(Nandiyanto et al., 2019)

Flavonoid compounds consisting of the functional groups O-H, C≡C, C=C, phenol or O-H bend, aromatic C-O, and C-C groups belong to the group of flavanone or dihydroflavonol compounds. This is supported by research by (Setiawan et al., 2016) that the functional groups in dried pineapple peel extract, namely the O-H, C=O, C=C, and C-O groups are flavanone or dihydroflavonol compounds. Flavonoid compounds in the flavanone group have the potential to act as antimalarials, this is supported by research conducted by (Fatmawati, Anggreini, Saputri, Tjahjandarie, & Tanjung, 2018; Hidayati et al., 2020) that flavanone compounds from the bark of *Erythrina fusca*. have antimalarial activity against *Plasmodium falciparum* with IC50 values of 1.18 and 0.82 µg/ mL.

The IR spectrum obtained from the FT-IR test can be influenced by the use of solvents. According to (Hemmateenejad, Yazdani, & Sharghi, 2012) the solvent used can influence the shape of the resulting spectra because differences in the polarity level of the solvent can result in varying shapes of UV-Vis absorption spectra. This statement is in accordance with research regarding *Eucheuma cottonii* seaweed which is extracted with other solvents. In another study conducted by (Madjid, Rahmawati, & Fasya, 2020) the petroleum ether extract of Eucheuma cottonii also obtained different FT-IR analysis results in the form of forsteroids with the functional groups – OH, C=C, CO, CH 2, -C (CH 3) 2 and obtained

10.21107/jps.v11i2.25437 Setyaningrum et al. E-ISSN: 2527-7634

triterpenoids with the functional groups –OH, C=O, C=C, CH 2,-C(CH 3) 2.

Apart from that, in the research of (Veronika, Mappiratu, & Sumarni, 2017) different results were also obtained, in this research grass *Eucheuma cottonii* was extracted using a phosphate buffer solution to obtain functional groups in the form of OH, C=O, and NH which indicated the presence of alkaloid compounds. Further research will be needed to test the activity of the findings of flavonoid content in *Eucheuma cottonii*. In vitro and in vivo testing as well as its synergistic effect with other compound formulations and its toxicity to non-target cells will help determine the efficacy of the initial screening findings in this study.

Conclusion

This research aims to screen the profile of secondary metabolite compounds from extracts of the algae *Eucheuma cottoni* and its potential as an antimalarial candidate. Findings indicated that the functional groups formed in the 70% methanol extract of *Eucheuma cottonii* seaweed are the O-H, C≡C, C=C, phenol or O-H bend, aromatic C-O, and C-C groups which are flavonoid compounds in the flavanone group which have the potential to be antimalarial.

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