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Original Research Article

Phytochemical screening and cytotoxic activities of *Enhalus acoroides* (L.f.) Royle and *Halimeda macroloba* Decaisne on cervical cancer cell lines

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Abstract

Purpose: *To determine the cytotoxic effects of phytochemical compounds in Enhalus acoroides and Halimeda macroloba crude extracts on cervical carcinoma and normal kidney tissue cells.*
Methods: *Dried Enhalus acoroides and Halimeda macroloba were macerated in ethanol. Then, phytochemical screening was done using thin-layer chromatography (TLC). The cytotoxic activities of extracts against HeLa and SiHa cervical cancer cells were performed using MTT assay.*
Results: *The H. macroloba extract was highly active against SiHa cells, with an IC_{50} of 17.22 ± 3.93 µg/mL, and moderately active against HeLa cells, with an IC_{50} of 36.57 ± 7.26 µg/mL. The E. acoroides extract was moderately active against both HeLa and SiHa cells. Moreover, both extracts exhibited lower toxicity against HEK293 normal kidney cells, with IC50 values of > 80 µg/mL.*
Conclusion: *These results suggest that E. acoroides and H. macroloba exert potent cytotoxic activity on cervical cancer cells. The mode of action associated with the cytotoxicity of these extracts, and the effect of the combination of the extracts with chemotherapy, should be investigated in subsequent studies.*

Keywords: *Cytotoxicity, Cancer cell, Phenolic compounds, Seagrass, Seaweed*

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INTRODUCTION

Seagrasses and seaweeds are photosynthetic organisms that provide food, nursery, and shelter for marine organisms, and they contain many biologically active compounds [1,2]. They are considered natural sources of secondary metabolites such as phenolics, flavonoids, saponins, steroids, alkaloids, and polysaccharides, some of which have antioxidant, anticancer, anti-inflammatory, anti-microbial, and antiviral properties [3,4].

Natural products derived from seagrasses and seaweeds have been extensively commercialized in the pharmaceutical, medical, health supplement, and cosmetic industries [3]. Biological assays have shown that some of the

bioactive antioxidant secondary metabolites of seagrasses and seaweeds have the potential for use in cancer treatment [5]. Twenty phytocompounds identified from a hydroalcoholic extract of *Enhalus acoroides* were found to have antioxidant, anticancer or anti-hepatotoxic activities [4]. Studies on the seagrasses *Posidonia spp*. and *Syringodium isoetifolium* have also revealed the presence of biologically active compounds that have anticancer activities [3, 6].

The seagrass *Enhalus acoroides* (L.f.) Royle is common and abundant along the Andaman Coast of Thailand. The largest bed of the species is found in Trang Province [7]. The seaweed *Halimeda macroloba* Decaisne is a very common and dominant species with high density at the intertidal zone of Lidee Island, Satun Province [8]. Both species have been used to reinforce biodegradable composite materials [9,10]. Recently, the bioactivity of fucoidan extracted from *S. plagiophyllum* from Thailand was reported for the first time, along with its nutritional composition [2]. Previously, the anti-proliferative effect of *S. oligocystum* against Adriamycin-resistant human small cell lung carcinoma cell line (GLC4 / Adr), was reported [1]. In addition, the latest study on phenolic compounds extracted from *Sargassum plagiophyllum* revealed that these compounds exerted potent anticancer activity against colon (HCT116 and PMF-k014) and cervical cancer cells (HeLa and SiHa) [11].

Nonetheless, *E. acoroides* and *H. macroloba* have not been utilized as sources of metabolites for alternative cancer treatment, notwithstanding that they are rich sources of valuable compounds. Therefore, this study was carried out to evaluate the potential of *E. acoroides* and *H. macroloba* as functional foods that may serve as natural medicines for cancer treatment. To this end, phytochemical screening of crude extracts of the two species and assessing their cytotoxic effects on cervical carcinoma cell lines was done.

EXPERIMENTAL

Collection of plant samples

Whole plants of *E. acoroides* (Ea) and *H. macroloba* (Hm) were collected from Koh Mook, Trang Province, and Koh Lidee, Satun Province, respectively. The plants were identified by Jaruwan Mayakun and samples were deposited in the herbarium of the Seaweed and Seagrass Research Unit, Prince of Songkla University, Songkhla, Thailand and herbarium numbers PT30 and SP500 were assigned, respectively. The plant samples were put in clean bags under dark and cold conditions and were immediately transferred to the laboratory. After washing to remove dirt and unwanted materials, the plants were oven-dried at 60 °C for 96 h. The dried samples were finely ground, and the powders were subjected to phytochemical screening.

Preparation of crude extracts

The dried and ground aerial parts of *E. acoroides* (164.95 g) and *H. macroloba* (52.13 g) were extracted with ethanol at room temperature, and the extracts were dried under low pressure. The dried materials were preserved in sterile containers, prior to screening or phytochemical compositions.

Phytochemical analysis

Using standard methods, qualitative phytochemical analysis was done to determine the main phytochemical compounds in the extracts [12]. Thin-layer chromatography analysis was done using the procedures outlined by Wagner *et al* [13]. Each sample was analyzed with thin-layer chromatography using aluminum-coated silica gel 60 GF 254 TLC plate. Sample spots were identified in a TLC visualizer at 254 and 366 nm using a detecting reagent of vanillin-sulfuric acid.

Cell culture

In this study, the *homo sapiens* cervix carcinoma cells SiHa (ATCC® HTB-35™) and HeLa (ATCC® CCL-2™) were used. The HEK293 embryonic kidney cell line served as normal cells. The cell lines, which were obtained from the American Type Culture Collection ATCC), Manassas, VA, were maintained in DMEM containing 10 % FBS, penicillin (100 U/mL), L-glutamine, and streptomycin (0.1 mg/mL). Incubation of the cells was done in humidified atmosphere containing 5 % carbon dioxide at 37 °C.

Cytotoxicity assay

The MTT assay was used to measure the cytotoxicity of crude extracts from *E. acoroides* and *H. macroloba* on cervical cancer and normal kidney cells. The HeLa, SiHa and HEK293 lines were separately seeded at a density of 5×10^3 cells in 96-well plates containing 150 µL of complete medium per well. Following 24-h adherence, the cells were exposed to the extracts which were diluted to concentrations of 5 – 80 µg/mL in medium, with DMSO and doxorubicin as negative and positive controls, respectively. After a 72-h incubation, the cells

were rinsed in PBS, and 100 µL of MTT solution (0.5 mg/mL) was added to each plate well. The resultant formazan crystals were solubilized with DMSO, and the absorbance of each formazan solution was measured in a multi-mode microplate instrument at wavelengths of 570 and 650 nm. Using the data, the determination of cell viability was carried out as outlined earlier, and the values of IC_{50} were derived using the procedure reported previously [14]. Cytotoxic activity is categorized into four groups as follows: highly active (IC_{50} ≤ 20 µg/mL), moderately active (IC_{50} > 21 – 200 µg/mL), weakly active (IC_{50} > 201 – 500 µg/mL), and zero cytotoxicity (IC_{50} > 501 µg/mL).

Statistics

The results are presented as mean ± standard deviation (SD) of 3 separate assays. Statistical comparisons of mean values were done with one-way ANOVA. Values of $p < 0.05$ were taken as indicative of statistical significance.

RESULTS

Phytochemical screening

The phytochemical constituents of the screened extracts are shown in Table 1. Alkaloids, steroids, saponins, phenolic compounds and flavonoids were present in both species, whereas anthraquinones, tannins, glycosides and coumarin were absent. Only the *H. macroloba* extract contained terpenoids.

Cytotoxicity of extracts against cervical cancer cells

The assay of cytotoxic activities of *E. acoroides* and *H. macroloba* extracts on the cancer cells and normal kidney cells revealed that the extracts produced significant and dose-reliant toxicity on HeLa and SiHa cells (Figure 1). The viable carcinoma cell population decreased significantly with increase in extract dose. The IC_{50} values after 72 h (Table 2) showed that *H. macroloba* extract was highly active against SiHa, with IC_{50} of 17.22 ± 3.93 µg/mL, but moderately active against HeLa, with IC_{50} of 36.57 ± 7.26 µg/mL. In contrast, *E. acoroides* extract exerted moderate cytotoxicity on the two cell lines. However, *E. acoroides* and *H. macroloba* extracts exhibited weak cytotoxic effects on HEK293 normal kidney cells, with IC_{50} values > 80 µg/mL.

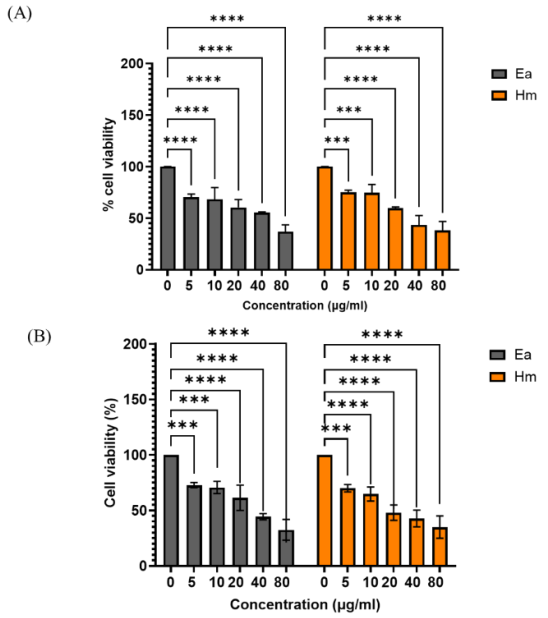

Figure 1: Cytotoxicity of *Enhalus acoroides* (Ea) and *Halimeda macroloba* (Hm) on cervical carcinoma cells. Viabilities of HeLa (A) and SiHa (B) cells were investigated using MTT method after 72-h exposure to crude extracts of *E. acoroides* and *H. macroloba* at graded concentrations. Results are mean ± SD of three assays. *** $p < 0.001$, **** $p < 0.0001$, vs control

DISCUSSION

The phytochemical screening of extracts from *Enhalus acoroides* and *Halimeda macroloba* revealed the presence of several bioactive compounds such as alkaloids, steroids, saponins, phenolic compounds and flavonoids, which possess a variety of pharmacological properties such as antioxidant, anti-tumor, anti-inflammatory and antibacterial activities [15,16]. These compounds have been found in green algae such as *Halimeda* and *Caulerpa,* and seagrasses such as *E. acoroides* and *Cymodocea rotundata*. The alkaloids, steroids, saponins, phenolic compounds and flavonoids identified in *E. acoroides* have potential as free radical scavengers, antioxidants, as well as antibacterial and anticancer agents [16,17]. Alkaloids induce apoptosis and donate H atoms to free radicals. Flavonoids also affect oxidative processes and increase apoptosis.

A recent study by Setyoningrum *et al* [16] showed that an extract of *E. acoroides* had antibacterial activity against *Staphylococcus aureus* similar to that of amoxicillin. However, the phytochemical contents of seaweed and seagrass are influenced by species, life stages, seasons, locations, and environmental factors [15-17].

Table 1: Phytochemical constituents of crude extracts of *E. acoroides* and *H. Macroloba*

Constituent	*E. acoroides*	*H. macroloba*
Terpenoids	-	+
Alkaloids	+	+
Steroids	+	+
Saponins	+	+
Anthraquinones	-	-
Phenolic compounds	+	+
Tannins	-	-
Flavonoids	+	+
Glycoside	-	-
Coumarin	-	-

+ = present; - = absent

Table 2: Cytotoxic activity (IC_{50}) of crude extracts from *Enhalus acoroides* (Ea) and *Halimeda macroloba* (Hm) on cervical cancer cell lines and an embryonic kidney cell line

Cell type	Cell line	IC_{50} (µg/mL)	
		Ea	*Hm*
Cervical cancer	HeLa	54.55±0.64	36.57±7.26
	SiHa	29.67±0.47	17.22±3.93
Normal kidney	HEK293	> 80	> 80

Navakanitworakul *et al* [11] found that there were differences in phenolic compounds amongst various life stages and within-thallus, with the highest concentration of phenolic compounds in the blades of adult plants. Results of cytotoxicity study indicated that *H. macroloba* extract exhibited high cytotoxicity against cervical cancer cells. This result is consistent with the findings of Husni *et al* [15], who reported that flavonoids and steroids from *H. tuna* crude extract exhibited cytotoxicity against lung cancer cells. In addition, Widiastuti *et al* [18] found that extracts of *E. acoroides* exerted cytotoxic activity against HeLa cervical cancer cells at a concentration of 122 ppm.

However, a crude extract of *E. acoroides* produced no toxic symptoms or histopathological lesions in acute and sub-acute toxicity studies on male Wistar albino rats [17]. These results support the findings of earlier studies showing that *E. acoroides* extract has potent cytotoxic activity and may be considered safe at normal therapeutic doses for use in pre-clinical trials.

CONCLUSION

This study has revealed that crude extracts from *E. acoroides* and *H. macroloba* exerts high cytotoxic activity on cervical carcinoma cell lines and low toxicity on healthy kidney cells. *Halimeda macroloba* extract is active against the aggressive SiHa cell line by inhibiting cell growth. However, the mode of action associated with the cytotoxicity of these extracts, and the effect of combination of the extracts with chemotherapy, should be investigated in subsequent studies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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