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Isolation, identification and biochemical studies of gallic acid from *Turbinaria decurrens* Bory

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ABSTRACT

Cancer is one of the diseases that cause the biggest death in the world. The research for natural compounds derived from marine is very developed at this time, especially in algae. Brown algae have more phenolic content than other types of algae, *T. decurrens Bory* is one of the brown algae that had taken from the Indonesian island of Dutungan, South Sulawesi. Phenolic groups are also known to be able to inhibit the growth of cancer cells. The purpose of this study is to isolate and identify compounds using spectrophotometer instruments. The isolates obtained were tested for anticancer activity against H460 and MCF-7 cells. Extraction was carried out by maceration method, fractionation was done by chromatography column eluent n-hexane:EtOAc: MeOH by increasing polarity. The structure determination is done based on the interpretation of FT-IR, 1D-NMR 1H,13C, and ESI-LCMS spectra data and anticancer activity test used MTT bioassay. The isolate is known as gallic acid, and the isolate was tested for anticancer activity against H460 and MCF-7 cells. The result is obtained IC_{50} value for H460 cells 5.69 µg/ml and MCF-7 cells 4.63 µg/ml. As a positive control used cisplatin with an IC_{50} value of 5.81 µg/ml against H460 cells and MCF-7 cells with a value of 5.59 µg/ml. Gallic acid has a higher toxic effect compared to cisplatin. One phenolic compound has been found in *T. decurrens* Bory which is gallic acid. Gallic acid has a higher toxic effect than cisplatin, this compound can be used as an anticancer agent.

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1. Introduction

Dutungan Island is located in Barru Regency, South Sulawesi, a rocky coastline, sandy bay and is a small coral island, providing special habitat for seaweed growing in this area. The last few years of research into the investigation of anticancer compounds from marine natural materials showed its activity as an anticancer (Khalifa et al., 2019; Simmons et al., 2005). *Turbinaria* is included in the genus brown seaweed (Phaeophyta), the brown color of this type is influenced by color pigments such as fucoxanthin, xanthophyl, carotenoids, and chlorophyll (Islami et al., 2014). The genus of *Turbinaria* has been spread in many Indonesian waters, one of which is found in the waters of Dutungan Island, South Sulawesi. Utilization of marine natural material as a source of new bioactive compounds has been developed, including as an antimicrobial, antiviral, and anticancer (Barzkar et al., 2019; Satari, 1996).

The genus *Turbinaria* has several potentials in the health field such as antibacterial (Kantida and Asha, 2012; Sridharan and Dhamotharan, 2012; Vijayabaskar and Shiyamala, 2011) antitumor (Fajarningsih et al., 2008), and antioxidants (Sami et al., 2019a). Thus, *Turbinaria* is a good candidate to be developed in the investigation for anticancer drugs. Phytochemical screening of *T. decurrens* Bory contains several secondary metabolites including phenolics, steroids, terpenoids, flavonoids (Sami et al., 2019a). It is known that the brown algae have more phenolic content than other algae, the phenolic content that is abundant in this type is the derivate of phlorotannin (Girija et al., 2013; Stiger et al., 2004).

In terms of the economic potential of *T. decurrens* Bory there has not been much research for bioactive compounds, even though this species actually has a considerable opportunity to be used as raw material in the pharmaceutical industry. Consequently, the purpose of this study was to isolate the phenolic group of *T. decurrens* Bory from ethyl acetate extract, and test isolates against breast cancer cells (MCF-7) and lung cancer cells (H460).

2. Materials and methods

2.1. Materials

All solvent methanol, ethyl acetate, n-hexane, acetonitrile, formic acid, were commercial grade Merck. The chromatography used silica gel 60 (0.040–0.063 mm), and thin-layer chromatography (TLC) used silica gel 60 F_{254} (Merck, Germany). MTT assay used RPMI-1640 (Sigma-Aldrich), fetal bovine serum (FBS, Sigma-Aldrich), DMSO (Merck, Germany), trypan blue (Sigma-Aldrich), trypsin-ethylenediaminetetraacetic acid (Sigma-Aldrich), cisplatin (Sigma-Aldrich), and MTT reagent (Sigma-Aldrich). Two human cancer cell lines were used: MCF-7 breast adenocarcinoma cancer cells (ECACC), and H460 lung cancer cells (ATCC HTB-177, Summit Pharma).

The sample used in this study was *Turbinaria decurrens* Bory brown algae collected from Dutungan Island, Barru Regency, South Sulawesi, Indonesia.

2.2. Methods

2.2.1. Extraction and isolation

Brown algae *T. decurrens* Bory (1 Kg) was extracted with ethyl acetate to obtain a crude extract (10 g). The crude extract was fractionated using silica gel vacuum liquid chromatography by increasing polarity of n-hexane-EtOAc-MeOH to produce 5 fractions. Subsequent purification of the D fraction (2 g) was carried out using flash chromatography eluted with 80:20 n-hexane-EtOAc to producing 12 sub-fractions. Subfraction 11 was purified in the same manner as the n-hexane-EtOAc-MeOH eluent by increasing polarity to produce compound 1 (50 mg).

2.2.2. Cytotoxic effect assay

Cytotoxic activity of the isolate was determined by the MTT colorimetric assay method (3- [4, 5-dimethylthiazol-2-yl] -2.5 diphenyltetrazolium bromide). H460 and MCF-7 cell culture needed 90 µL in wells with a density of 10^4 cells per well in 96 wells and incubated for 24 hours at 37°C in 5% CO_2. 10 µL isolate pipette containing various concentrations of 2, 4, 6, 8, and 10 µg/ml and cisplatin as a positive control was inserted into each well and incubated 72 hours at 37°C in 5% CO_2. The media was removed and add 100 µL MTT and the cells are incubated again for 3 hours under the same conditions. The MTT solution was removed and the formazan crystals formed were re-dissolved with DMSO, and incubated for 15 minutes. The absorbance is read at a wavelength of 570 nm. Inhibition of cancer cell growth was calculated by the equation below and IC_{50} values were determined using linear regression analysis of an average of three replications (Aslantürk, 2018).

$$\text{Cytotoxic \%} = 1 - \left(\frac{Abs\ sample - Abs\ medium}{Abs\ solvent - Abs\ medium}\right) \times 100\%$$

2.2.3. Analysis data

The isolated compound was identified using FT IR (Shimadzu), ESI LC-MS (Bruker) and 1D NMR spectrophotometer (JEOL). ^{1}H NMR was measured in 400 MHz while ^{13}C in 125 MHz using CD_3OD solvent.

3. Results and discussion

3.1. Results

3.1.1. Tests for phenol

The isolated compound 1 gave a positive test to phenol by using $FeCl_3$, the isolate gave blue color. Ethyl acetate extracts had more phenolic content compared to n-hexane and methanol extracts in *T. decurrens* Bory (Sami et al., 2019b).

Compound 1 was isolated as a white powder with the molecular formula $C_7H_6O_5$, using ESI-LC-MS [M-H] 169.17 m/z, melting point 248-250°C. FT-IR spectrum showed several functional groups including hydroxyl groups (3369.64 cm^{-1}), alkene (= CH) unsaturated (3001.24 cm^{-1}), carbonyl groups (1705.07 cm^{-1}), aromatic alkene groups (1541.12 cm^{-1}).

Table 1. ^{1}H-NMR and ^{13}C-NMR (400 MHz) of the isolate and gallic acid reference (CD_3OD, in ppm) (Dao et al., 2018)

Position of C	Isolate		Gallic acid reference	
	^{1}H NMR[a]	^{13}C NMR[a]	^{1}H NMR[b]	^{13}C NMR[b]
1		122.4		122.1
2	7.03 1H s	110.7	7.06 1H s	110.4
3		146.7		146.3
4		139.9		139.5
5		146.7		146.3
6	7.03 1H s	110.7	7.06 1H s	110.4
7		170.8		170.6

3.1.2. MTT assay

The anticancer activity of isolate was evaluated using MTT assay, with each concentration 2, 4, 6, 8, and 10 µg/ml. The experiment was done in triplicate and the absorbance was measured at 570 nm by using 96 well microplate reader. Cisplatin was used as a positive control with the same procedure

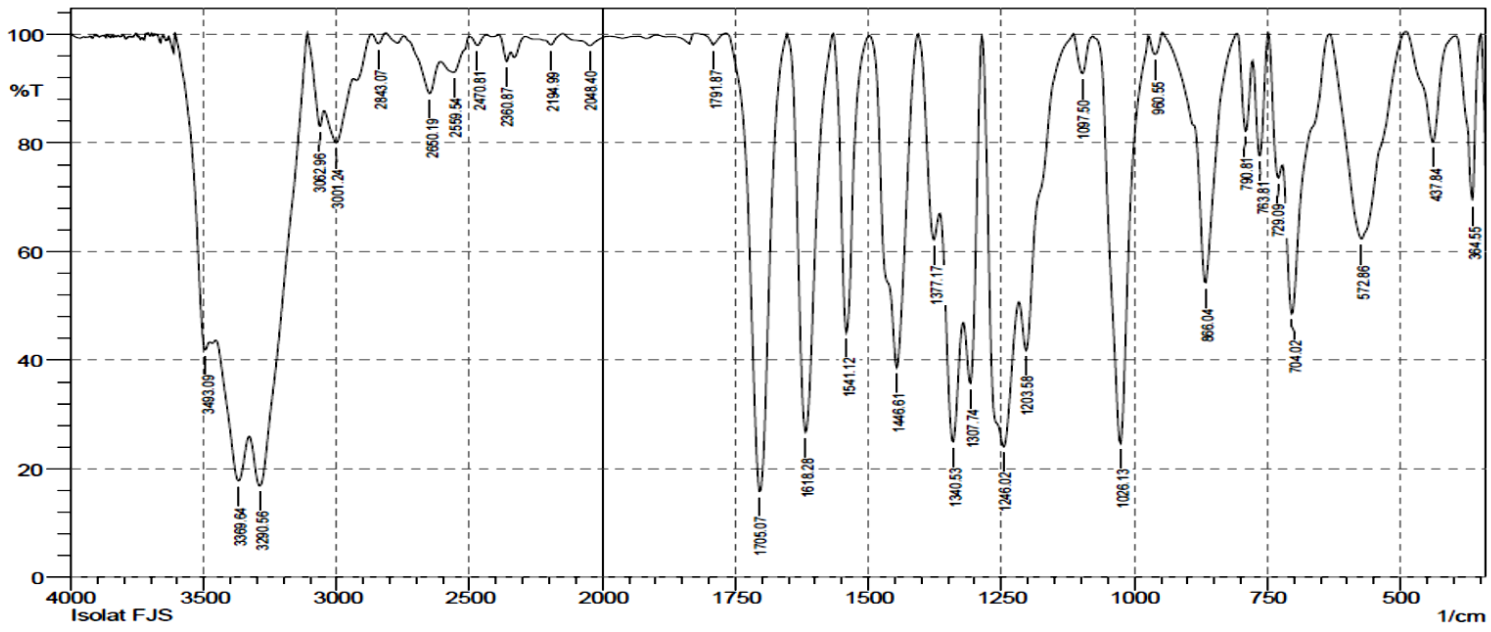

Fig. 1. FT-IR spectrum of gallic acid

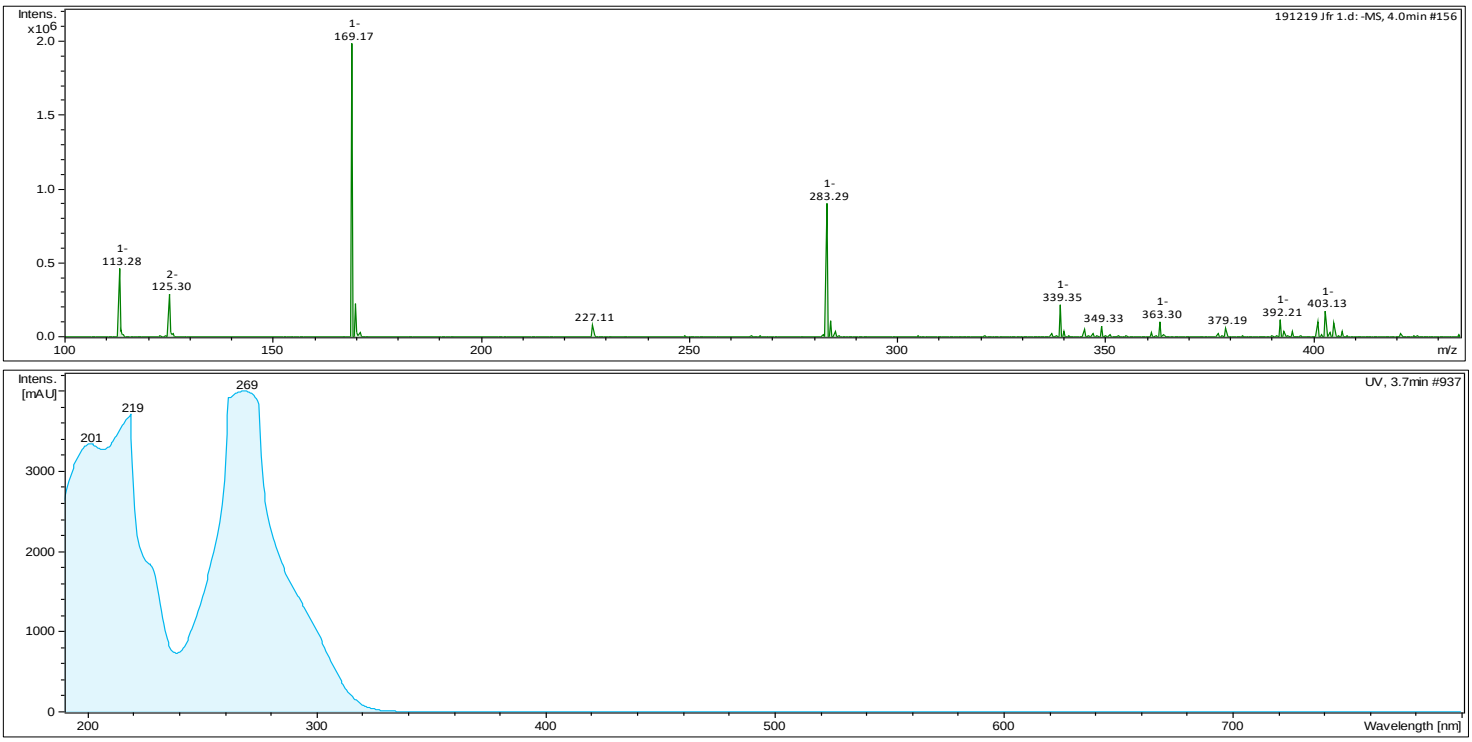

Fig. 2. ESI MS (above) and UV (below) spectra of gallic acid

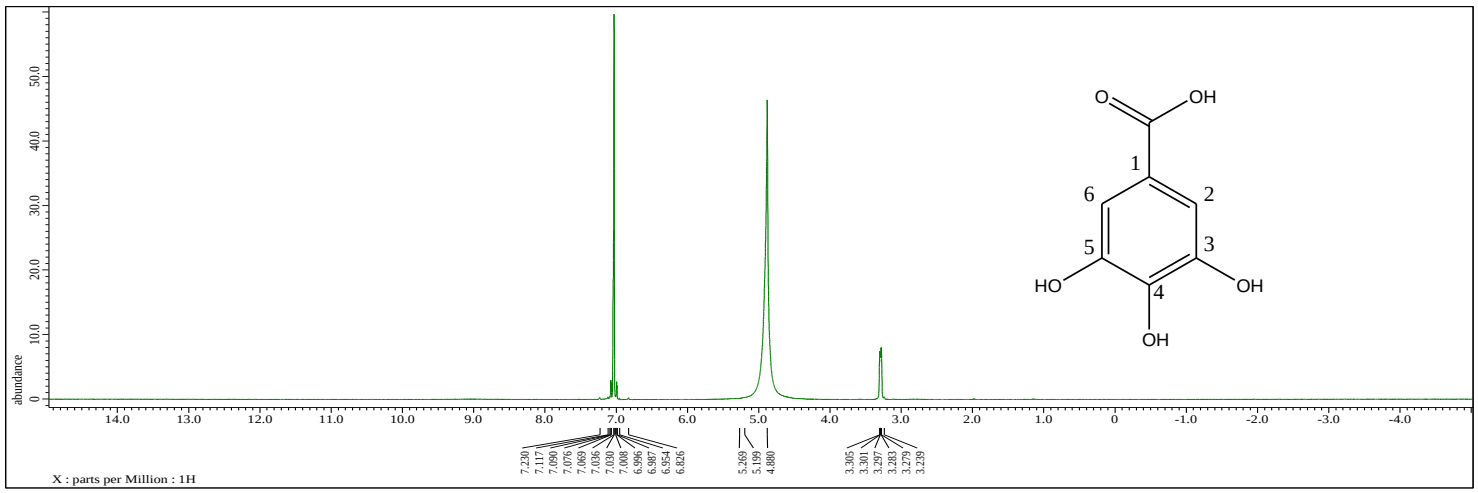

Fig. 3. [1]H NMR spectrum of gallic acid

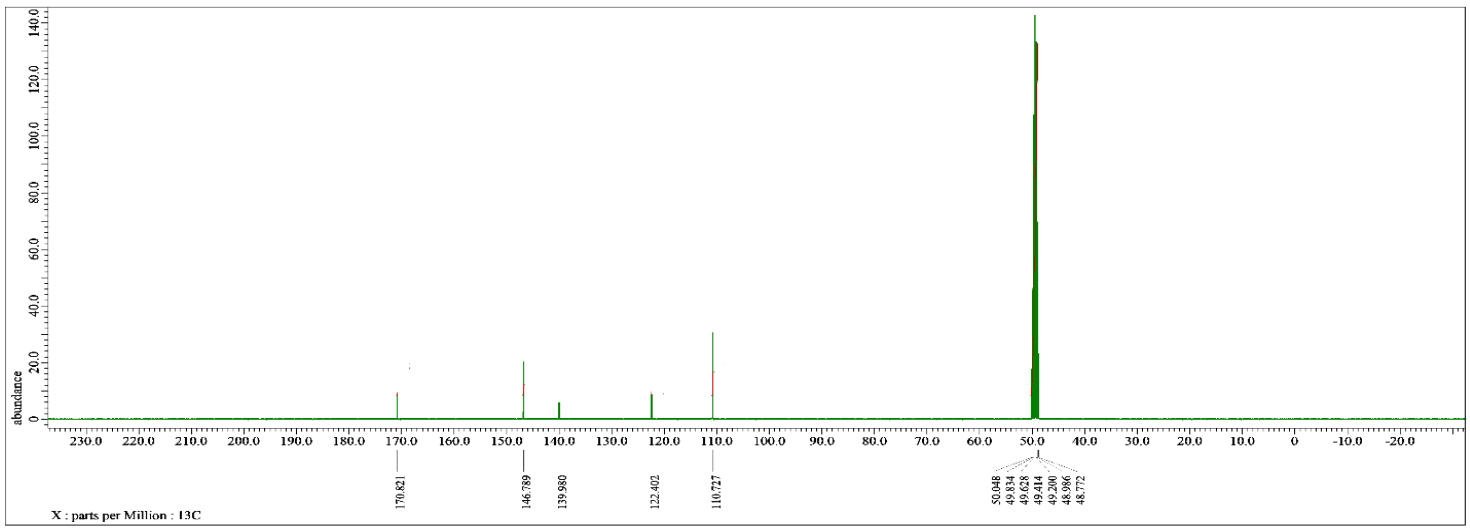

Fig. 4. [13]C NMR spectrum of gallic acid

3.2. Discussion

Gallic acid: White powder, ESI-LCMS 169.17 m/z [M-H]. ^{1}H-NMR (CD_3OD, 500 MHz) δ (ppm): 7.03 (1H, s, H-2, H-6); ^{13}C-NMR (CD_3OD, 125 MHz), δ (ppm): 122.4 (C-1), 110.7 (C-2, C-6), 146.7 (C-3, C-5), 139.9 (C-4), 170.8 (C-7). In the ^{1}H-NMR spectrum the strong signal δ 7.03 confirmed an aromatic (1H, s, H-2, H-6) of this compound. In the ^{13}C-NMR signal of aromatic ring δ 122.4 (C-1), 110.7 (C-2, C-6), 146.7 (C-3, C-5), and 139.9 (C-4). The most downfield signal at 170.8 comes from carbonyl carbon (C-7). The spectra were identified as gallic acid with 6 carbon signals and one carbonyl group signal which was compared with the literature (Dao et al., 2018).

Table 2. Anticancer activity of isolate against H460 and MCF-7 by MTT assay

Sample	Concentration, µg/ml	Cytotoxic % H460 cells	IC_{50}, µg/ml	Cytotoxic % MCF-7 cells	IC_{50}, µg/ml
Gallic acid	2	-6	5.69	34	4.63
	4	22.2		47.3	
	6	72.1		57.7	
	8	82.4		69	
	10	100		83	
Cisplatin	2	0.2	5.81	41.9	5.59
	4	24.5		66.5	
	6	60.6		75.5	
	8	76.5		83.3	
	10	100		91.1	

MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) is a laboratory test based on discoloration (colorimetric) to measure cancer cell proliferation. Application MTT test is very useful, MTT test can be done by measuring the activity of the mitochondrial enzyme succinate dehydrogenase in 96 well plates. This trial was carried out for in vitro a substance which potential as cytotoxic. In this study we applied the MTT test to evaluate the potential of isolates as anticancer using breast and lung cancer cells in vitro. MTT measures cell respiration and the amount of formazan formed is proportional to the number of living cells present in culture. An increase or decrease in the number of cells results in a simultaneous change in the amount of formazan formed, the effect of the level of cytotoxicity caused by the substance (Nur et al., 2021; 2022).

The cytotoxic activity of MCF-7 breast cancer cells and H460 lung cells showed with IC_{50} values MCF-7 of 4.63 µg/ml and H460 lung cells of 5.69 µg/ml. Cisplatin is used as a positive control test for MCF-7 breast cancer cells having an IC_{50} value of 5.59 µg/ml and testing of H460 lung cells IC_{50} value of 5.81 µg/ml. IC_{50} is the concentration of the drug being tested that can cause 50% cell death and can predict the level of cytotoxic effects, the lower the value, the more cytotoxic it is. Cisplatin is a chemotherapy agent that is widely used in the treatment of cancer. Thus, isolate (gallic acid) has more toxic activity compared to cisplatin, this means that isolate has the potential as an anticancer candidate compound.

4. Conclusions

Isolation and identification of secondary metabolites of *T. decurrens* Bory from ethyl acetate extract got a phenolic compound, gallic acid. Gallic acid has been tested for anticancer activity against H460 and MCF-7 cells with IC_{50} of 5.69 and 4.63 µg/ml, respectively.

Conflict of interest

Authors declare no conflict of interest.

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