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Original Article

POTENTIAL CELL PROLIFERATION INHIBITOR ISOLATED FROM INDONESIAN BROWN ALGAE (PHAEOPHYTA)

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ABSTRACT

Objective: The objective of this study is to determine the toxic activity of n-hexane and ethyl acetate extracts of brown algae as anticancer candidates.

Methods: The brown algae were collected from West Java south coast, identified and then dried. The dry algae was then extracted by using n-hexane and ethyl acetate, filtered, then dried. The toxic activity of n-hexane and ethyl acetate extracts of five species brown algae was screened by using the brine shrimp lethality test (BSLT). The detection for chemical compound was carried out by placing the extracts on a Thin Layer Chromatography (TLC) plate and spraying them with several spray reagents such as Dragendorff, Citro boric acid, and vanillin-sulfuric acid.

Results: We identified five species of brown algae i.e.: *Sargassum* sp., *Sargassum duplicatum* J. G. Agardh, *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh, and *Turbinaria decurrens* Bory. Four of ten extracts had toxic activities, i.e.: n-hexane extract of *Sargassum myriocystum* J. G. Agardh (LC_{50}= 273.28 µg/ml), n-hexane extract of *Turbinaria ornata* (Turner) J. G. Agardh (LC_{50}= 320.4 µg/ml), n-hexane extract of *Turbinaria decurrens* Bory (LC_{50}= 579.33 µg/ml), and the ethyl acetate extract of *Sargassum* sp. (LC_{50}= 743.98 µg/ml), whereas *Sargassum duplicatum* J. G. Agardh was found to be less toxic (nontoxic) (LC_{50}>1000 µg/ml). The active compound of this alga was detected in TLC is terpenoid.

Conclusions: N-hexane extract of *Sargassum myriocystum* J. G. Agardh showed the highest toxicity in the brine shrimp assay (LC_{50}= 273.28 µg/ml).

Keywords: Brown algae, Phaeophyta, Brine shrimp lethality assay, Anticancer.

INTRODUCTION

Algae are autotrophic organisms that do not have leaves, trunks and roots. Algae are classified within Thallophyta because of their morphology. Algae divide into micro algae and macro algae. Microalgae are found in benthic and littoral habitat, and are also found in marine waters as phytoplankton. Macro algae presented in littoral regions, grown in intertidal and sub-tidal areas. Macro algae are divided into green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) on the basis of their color chemical composition. Algae have been considered as a source of bioactive compounds as they may be used as food, material for industry and herbal medicine [1].

Traditionally, algae have been used as a supplement and herbal medicine because of their metabolites and minerals that are useful as antibacterial, antioxidant, anticancer, anti-inflammatory agents and they have also a cytotoxic activity [2]. Chinese and Japanese traditional medicines have been using algae as a medicinal herb for a long time. *Laminaria* sp., *Ecklonia* sp., and *Sargassum* sp. are used for tumor therapy [3]. The Vietnamese who live on the seashore consume algae as vegetable salads, pickles, jellies and soups. They also use algae as herbal medicine to heal cough, asthma, abdominal pain, headache and to suppress tumor growth [4].

Cytotoxicity of plant materials, including marine algae, indicates the presence of anticancer compounds. BSLT has successfully been used as prescreening of bioactive compounds having anticancer activity [5]. This bioassay has been established as a safe, practical and economical method for determining the bioactivity of marine products as well as of higher plant products [6, 7]. There is a significant correlation between BSLT and cytotoxicity in human carcinoma cell lines [8].

The metabolites synthesized by the marine brown algae are well known for their cytotoxic properties. Cytotoxic properties by plant materials are due to the presence of antitumor compound [5]. The sulphated polysaccharide of brown algae has potentiality to inhibit cell proliferation and to induce apoptosis. It has been reported that the polysaccharides are able to inhibit the proliferation of human lymphoma cell lines [9]. Other studies showed that the polysaccharides of various types of brown algae are able to inhibit angiogenesis, cell proliferation and induce apoptosis as well [10-12]. Carotenoids of brown algae are likewise induced apoptosis and inhibit cell proliferation [13].

A previous study of *Sargassum* sp. showed its cytotoxic effect on several cancer cell lines; however, the effect on the non-cancerous cells was not discussed in these studies. Aqueous extract of red algae *Gracilaria corticata*, from the Persian Gulf seashore, showed cytotoxic activities against Jurkat and Molt-4 human cancer cell lines [14]. The organic solvents extracts of brown algae *Sargassum swartzii, Cystoseira myric* and *Colpomenia sinuosa* collected from the Persian Gulf demonstrated a cytotoxic effect against a panel of cell lines including HT-29, Caco-2, T47D, MDA-MB468 and NIH 3T3 cell lines [15].

A polysaccharide isolated from macro alga *Sargassum stenophyllum* also showed antiangiogenic and antitumor activities [16]. The authors suggested a role of the polysaccharide in modulation of the activity of angiogenic growth factors.

Indonesia has a large size of ocean containing very high biodiversity of algae. Indonesian algae are generally divided into three divisions, i e.: red algae (Rhodophyta) consist of 452 species, green algae (Chlorophyta) consist of 196, species and brown algae (Phaeophyta) consist of 134 species [17]. Unfortunately, the pharmacological study of Indonesian brown algae are limited, therefore we perform this study.

Toxicity tests of n-hexane and ethyl acetate extracts of Indonesian brown algae have not been reported. This study was conducted to explore the secondary metabolite of brown algae from the southern coast of West Java Indonesia which has the potential to be developed into anticancer.

MATERIALS AND METHODS

Collection of brown algae

The brown algae was collected from Santolo Beach, Pameungpeuk, West Java. The algae was then identified and determined in the Research Center of Oceanography-Indonesian Institute of Sciences. Samples of each species were then washed in running fresh water to remove salt, sand, epiphytes and other adhering detritus matters. Cleaned brown algae were air dried under shade. The dried algae was finely ground in an electric grinder.

Extraction

Five hundred (500) grams of dried brown algae powder were extracted three times by using n-hexane and ethyl acetate for three days and then filtered. The filtrates were pooled and concentrated on a rotary vacuum evaporator.

Hatching the brine shrimp cysts

One (1) g of *Artemia salina* (L) cyst (Sanders TM Great Salt Lake, Brine Shrimp Company L. C., U. S. A.) was hatched in sea water at 27-30^{o}C with aeration. Plastic chamber with two unequal compartments connected by holes on the divider was used for hatching. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 h incubation at room temperature (25-19 ^{o}C), larvae were collected by pipette from the illuminated side and used for the brine shrimp lethality test.

BSLT

BSLT was used to determine the toxicity of n-hexane or ethyl acetate extracts of brown algae. Various concentrations of n-hexane and ethyl acetate extracts (1, 10, 100, 1000 µg/ml) were prepared in glass vials. Ten brine shrimp larvae were moved into each glass vial containing the extracts and supplemented with sea water. The vials were incubated for 24 h under constant illumination at room temperature (25-29 ^{o}C). After 24 h, the dead larvae were counted. LC_{50} was determined by using probit analysis.

Phytochemical screening by TLC

TLC was utilized to fractionate the extract and investigate the composition of the extracts. The detection of chemical compounds was done by spraying the chromatograms with several spray reagents such as Dragendorff for detection of alkaloids, Citroboric acid for detection of flavonoid, and vanillin-sulfuric acid for detection of terpenoid.

RESULTS

Brown algae collection

The samples of brown algae were collected from the Santolo Beach, Pameungpeuk, Garut, West Java. There were eight (8) types of algae (sample code: A, B, C, D, E, F, G, H) identified and determined by The Research Center of Oceanography-Indonesian Institute of Sciences, and we found five (5) species of brown algae, i.e.: *Sargassum* sp., *Sargassum duplicatum* J. G. Agardh, *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory as shown in table 1.

Extraction yield

The brown algae (*Sargassum* sp., *Sargassum duplicatum* J. G. Agardh, *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory were extracted by maceration method in n-hexane and ethyl acetate. As expected, the type of solvent affected the amount of the yield according to the solubility of extractive compounds. The yield of extraction ranged between 0.05%-0.89%. The yield of the ethyl acetate extract was higher than that of n-hexane extracts (table 2).

BSLT RESULTS

The extract of a plant was considered toxic to *Artemia salina* if its LC_{50} value was less than 1000 ppm [18]. Our results showed that four extracts out of ten examined extracts had toxic activities, i.e.: n-hexane extract of *Sargassum myriocystum* J. G. Agardh (LC_{50}= 273.28 µg/ml), n-hexane extract of *Turbinaria ornata* (Turner) J. G. Agardh (LC_{50}= 320.4 µg/ml), n-hexane extract of *Turbinaria decurrens* Bory (LC_{50}= 579.33 µg/ml) and ethyl acetate extract of *Sargassum* sp. (LC_{50}= 743.98 µg/ml), whereas *Sargassum duplicatum* J. G. Agardh was found to be nontoxic (LC_{50} =>1000 µg/ml). The results revealed that the n-hexane extracts were more toxic than ethyl acetate extracts. The results of BSLT of those five brown algae (Phaeophyta) are shown in table 3.

Phytochemical screening of n-hexane extracts of *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory was carried out by TLC spray with reagents selected to predict presence of active compounds. We found that the active compounds in brown algae are terpenoids (table 4).

DISCUSSION

We found that five species of algae were abundant in the samples collected from the sea at the Santolo beach, Pameungpeuk, Garut, West Java, i.e.: *Sargassum* sp., *Sargassum duplicatum* J. G. Agardh, *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory. These algae attached to rocks or coral by flattened disks and can be torn off from the substrates during massive waves and washed up on the beach. Their color ranges from light brown to dark brown. *Turbinaria* grows in large areas both in the intertidal and sub tidally. This alga has cylindrical branches with a diameter of 2-3 mm and short lateral branches of 1-1.5 cm in length. There are three species Turbinaria in Indonesia, namely *Turbinaria conoides, Turbinaria ornata* and *Turbinaria decurrens*. However, the first one was missing in the collected samples originating from the Santolo beach.

Semi polar compounds dissolved in ethyl acetate, while the non-polar compounds dissolved in n-hexane. The yield of the ethyl acetate extract of *Sargassum myriocystum* J. G. Agardh (0.89%) is the highest one among the yields of the brown alga extracts. This demonstrates that the content of semi polar compounds in brown algae is higher than non-polar compounds. Generally, several factors may affect the yield of the extract such as extraction method, extraction time, type of the solvent, concentration of solvent and drying method [19-21].

In this study, n-hexane extract of *Sargassum myriocystum* J. G. Agardh, n-hexane extract of *Turbinaria ornata* (Turner) J. G. Agardh and n-hexane extract of *Turbinaria decurrens* Bory showed the toxic activity to the brine shrimp. The highest toxicity was found in n-hexane extract of *Sargassum myriocystum* J. G. Agradh (LC_{50}= 273.28 µg/ml), whereas n-hexane and ethyl acetate extracts of *Sargassum duplicatum* J. G. Agardh had no toxic activity in this assay. Therefore, it may be hypothesized that some secondary metabolite dissolved in then-hexane extract may exert the toxic effect on brine shrimp. The concentration of secondary metabolites determines the level of toxicity. The secondary metabolite of *Sargassum myriocystum* J. G. Agardh, *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory is terpenoid (table 4). Higher terpenoid concentration of *Sargassum myriocystum* J. G. Agardh may cause higher toxicity of this species compared to the *Turbinaria ornata* (Turner) J. G. Agardh and *Turbinaria decurrens* Bory.

Terpenoid is known as a potent compound active against cancer [22]. Meroditerpenoid from *Stypopodium flabelliforme* (Phaeophyta) has been reported to have specific antiproliferation activity in several cell lines [23]. Diterpenes from methanol extracts of *Padina pavonia* collected from the Red Sea at Hurghada, Egypt, also showed anti-tumor activities against lung carcinoma (H460) and liver carcinoma (HepG2) human cell lines (*in vitro*) [24]. Diterpenes in the species of *Sargassaceae* also showed anti-tumor activities on Daudi, Jurkat and K562 cell lines [25].

Previous research has revealed that sterols of brown algae had cytotoxic activity. The isolated sterols from *Turbinaria conoides* exhibit cytotoxicity against various cancer cell lines [26]. Sterol from *Sargassum carpophyllum* also inhibited proliferation in several human cancer cell lines [27]. Terpenoid is also known as larvicide active against several insects. Terpenoid from plant *Copaifera reticulata* had larvicidal activity on *Aedes aegypti* larvae [28]. In other insect (Lepidoptera) larvae, terpene blocks the stimulatory effect of glucose and inositol on chemosensory receptor cells located on the mouth [29]. It is possible that terpenoids could kill Artemia larvae in the same way.

Table 1: Classification of brown algae collected from Santolo Beach, Pameungpeuk, Garut, West Java

Samplecode	Order	Family	Genus	Species
A	Fucales	Sargassaceae	Sargassum	*Sargassum* sp.
B	Fucales	Sargassaceae	Sargassum	*Sargassum duplicatum* J. G. Agardh
C	Fucales	Sargassaceae	Sargassum	*Sargassum myriocystum* J. G. Agardh
D	Fucales	Sargassaceae	Sargassum	*Sargassum duplicatum* J. G. Agardh
E	Fucales	Sargassaceae	Sargassum	*Sargassum duplicatum* J. G. Agardh
F	Fucales	Sargassaceae	Sargassum	*Sargassum duplicatum* J. G. Agardh
G	Fucales	Sargassaceae	Turbinaria	*Turbinaria ornata* (Turner) J. G. Agardh
H	Fucales	Sargassaceae	Turbinaria	*Turbinaria decurrens* Bory

Table 2: Yield of brown algae extraction

Species	Extract	Yield (%)
Sargassum sp.	N–hexane	0.21
	Ethyl acetate	0.71
Sargassum duplicatum J. G. Agardh	N–hexane	0.40
	Ethyl acetate	0.10
Sargassum myriocystum J. G. Agardh	N–hexane	0.17
	Ethyl acetate	0.89
Turbinaria ornata (Turner) J. G. Agardh	N–hexane	0.05
	Ethyl acetate	0.10
Turbinaria decurrens Bory.	N–hexane	0.14
	Ethyl acetate	0.30

Table 3: Toxic activities of brown algae extracts on brine shrimp

Brown algae species	Extracts	LC_{50} (µg/ml)
*Sargassum*sp.	N-hexane	>1000
	Ethyl acetate	743.98
Sargassumduplicatum J. G. Agardh	N-hexane	>1000
	Ethyl acetate	>1000
*Sargassumm myriocystum*J. G. Agardh	N-hexane	275.28
	Ethyl acetate	>1000
Turbinaria ornata (Turner) J. G. Agardh	N-hexane	320.4
	Ethyl acetate	>1000
Turbinaria decurrens Bory	N-hexane	579.33
	Ethyl acetate	>1000

Table 4: Phytochemical screening of brown algae extracts

Species	Extract	Alkaloid	Flavonoid	Terpenoid
Sargassum myriocystum J. G. Agardh	N-hexane	-	-	+
	Ethyl acetate	-	-	+
Turbinaria ornata (Turner) J. G. Agardh	N-hexane	-	-	+
	Ethyl acetate	-	-	+
Turbinaria decurrens Bory	N-hexane	-	-	+
	Ethyl acetate	-	-	+

-= not present, += present

Our study showed that brown algae extracts had toxic activity on brine shrimp. According to the interpretation of the brine shrimp assay, this toxic effect may be considered as an anticancer activity of the brown algae extracts. However, it should be clarified whether this effect is generally cytotoxic or whether it is specific towards the cancer cells. Further studies are also required to isolate and characterize the active compounds from these extracts. It remains to be proved that the active compounds could play antiproliferative role as e. g. induce apoptosis in the cancer cell lines.

CONCLUSION

We found that n-hexane extract of *Sargassum myriocystum* J. G. Agardh showed the highest toxicity on brine shrimp (LC_{50}= 273.28 µg/ml). The extract of *Sargassum myriocystum* J. G. Agardh also contained terpenoids. Further isolation of such active compounds and characterization of their biological activity may lead to the discovery of new anticancer agents.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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