Biotransformation of bioactive compounds in seaweed through fermentation with marine endophytic fungi as antioxidant

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Abstract. *Sargassum polycystum* is a brown seaweed with potential antioxidant properties due to its bioactive components. These bioactive components can be altered through biotransformation by fermentation. This fermentation can be carried out using marine endophytic fungi. The purpose of this study is to determine effect of fermentation process using the marine endophytic fungi *Trichoderma harzianum* KTR3 and KTR4 on the bioactive components and antioxidant activity. *Sargassum polycystum* and fermented *Sargassum polycstum* will be macerated using ethyl acetate solvent for 72 hours. *Sargassum polycstum* extract and *Sargassum polycstum* fermented with KTR3 and KTR4 showed the presence of tannins, saponins, steroids, and terpenoids. Fermentation treatment with *Trichoderma harzianum* KTR3 resulted a non-significant increase in antioxidant activity, indicated by a decrease in IC_{50} values in the fermented *Sargassum polycstum* extract. Metabolite profiling revealed an increase in the composition of compounds in *Sargassum polycstum* fermented with the KTR3 isolate, identifying 52 compounds, of which 36 were new biotransformation products. Marker compounds in fermented *Sargassum polycstum* extract are citrinin and pheophorbide A.

1 Introduction

Seaweed is one of the biological resources with very high diversity in Indonesian. This seaweed is known to have an abunandt amount, which is 8.6% of the total marine biota. The diversity of seaweed types in Indonesia consists of 911 species, 268 genera, and 89 families. These diverse types of seaweed are grouped into 3 classes, *rhodophyta* (red algae), *phaeophyta* (brown algae), and *chlorophyta* (green algae) [1]. *Sargassum* is widely found in the subtidal and intertidal zones with a species diversity of 537 species, of which 358 species

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have been successfully classified taxonomically [2]. *Sargassum polycystum* is one type of *phaeophyta* seaweed that generally lives wild in Indonesian, such as in the waters of Sukabumi, West Java. *Sargassum polycystum* is known contain various primary and secondary metabolite compounds. Primary metabolites are chemical compounds that have important role in the physiological processes and growth of organisms, while secondary metabolites are chemical compounds produced by an organism as a form of response to changing environmental conditions or stress [3].

The primary metabolite components contained in seaweed include protein, carbohydrates, and fat. *Sargassum polycystum* contains 3.65% protein, 53.66% carbohydrates, and 0.50% fat. The secondary metabolite content also found in *Sargassum polycystum* consists of bioactive components flavonoids, saponins, steroids, alkaloids, phenols, and triterpenoids [4]. The bioactive compounds found in *Sargassum polycystum* are known to have various biological activities, one of which is antioxidants. Antioxidants themselves are defined as substances or molecules that can delay or prevent oxidative damage or oxidative stress by counteracting the production of Reactive Oxygen Species (ROS). ROS includes various types of molecules such as free radicals [5].

The need and role of antioxidants continues to increase. This is driven by the statement of the World Health Organization (WHO), which reported that some of the leading causes of death adjusted for (DALYs) in 2019 include heart disease, cancer, diabetes, and neurodegenerative disorders. These diseases in some conditions are related to oxidative stress. Oxidative stress is a state where there is an imbalance between the production of free radicals and the body's ability to neutralize or fight free radicals itself. Prevention of oxidative stress can be done by increasing the need for antioxidants in the body [6]. Natural sources of antioxidants can be obtained from aquatic resources, such as bioactive compounds in seaweed and marine endophytic fungi.

Antioxidant activity in several natural sources such as seaweed can be increased through biotransformation process. Biotransformation is a chemical transformation method that uses a biological system as a catalyst in its process [7]. The fermentation method has developed into one part of the biotransformation process. The biotransformation process used to increase bioactive components and biological activity requires the role of microorganisms. These microorganisms act as agents that break down bioactive components in the fermentation process. Microorganisms that are widely used in the fermentation process are fungi [8]. Exploration of variety fungus continues to develop, especially in fungus obtained or isolated from waters or known as marine endophytic fungi. The Aquatic Product Technology Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, IPB University has a collection of marine endophytic fungi isolated from various sources of aquatic products, such as mangroves, seagrass, and seaweed. One of the collections marine endophytic fungi that have been successfully isolated and cultivated is the *Trichoderma* species.

Trichoderma is generally classified as an endophytic fungi that has been widely isolated from both terrestrial and marine environments [9]. This fungi produces several secondary metabolites, such as polysaccharides, terpene compounds, and phenolic compounds and has antioxidant and antimicrobial biological activities [10]. The biotransformation process involving the role of fungi needs to be carried out to obtain information related to increasing the abundance of bioactive compounds and antioxidant activity in seaweed. Therefore, it is necessary to conduct research on the biotransformation of bioactive compounds in seaweed with fermentation of marine endophytic fungi which aims to determine the effect of the fermentation process on bioactive components and increasing the antioxidant capacity of seaweed.

2 Materials and methods

2.1 Materials and equipment

The raw material used in this study was *Sargassum polycystum*, obtained from Sukabumi Waters, West Jawa. Marine endophytic fungi were obtained from collection of the Laboratory of Aquatic Product Microbiology, Department of Aquatic Products Technology, Bogor Agricultural University. The type of marine endophytic fungi used was KTR3 (isolated from *Vaucheria* sp.) and KTR4 (isolated from *Halimeda* sp.). Other ingredients used include *Potato Dextrose Agar* (DIFCO, PDA), *Potato Dextrose Broth* (DIFCO, PDB) media, peptone (DIFCO), NaCl (SIGMA), aquades, methanol (SMARTLAB), ethyl acetate (SMARTLAB), chloroform (SMARTLAB), 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent (Tokyo Chemical Industry), $FeCl_3$ 5% (Merck), and P-anisaldehyde (Merck).

The tools used include The tools used in the study were glassware (Iwaki Japan), separating funnel (Iwaki Jalan), autoclave (Yamato SM52), shaker (DLAB SK-0330-PRO), TLC plate silica gel GF254 (Merck), rotary vacuum evaporator (BUCHI), chamber, micropipette (Nichipet Ex II), UV lamp 254 nm and 366 nm, UV-Vis Spectrophotometer (Rayleigh), UHPLC Vanquish Tandem Q Exactove Plus Orbitrap HRMS ThermoScientific, cuvette and Whatman paper number 42 (cytiva).

2.2 *Sargassum polycystum* preparation

Dried *Sargassum polycystum* seaweed was washed with freshwater to remove impurities and residual sand. The cleaned seaweed was dehydrated for ± 4-5 hours at 60℃. The dried seaweed will be ground or reduced in size using a blender.

2.3 Cultivation of *Trichoderma harzianum*

Refreshing the KTR 3 and KTR 4 was carried out using PDA media. Media was prepared with sterilizing 7.8 g of PDA media in 200 mL of aqueous. PDA media will be sterilized using an autoclave at 121℃ for 1 hour. The sterilized media will be poured into a plate dishes as much as 10-15 mL.

2.4 Preparation of fungi starter

Preparation of fungi starter begins with the transfer of acclimatized fungi to PDB media. Liquid media is made from 2.4 PDB powder dissolved in 100 mL of aqueous, then the media is sterilized using an autoclave at a temperature of 121 °C for 1 hour. 3 pieces of acclimatized fungi x 1 cm will be transferred to PDB media and incubated for 7 days at room temperature. Fungi that have grown in PDB media will then be acclimatized to a new medium, namely acclimatization media made from seaweed. 20 g of dried seaweed is added with 225 mL of aqueous and then heated at a temperature of 60 °C for 20 minutes. The acclimatization media is delignified and sterilized using an autoclave. 25 mL of fungi from PDB media will be added to the media with the addition of 0.5% peptone and 3% NaCl. Starter preparation was carried out at room temperature using a shaker at a speed of 120 rpm for 7 days.

2.5 Fermentation

Fermentation of *Sargassum polycystum* was conducted using the fungi KTR3 and KTR4. *Sargassum polycystum* was added with 0.5% peptone and 3% NaCl then sterilized.

Sterilization was done in an autoclave at 121°C for 1 hour then drained to room temperature. The KTR3 and KTR4 cultures were then inoculated from the acclimatization media into the fermentation media as much as 25 mL. Fermentation was carried out for 7 days with the help of a shaker with a speed of 120 rpm.

2.6 Extraction

Extraction of dried *Sargassum polycystum* was carried out using ethyl acetate solvent with a ratio (1:5, w/v) by maceration for 72 hours. The fermented seaweed was incubated for 7 days using a shaker and then filtered using Whatman 40 paper. The filtration results in the form of liquid and solids were then weighed and extracted using ethyl acetate solvent. The ratio of solvent use in solid extraction is 1:5 (w/v), while for liquid filtrate using a ratio of 1:1 (v/v). The extracted filtrate was then evaporated with a rotary vacuum evaporator at a temperature of 40°C at a pressure of 378 mBar to remove the ethyl acetate solvent.

2.7 Analyses

2.7.1 Phytochemical assay of dried seaweed

Qualitative phytochemical assay of dried seaweed was carried out to identify several bioactive compounds, including alkaloids, phenolic compounds (flavonoids, tannins, saponins), triterpenoids, steroids, and hydroquinones.

2.7.2 Evaluation of antioxidant activity

The antioxidant activity assessment was perfomed using the DPPP (1,1-diphenyl-2-picrylhydrazyl) method. The test samples included extracts from *Sargassum polycystum* seaweed extract, KTR3 and KTR4 fungi extracts, 2% acclimatized fungi extracts and fermented fungi seaweed extracts. DPPH solution (0.2 mmol/L) was prepared in methanol solvent, and the sample extracts were diluted using methanol as well. Stock samples were diluted to concentrations of 90, 120, 150, 180, 210, 240, and 270 ppm. 160 µL of DPPH solution was pipetted into 500 µL of sample solution, then the mixture was incubated for 30 minutes at room temperature. The incubated samples were then subjected to absorbance measurements at a wavelength of 517 nm. Antioxidant activity was measured in the form of the percentage of inhibition and IC_{50} value. The percentage of inhibition was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{AB - AS}{AB} \text{ x } 100\%$$

Description:
AB = Blank Absorbance
AS = Sample Absorbance

2.7.3 Thin Layer Chromatography, antioxidant bioautography, and phytochemical assay

The antioxidant bioautography was conducted using Thin Layer Chromatography (TLC) with a stationary phase of silica gel GF254 and a mobile phase of ethyl acetate mixture chloroform eluent, with a ratio of 1:6 (v/v) for *Sargassum polycstum* extract and 1:9 (v/v) for *Sargassum polycstum* fermented extract. Both extracts were applied to the TLC plate, eluted, and examined under UV light at 254 and 366 nm. After that, the plate was sprayed with

DPPH (0.4 mM) to detect antioxidant activity, which was indicated by a change in spot colour from purple to yellowish white. Determination of phytochemical compounds was carried out by spraying $FeCl_3$ reagent to detect polyphenols, and sulfuric acid anisaldehyde to detect terpenoids or steroids.

2.7.4 Profiling bioactive compound using UHPLC-MS

The process of determining the metabolite compound profile involved *Sargassum polycystum* extract, marine endophytic fungi extract KTR3, and fermented *Sargassum polycystum* extract of KTR3 fungi. The extract was produced through ethyl acetate extraction and evaporation, then analyzed using UHPLC-MS Vanquish Tandem Q Exactive Plus Orbitrap HRMS. The paste extract (5 mg) was dissolved in 1 mL of methanol, filtered, and injected (2 μL) onto the Accucore C18 column. The mobile phase used water with 0.1% formic acid and acetonitrile with 0.1% formic acid in a 5%-95% gradient program for 25 minutes. The column temperature was maintained at 30 °C, and the analyzed mass range was 100-1500 m/z with positive ionization mode. The analysis results were identified using the ChemSpider, mzCloud, and Human Metabolite Database databases.

3 Results and discussion

3.1 Characteristics of *Sargassum polycystum*

Sargassum polycystum in this study had morphology, with characteristics of oval to long leaf thallus and dark brown with brown spots. The leaf thallus of *Sargassum polycystum* consists of several branches attached to the main stem branches. The stem of *Sargassum polycystum* has a cylindrical shape with a length of 10-20 cm. *Sargassum polycystum* has round vesicles. Phytochemical identification is carried out to identify and evaluate bioactive compounds in seaweed. The components of the bioactive compounds of *Sargassum polycystum* can be seen in Table 1.

Table 1. Components of bioactive compounds of dried *Sargassum polycystum*.

Phytochemicals	*Sargassum polycystum*	*Sargassum* sp. [12]
Alkaloid	-	-
Flavonoid	-	-
Hydroquinone phenol	-	+
Steroid	+	+
Triterpenoid	-	+
Tannin	-	+
Saponins	+	+

Noted: positive contain the bioactive (+) and negative contain the bioactive (-)

Dried *Sargassum polycystum* in this study positively contained saponins and steroid components. However, the identified active compunds were fewer compared to those found in dried *Sargassum* sp. [12]. Variations in the composition of these active compounds can be influenced by several factors, such as differences in environmental conditions of the habitat, type of seaweed, harvest age, geographical location, time and method of harvest, current, season, year, physiological factors, and drying methods [11].

3.2 Extract and Yield

Sargassum polycystum, endophytic fungi *Trichoderma harzianum*, and *Sargassum polycystum* with fungi fermentation will be extracted using ethyl acetate solvent and then the yield analysis is carried out on the extract obtained. Ethyl acetate solvent was used in this study because it is semi-polar with low toxicity [13]. The use of ethyl acetate has also shown in several previous studies the results of fractions that have antioxidant activity, due to its ability to extract polyphenols and carotenoids [14]. The results of the yield of samples extracted using ethyl acetate solvent are presented in Table 2.

Table 2. Ethyl acetate extract yield.

Treatment	Type	Code	Extract (g)	Yield (%)
Raw material	*Sargassum polycystum*	STF	0.127±0.05	0.0080
Fermentation	*Sargassum polycystum* feremented KTR3	SF-KTR3	0.023±0.01	0.0400
	Fermented filtrate *Sargassum polycystum* KTR3	FF-KTR3	0.043±0.03	0.0003
	Sargassum polycystum feremented KTR4	SF-KTR4	0.071±0.05	0.1300
	Fermented filtrate *Sargassum polycystum* KTR4	FF-KTR4	0.118±0.04	0.0007
Endophytic fungi	Fungi broth KTR3	BKT-KTR3	0.113±0.07	0.0007
	Fungi broth KTR4	BKT-KTR4	0.078±0.07	0.0005

The yield obtained was relatively low. The amount of yield produced can be influenced by the composition and content of the compounds. The yield value is associated with the amount of bioactive content extracted from the sample during process [15]. The extraction outcomes can be influenced by the choice of solvents, as both the solvent selection and extraction methods impact the secondary metabolites. Solvent selection typically follows the principle of "like dissolves like", where polar compounds dissolve in polar solvents and non-polar compounds dissolve in non-polar solvents [16]. Several studies have shown that *Sargassum polycystum* extract compounds have polarity that approaches several solvents, such as methanol. The yield of the extract is also influenced by the number of raw materials, solvents, and extraction methods used [17]. Other factors that can affect yield of fungi extracts, especially metabolite compounds, are the conditions of the growth media and incubation conditions [18]. Several factors that can affect the production of extracellular enzymes in *Trichoderma* fungi isolates, including environmental conditions and culture conditions. Environmental factors that affect fungi growth include pH, temperature, humidity, and nutrient availability in the media, while culture conditions include the initial density of the fungi inoculum [19].

3.3 Antioxidant activity of ethyl acetate extract

Antioxidant activity evaluation showed that the ethyl acetate extract of *Sargassum polycystum* had moderate antioxidant activity. The IC_{50} value of the ethyl acetate extract of *Sargassum polycstum* decreased after fermentation with KTR3, but the antioxidant activity was still moderate. Interestingly, the IC_{50} value of the ethyl acetate extract of *Sargassum polycstum* increased after fermentation with KTR4 (Table 3). The antioxidant activity of the fermentation filtrate of *Sargassum polycstum* and *Sargassum polycstum* fermented with KTR3 had respectively values of 174±36.67 μg/mL and 155±16.2 μg/mL. The antioxidant activity value showed a non-significant increase in antioxidant activity in fermented *Sargassum polycstum*, which was 16%, but the antioxidant activity remained in the moderate group.

Table 3. Antioxidant activity of ethyl acetate extract.

Treatment	Type	Code	IC_{50} (ppm)
Raw material	*Sargassum polycystum*	STF	184±14.01
Fermentation	*Sargassum polycystum* feremented KTR3	SF-KTR3	155±16.20
	Fermented filtrate *Sargassum polycystum* KTR3	FF-KTR3	174±13.67
	Sargassum polycystum feremented KTR4	SF-KTR4	226±15.01
	Fermented filtrate *Sargassum polycystum* KTR4	FF-KTR4	238±17.78
Endophytic fungi	Broth KTR3	BKT-KTR3	190±215.2
	Broth KTR4	BKT-KTR4	270±177.6

Antioxidant activity is influenced by the components of bioactive compounds contained in the extract [20]. The decrease in antioxidant activity in *Sargassum polycstum* fermented using KTR4 can occur due to variations in secondary metabolites. Secondary metabolites produced by a species depend on external conditions and competition with other species. The resulting response to this competition causes the species to adjust the production of certain metabolites, either by increasing or decreasing their production [21]. Fermentation treatment using KTR3 on *Sargassum polycystum* causes an increase in antioxidant activity. The fermentation process can increase antioxidant capacity associated with an increase in bioactive compounds. Antioxidant capacity can be increased through the fermentation process because it allows an increase in bioactive such as polysaccharides and phenolic compounds [22]. Fermentation using microorganisms can modify antioxidant compounds because it produces enzymes that cause damage to the structure of seaweed cell walls, thereby inducing and releasing various bioactive compounds that increase their bioavailability [23].

The antioxidant activity of both types of endophytic fungi *Trichoderma harzianum*, namely types KTR3 and KTR4, is classified as very weak when compared to several previous studies. The antioxidant activity of endophytic fungi is influenced by the presence of

bioactive compounds. The composition of bioactive compounds or secondary metabolites in endophytic fungi can be influenced by genetic variation, host species, and interactions with microorganisms. The interaction of endophytic fungi with their hosts can affect the biosynthesis of active compounds. Genetic differences between the endophytic fungi used also allow for variations in antioxidant activity [24].

The antioxidant activity produced in all samples tends to have low activity when compared to several literatures. This can be caused by solvents in the extraction process. The extraction process on raw materials that target bioactive compounds with high antioxidant activity is generally carried out using solvents with high polarity, such as methanol. The use of solvents with high polarity is known to attract phenolic compounds, alkaloids, and flavonoids, where some of these compounds have good antioxidant bioactivity [17]. The use of ethyl acetate solvent in the extraction process is usually carried out in the type of stepwise extraction, this aims to separate and purify semi-polar compounds that were previously not properly extracted by polar solvents. The use of ethyl acetate is not only expected to optimize the acquisition of compound profiles, but also has the potential to increase the antioxidant activity of the extract because semi-polar compounds extracted using ethyl acetate often have significant biological activity [25].

3.4 Phytochemical compounds and antioxidant bioautography

Phytochemical analysis and identification can be done using the Thin Layer Chromatography (TLC) method. This Thin Layer Chromatography can separate the compound fractions contained in the extract based on their polarity, which are then identified through the Retardation factor (Rf) value (Table 4). Identification of phytochemical compounds contained in the extract is done by spraying reagents on TLC. The reagents used in this study are $FeCl_3$ and anisaldehyde sulphate (Table 4). The $FeCl_3$ reagent is a reagent used to detect the presence of phenolic compounds which are indicated by a change in the colour of the spots to blue or black after the silica gel plate [26]. The anisaldehyde sulphate reagent is used to see the formation of coloured spots on the silica gel plate, namely purple, violet, and grey. The advantage of using the thin layer chromatography (TLC) method is that it can be used for bioautography analysis. The biological activity identified using TLC bioautography in this study is antioxidant. The TLC method can separate or form compound fractions which are then tested for antioxidants by spraying DPPH reagent (Table 4).

The five extracts produced spots with different Rf values in the Thin Layer Chromatography method. This is influenced by several factors, namely chemical composition, compound polarity, and the interaction produced between the stationary phase and the mobile phase (Table 4).

Spraying $FeCl_3$ reagent on the fractionated TLC silica gel plate gave positive results for the content of polyphenol compounds in the four extracts, namely STF, FF-KTR3, SF-KTR4, and SF-KTR3. The results of the determination of phytochemical compounds showed the appearance of spots that were suspected of containing phytochemical compounds according to the anisaldehyde sulphate reagent. Spraying anisaldehyde sulphate reagent on the fractionated TLC silica gel plate gave positive results for the content of saponins, steroid, and terpenoid compounds in the four extracts, namely STF, FF-KTR4, SF-KTR3, and SF-KTR4. The results of antioxidant bioautography in the five samples showed antioxidant activity. Qualitative evaluation of antioxidant activity using DPPH was observed through a change in colour from purple to yellow. The purple colour in DPPH is caused by the DPPH radical structure that has undergone electron delocalization. The electron delocalization process will stop, and DPPH will be reduced, the reduced form of DPPH will make it lose its purple colour and change to yellow. The decrease in the intensity of the purple colour in DPPH occurs

because of the capture of one electron by the DPPH radical compound from the antioxidant substance which causes no opportunity for electrons to resonate [27].

Table 4. *Rf* value, biocompound identity, and antioxidant bioautography

Sample	*Rf* value	Reagent		Antioxidant bioautography	Bioactive compounds
		$FeCl_3$	Anisaldehyde		
STF	0.17, 0.3, 0.5, 0.6, 0.83, 0.93	0.93, 0.5, and 0.3	0.17, 0.3, and 0.5	0.3 and 0.5 (yellow)	Tannin, terpenoid, and saponins
SF-KTR 3	0.17, 0.3, 0.4, 0.76, 0.83	0.17, 0.4, and 0.83	0.3, 0.4, and 0.83	0.4 and 0.76 (white-yellow)	Tannin, steroid, terpenoid, and saponins
FF-KTR 3	0.1 and 0.28	0.1	0.1 and 0.28	0.2 (white-yellow)	Tannin, steroid, and terpenoid
SF-KTR 4	0.18, 0.25, 0.35, 0.4, 0.75, 0.78	0.78 and 0.75	0.18, 0.35, 0.4, and 0.78	0.35 and 0.75 (white-yellow)	Tannin, steroid, terpenoid, and saponins
FF-KTR 4	0.13	-	0.13	-	Tannin and terpenoid

Information:
STF: Raw material *Sargassum polycystum*
FF-KTR: Fermented filtrate *Sargassum polycystum*
SF-KTR: Fermented *Sargassum polycystum*

3.5 Chemical compound profiling of extract *Sargassum polycystum,* marine fungi KTR3, and *Sargassum polycystum* fermented

Determination of metabolite profiles in this study was carried out on extracts of *Sargassum polycystum* samples, *Trichoderma harzianum* fungi, and fermented *Sargassum polycstum*. The fermented *Trichoderma harzianum* and *Sargassum polycstum* fermented extracts that were subjected to metabolite profile analysis were extracts with the best antioxidant activity, namely using the KTR3 isolate.

The data from determination of the metabolite profile in three samples are displayed in the form of a chromatogram showing the detected compounds (Figure 1). The results of the interpretation of the metabolite profile data from these three samples showed the presence of 100 metabolite compounds in each sample which were known in the form of their molecular formulas. Identification of compounds using molecular formulas was matched with the online databases ChemSpider, mzCloud, and Human Metabolome Database. The results of confirmation with the database obtained 31 compounds identified in *Sargassum polycstum*, 21 compounds identified in the fungi isolate KTR3, and 52 compounds identified in fermented *Sargassum polycstum* isolate KTR3.

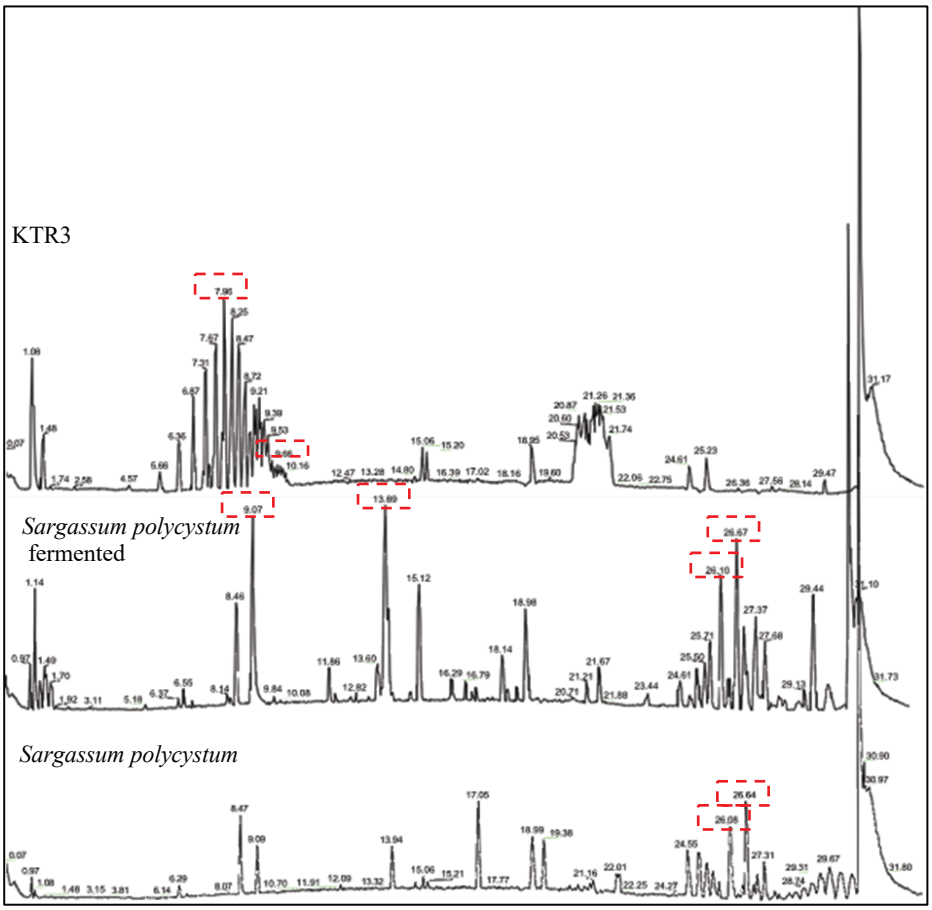

Fig. 1. UHPLC-MS chromatogram

Several compounds identified qualitatively in the ethyl acetate extract of *Sargassum polycstum* and fermented *Sargassum polycstum* using TLC, such as phenols (tannins), steroids, and terpenoids were also re-identified in the metabolite profile analysis using UHPLC-MS. The abundance of each detected compound was different, as indicated by the difference area of the sample in the peak formed in the chromatogram. Compounds with higher abundance compared to other compounds are called major compounds, while compounds with lower abundance are called minor compounds. The major compounds that can be identified from the three samples are as follows:

1. The major compound of *Sargassum polycystum* is Pheophorbide A;
2. The major compound of *Trichoderma harzianum* KTR3 isolate is Netilmicin; and
3. The major compound of fermented *Sargassum polycystum* isolate KTR3 is Citrinin

Table 5. Chemical compound profiling of extract fermented *Sargassum polycystum* from biotransformation

No	Chemical compound	Formula	MW	RT	Area sampel
1	Citrinin	$C_{13}H_{14}O_5$	250.08337	13.879	2348116956
2	Nabilone	$C_{24}H_{36}O_3$	372.26525	15.107	1152553436
3	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.27581	29.455	1133683740
4	7-ketodeoxycholic acid	$C_{24}H_{38}O_5$	406.27074	14.000	835917201,8
5	Amfepramone	$C_{13}H_{19}NO$	205.14627	13.602	584750742,9
6	Bufa-20,22-dienolide	$C_{24}H_{34}O_2$	354.25459	15.118	566343068,9
7	Ethyl docosahexaenoate	$C_{24}H_{36}O_2$	356.27065	18.151	556722361,8
8	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.15103	21.676	478567853,7
9	MFCD00083370	$C_{22}H_{44}O_3$	356.32799	26.995	405934604,5
10	Adenine	$C_5H_5N_5$	135.05428	1.344	379154983,7
11	MK 6	$C_{41}H_{56}O_2$	580.42912	28.926	366193437,7
12	Visnagin	$C_{13}H_{10}O_4$	230.05733	11.845	352045691,1
13	2'-Deoxyadenosine	$C_{10}H_{13}N_5O_3$	251.10108	1.540	330071929
14	Cobiprostone	$C_{21}H_{34}F_2O_5$	404.23744	9.431	315491091
15	Diisopromine	$C_{21}H_{29}N$	295.22914	27.575	299950312
16	Erucic acid	$C_{22}H_{42}O_2$	338.31752	27.01	292584405
17	Terodiline	$C_{20}H_{27}N$	281.21367	26.371	289690589
18	Thymine	$C_5H_6N_2O_2$	126.04273	1.711	271635072
19	SL9650000	$C_{29}H_{44}O_2$	424.33275	27.613	254453172
20	DEET	$C_{12}H_{17}NO$	191.13064	13.766	248172899
21	4-[3,5-di(tert-butyl)-1H-pyrazol-1-yl]benzoic acid	$C_{18}H_{24}N_2O_2$	300.18288	18.676	242292322
22	Orellanine	$C_{10}H_8N_2O_6$	252.03903	11.834	179998426
23	Mangostin	$C_{24}H_{26}O_6$	410.17159	23.435	179706372
24	Lovastatin	$C_{24}H_{36}O_5$	404.25508	14.138	172319651
25	Arg-asn	$C_{10}H_{20}N_6O_4$	288.15386	6.558	162153959
26	7alpha-Hydroxy-3-oxochol-4-en-24-oic acid	$C_{24}H_{36}O_4$	388.26015	13.938	160656395
27	3b-Hydroxy-5-cholenoic acid	$C_{24}H_{38}O_3$	374.28105	18.150	156091755
28	Uracil	$C_4H_4N_2O_2$	112.02728	1.170	128645450
29	Glycitein	$C_{16}H_{12}O_5$	284.06767	18.659	126062317
30	1-(2-Deoxy-alpha-D-erythro-pentofuranosyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxyli acid	$C_{10}H_{12}N_2O_7$	272.06542	13.879	123200429
31	2-[(5Z,8Z,11Z)-icosatrienoyl]-sn-glycero-3-phosphoethanolami	$C_{25}H_{46}NO_7P$	503.30211	23.959	121881852
32	TEP	$C_6H_{15}O_4P$	182.07045	9.832	119816585
33	e-Tochoferol	$C_{28}H_{42}O_2$	410.31722	27.683	114084595
34	Diethylene glycol	$C_4H_{10}O_3$	106.06303	9.061	109566766
35	Diflucortolone valerate	$C_{27}H_{36}F_2O_5$	478.25307	20.824	109071660
36	Octinoxate	$C_{18}H_{26}O_3$	290.18736	25.343	105369275

Noted: MW (Molecular Weight) and RT (Retention Time)

Sargassum polycystum fermented using endophytic fungi *Trichoderma harzianum* isolate KTR3 produced higher identified metabolite compounds compared to other samples, namely 52 metabolite compounds. As many as 69% of the compounds identified in fermented *Sargassum polycstum* extract were new compounds resulting from the biotransformation process (Table 5). Biocompounds identified in fermented *Sargassum polycystum* extract have

different retention time (RT) values. This RT value is related to the polarity of the compounds in the sample, generally the UHPL-Vanquish instrument uses a reverse-phase column type. The use of this type of reverse-phase column is inversely proportional to the normal-phase, where the stationary phase will interact more strongly with non-polar compounds, resulting in a higher retention time [28]. The results of metabolite data in fermented *Sargassum polycystum* extract show compounds with RT values between 1.5-28.9 min. A high RT value indicates that the compound is non-polar, the non-polar compounds produced can be caused by the use of ethyl acetate solvent in the extraction process.

Citrinin is one of the major compounds identified in fermented *Sargassum polycstum*. Citrinin itself is a secondary metabolite in the form of polyketide. Generally, this metabolite compound is often found in the fungi species *Penicillium* sp., this compound has been identified as having potential as an antitumor agent. Its potential as an antitumor is due to the antagonistic properties produced, such as antioxidants and cytotoxic [29]. *Sargassum polycystum* fermented using KTR3 produces several bioactive compounds that have bioactivity as antioxidants, namely steroids, phenols, and isoflavones. Compounds included in the steroid derivative group that were also identified in fermented *Sargassum polycstum* samples include 7-ketodeoxycholic acid, Bufa-20,22-dienolide, 7alpha-Hydroxy-3-oxochol-4-en-24-oic acid, and 3b-Hydroxy-5-cholenoic acid. Other derivative compounds that were also identified were SL9650000 and glycitein which are classified as phenol compounds. Derivative compounds themselves are chemical compounds produced from changes or modifications of the original (parent) compound through various chemical reactions. The increase and formation of these new compounds can be caused by the biotransformation process. Gateta [30] and Prieto [23] in their research which carried out the fermentation process using microorganisms showed an increase in the accumulation and modification of phenol compounds.

The fermentation process involving the role of fungi can cause the breakdown or simplification of complex compounds into simple ones. Microorganisms in this fermentation process can produce enzymes that cause damage or breakdown of the wall structure of certain organisms, thereby inducing and releasing various bioactive compounds. The breakdown and release of various compounds during the fermentation process causes an increase in the concentration of certain compounds and the formation of new compounds [22]. Conditions during the fermentation process can also affect an organism in producing metabolite compounds. Microorganisms are known to have the ability to metabolize a compound through the production of certain enzymes. These enzymes during fermentation can break down or hydrolyse compounds, allowing for an increase in compounds or the formation of modifications to the parent compound [23].

4 Conclusion

Extract *Sargassum polycystum* and *Sargassum polycystum* that have been fermented using marine endophytic fungi showed the presence of tannin, saponins, steroid, and terpenoid compounds based on qualitative testing using TLC. Antioxidant activity increased in the fermentation treatment of *Sargassum polycstum* using marine endophytic fungi *Trichoderma harzianum* KTR3, namely by 16%, but antioxidant activity was still classified as moderate with an IC_{50} value of 155 ± 16.20 ppm. The biotransformation process showed an increase in the number of metabolite compound profiles in *Sargassum polycystum* fermented using KTR3, namely 52 compounds. The composition of bioactive compounds after biotransformation showed the presence of 36 new compounds.

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References

1. C. Litaay, H. Arfah, F. Pattipeilohy, Potensi sumber daya hayati rumput laut di Pantai Pulau ambon sebagai bahan makanan. JPHPI. **25**(3), 405-417 (2022).
2. M.I. Rushdi, I.A. Rahman, H. Saber, E.Z. Attia, W.M. Abdelraheem, H.A. Madkour, H.M. Hassan, A.H. Elmaidomy, U.R. Abdelmohsen, Pharmacological and natural products diversity of the brown algae genus *Sargassum*. RSC Adv. **10**, 24951-24972 (2020).
3. G.P. Rosa, W.R. Tavares, P.M. Sousa, A.K. Pages, A.M. Seca, D.C. Pinto, Seaweed secondary metabolites woth beneficial health effects: an overview of successes in in vivo studies and clinical trials. MDPI. **18**(8), 1-35 (2020).
4. S.H. Manteu, Nurjanah, Nurhayati, Karakteristik rumput laut cokelat (*Sargassum policystum* and *Padina minor*) dari perairan Pohuwato Provinsi Gorontalo. JPHPI. **21**(3), 396-405 (2018).
5. B. Akbari, N.B-Yazdi, M. Bahmaie, F.M. Abhari, The role of plant-derived natural antioxidant in reduction of oxidative stress. BioFactors. **1**-23 (2022).
6. A.M. Pisoschi, A. Pop, F. Iordache, L. Stanca, O.I. Geicu, L. Bilteanu, A.I. Serban, Antioxidant, anti-inflammantory and immunomodulatory roles of vitamins in COVID-19 therapy. EJMECH. **232**, 1-24 (2022).
7. M.R. Pimentel, G. Molina, A.P. Dionisio, M.R. Junior, G.M. Pastore, The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol. Res. Int. **1** (2011)
8. S.B-Isasi, C. Caru, M.E. Lienqueo, Valorization of the green seaweed *Ulva rigida* for production of fungal biomass protein using a hypercellulolytic terrestrial fungi. Algal Res. **59**, 1-9 (2021)
9. J.C. De Padua, T.E. de Cruz, Isolation and Characterization of nickel-tolerant *Trichoderma* strains from marine and terrestrial environments. J. Fungus. **7**(591), 1-15 (2021).
10. D. Handayani, N. Ananda, M.A. Artasasta, R. Ruslan, O. Fadriyanti, T.E. Tallei, Antimicrobial activity screening of endophytic fungi extracts isolated from brown algae *Padina* sp. JAPS. **9**(3), 9-13 (2019).
11. S. Diachanty, Nurjanah, A. Abdullah, Aktivitas antioksiand berbagai jenis rumput laut coklat dari Perairan Kepulauan Seribu. JPHPI. **20**(2), 305-318 (2017).
12. S.D. Hardiningtyas, N. Jauharah, K. Tarman, Characteristics of bioactive components in fermented *Sargassum* and *Ulva* seaweed using SCOBY as potential anti-diabetic candidates. BIO Web of Conferences. 106, 1-11 (2024)
13. Z. Zainuddin, S. Hafsah, G. Erida, Uji efektivitas bioherbisida etil asetat babadotan (*Ageratum conyzoides* L.) dari berbagai ketinggian tempat and konsentrasi terhadap pertumbuhan gulma bayam duri (*Amaranthus spinosus* L.). JIM Pertanian. **3**(4), 34-42 (2018).
14. E.A. Sianipar, S.I. Gunardi, Anti-inflammatory and antioxidant properties of *Sargassum polycystum* ethyl acetate extract from Indonesia. J Herbmed Pharmacol. **12**(3), 407-412 (2023).

15. W.F. Dewatisari, L. Rumiyanti, I. Rakhmawati, Rendemen and skrining fitokimia pada ekstrak daun *Sanseviera* sp. Jurnal Penelitian Pertanian Terapan. **17**(3), 197-202 (2017).
16. T. Hidayat, Nurjanah, M. Nurilmala, E. Anwar, Karakterisasi rumput laut tropika dari Kepulauan Seribu sebagai sumber bahan baku kosmetik. CR Journal. **4**(2), 49-62 (2019).
17. I. Savitri, L. Suhendra, N.M. Wartini, Pengaruh jenis pelarut pada metode maserasi terhadap karakteristik ekstrak *Sargassum polycystum*. Jurnal Rekayasa and Manajemen Agroindustri. **5**(3), 93-101 (2017).
18. J.C. Frisvad, Media and growth conditions for induction of secondary metabolite production. Fungal Secondary Metabolite. **944**, 47-58 (2012).
19. K.L. Rao, K.S. Raju, H. Ravisankar, Cultural conditions on the production of extracelluler enzymes by *Trichoderma* isolates from tobacco rhizosphere. Braz. J. Microbiol. **47**, 25-32 (2016).
20. F.J. Sami, N.H. Soekamto, Firdaus, J. Latip, Uji aktivitas antioksiand ekstrak alga coklat *Sargassum polycystum* and *Turbinaria deccurens* asal pulau Dutungan Sulawesi Selatan terhadap radikal DPPH. Jurnal Kimia Riset. **4**(1): 1-6 (2019).
21. L. Losada, O. Ajayi, J.C. Frisvad, J. Yu, WC Nierman, Effect of competition on the production and activity of secondary metabolites in *Aspergillus species*. Med. Mycol. J. **47** : 88-96 (2009).
22. R.M. Camacho, S.G. Morales, J.A. Fuentes, R.M. Jasso, A.B.Mendoza, A.V. Rodriguez, A.R. Olivo, Characterization of *Sargassum* spp. from the Mexican Carribbean and its valorization through fermentation process. MDPI. **11**(685), 1-15 (2023).
23. S. Prieto, M. Diaz, F. Barbieri, G. Tabanelli, F. Gardini, M.J. Valera, A.R. Bravo, V. Verardo, A.M. Caravaca, Impact of lactic acid bacteria fermentation on phenolic compounds and antioxidant activity of avocado leaf extracts. MDPI : Antioxidants. **12**(2), 1-17 (2023).
24. M. Jia, L. Chen, H. Xin, C. Zheng, K Rahman, T.Han, L Qin, A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol. **7**(906), 1-14 (2016).
25. I.C.Guaranda, R.V. Espinoza, A.B. Lucas, M.Q. Aviles, P. Manzano, Effect of solventsolvent partition on antioxidant activity and GC-MS profile of *Ilex guayusa* Loes. leaves extract and fractions. Nat. Prod. Res. **36**(6), 1570-1574 (2021).
26. Y. Alen, F.L. Agresa, Y. Yuliandra, Analisis kromatografi lapis tipis (KLT) and aktivitas antihiperurisemia ekstrak rebung *Schizostachyum brachycladum* Kurz (Kurz) pada mencit putih jantan. Jurnal Sains Farmasi & Kimia. **3**(2), 146-152 (2017).
27. M.U. Fauziah, A. Supriadin, N.T. Berghuis, Aktivitas antioksiand ekstrak metanol pada ekstrak virgin minyak zaitun kemasan. Al-Kimiya. **4**(2), 61-69 (2017).
28. A. Jankevics, A. Jenkins, W.B. Dunn, L. Najdekr, An improved strategy for analysis of lipif molecules utilising a reversed phase C30 UHPLC column and scheduled MS/MS acquisition. Talanta. **229**, 1-8 (2021).
29. J.W. Filho, T. Andrade, R.M. Lima, D.H. Silva, A.C. Reis, J.V. Santos, Cytogenotixic evaluation of the acetonitrile extract, citrinin and dicitrinin-A from *Penicillium citrinum*. Drug. Chem. Toxicol. **45**(2), 688-697 (2020).
30. T. Gateta, S. Nacoon, W. Seemakram, J. Ekprasert, P. Theerakulpisut, J. Sanitchon, N. Suwannarach, S. Boonlue, The potential of endophytic fungi for enchancing the growth and accumulation of phenolic compounds and anthocyanin in Maled Phai Rice (*Oryza sativa* L.). MDPI: Journal of Fungi. **9**(9), 1-19 (2023).