Research Article

ANTITUMOR POTENTIAL, ANTICANCER PROPERTY AND PHYCOCHEMICAL SCREENING OF *PADINA MINOR* YAMADA EXTRACTS

Bryan John J. Subong* and Karlo H. Primavera
Department of Chemistry, University of the Philippines - Visayas Miagao, Iloilo, Philippines.
*Corresponding author's E-mail: bryanjohnsubong@yahoo.com

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ABSTRACT

The present study was conducted to evaluate the antitumor potential, anticancer property and phycochemical screening of *Padina minor* Yamada using different solvent extracts namely hexane, dicholoromethane, ethyl acetate, methanol and aqueous. The antitumor potential was performed using the brine shrimp lethality assay. The anticancer activity made use of mechanism-based activity using mutant yeast strains namely 1138, 1140, and 1353 and the Sc7 yeast strain bioassay. It was found out that the methanol extract, hexane extract and ethyl acetate has LC_{50} at 48 hours closest to the standard set by the National Cancer Institute which is 30 ppm. All extracts have shown no significant difference in the number of brine shrimp mortality at 48 hours compared to anticancer drugs Vinblastine and Etoposide. Yeast bioassay revealed that the Hexane extract shows bioactivity on Topoisomerase I (1140 yeast strain) while Ethyl acetate shows on Topoisomerase II (1353 yeast strain). The said two extracts have shown significant difference with the positive control Nystatin, antifungal agent, suggesting that the activity is other than antifungal. The hexane, ethyl acetate, and crude methanol extract has shown inhibition against Sc7 yeast strain. Phycochemical screening showed that the crude methanol extract has the most number of compounds groups along with the ethyl acetate extract. The order of abundance of the metabolites in terms of mass is polar, medium-polar and non-polar metabolites, respectively.

Keywords: Antitumor potential, Anticancer, Marine Natural Products, *Padina minor* Yamada, Phycochemical Screening.

INTRODUCTION

In Asia, traditional medicines have used marine algae for treatment of cancer and for improved health[1]. Fitton suggested that brown algae could inhibit the occurrence of breast cancer, other inflammatory disorders and reduce cholesterols[2]. Their sulfated polysaccharides are reported to have anticoagulant, anti-mutagenic, anti-viral, hypolipidemic, and anti-inflammatory activities[3]. Fucoidan, a sulfated polysaccharide found in brown algae is known to exhibit antitumor effects and is found to induce apoptosis in HT-29 and HCT 116 human colon cancer cells[4].

Padina minor Yamada is a brown algae growing abundantly in Panay Island. In Iloilo, it is found in the towns of Ajuy, Concepcion, Estancia and Guimbal[5]. The said algae is characterized as having a blade flabellate, yellowish brown or light brown to whitish in color, grows up to 7 centimeters high, and is divided into several lobes, 1-3 centimeters wide and two-cell thick throughout[6].

P. minor is used as animal feed for swines and aquaculture. It is said to have antibacterial properties but with no reported antifungal activity[6]. Some studies showed that the said algae have hypotensive and antioxidant activties[7]. In addition, the aqueous extract has shown gastroprotective actvitiy[8]. Currently, it is being explored for possible nutraceutical and cosmeceutical properties[9]. Owing to its ethnopharmacological activities and its being brown algae, an evaluation of the antitumor and anticancer activity is needed to assess its potential as a possible source of medical drugs.

MATERIALS AND METHODS

Sample Collection

Seaweed samples were collected from the rocky intertidal region of Barangay Mambatad, Miagao, Iloilo throughout January 2011. A voucher specimen was deposited in the laboratory using 5% formaldehyde for taxonomic identification. The alga was greenish to yellowish brown in color, had a fan-shaped, small thallus, and 3-6 cm high (whole algae). The sample was identified by Ms. Soledad Garibay of the UPV Museum of Natural Sciences and Prof. Nestor Yunque of the Division of Biological Sciences, UPV using monographs and books.

The collected seaweeds were brought to the Chemistry Thesis Room Laboratory of UPV. It was washed thoroughly with tap water to remove sand particles, animal castings, epiphytes, epizoons, and attached detritus. The necrotic parts were also removed. The samples were then rinsed with distilled water to remove salt and other contaminants.

Preparation of Sample for Extraction

Cleaned samples were then air-dried under the shade for one week at room temperature to prevent photolysis and thermal degradation of metabolites. The percent moisture of the dried algal powder was between 8%-10%. The dried algal material was osterized to powder and sieved using a 0.2 mm mesh for homogeneity. The sample was then kept in a zip-bag prior to extraction.

The algal powder was then soaked with 80% methanol (Scharlau) in a solute-solvent ratio of 1:3 for one week. The mixture was then subjected to ultrasonic bath

extraction for 15 minutes at 15 khz for complete extraction. The extract was then concentrated via rotary evaporator (Heidloph VV 2000) at 40oC at reduced pressure at 1200 rpm.

Sequential Liquid-Liquid Extraction

The concentrated crude extract was subjected to liquid-liquid extraction using solvents of increasing polarity; namely, hexane, dichloromethane and ethyl acetate. The sequential extracts and crude extracts were then concentrated and were evaporated at room temperature until a stable and constant weight is achieved. The percent yield was noted.

Biological Assays

Five concentrations of the different extracts namely 1000 ppm, 500 ppm, 100 ppm, 50 ppm and 10 ppm were prepared for each extract. Ten percent dimethyl sulfoxide was used as carrier and solvent of the extracts. The biological assays were performed at the UPV- National Institute of Molecular Biology and Biotechnology Laboratory.

Antitumor potential

Antitumor screening was done using brine shrimp lethality assay[10-12]. One hundred microliter of each working solutions for extract was transferred to an individual well that contained 1 mL filtered seawater with ten nauplii placed in a 24-well microtiter plate. The mortality was determined after 6 hours (acute toxicity), 12 hours (nauplii in instar I/II), 24 hours (nauplii in II/III) and at 48 hours (nauplii in instar III/IV) of exposure[13]. The percentage mortality was calculated using the formula:

% mortality = percentage of survival in the negative control - percentage of survival in the treatment

The anticancer drugs Vinblastine (Korea United Laboratories) and Etoposide (Korea United Laboratories) were used as positive control. The activity was measured using LC50 values for every time interval.

Anticancer property

The anticancer property of the seaweed was investigated using topoisomerase I, topoisomerase II and rad52 deficient yeast cells, namely 1138, 1140, 1353 from the Virginia Polytechnic and State University. The yeasts were grown in yeast extract dextrose broth at 30°C. Inoculum was prepared by suspending the yeast culture and adding a portion to sterile distilled water to an optical density (OD) of 0.12 at 620 nm. One hundred microliters of YEPD, 100 microliter of solvent extract and 50 microliter of inoculums were received in each well using 96-microwell plates (Falcon). The plates were then incubated for 48 hours. The percent inhibition was calculated using the formula:

Percent inhibition = [(OD blank - OD test well) / (OD blank)] x 100

The inhibition concentration 50 percent (IC50) was used to determine activity. 40 microgram/mL of Nystatin was used as positive control.

The sensitivity to potential antitumor and cytotoxic activity was also determined using the Sc7 yeast cells. The yeasts were grown and assayed using the same procedure as that of 1138, 1140, and 1353 assay was performed except that Yeast Morphology broth was used instead of the yeast extract peptone dextrose broth.

The IC_{50} or inhibition concentration 50% was used to determine activity. The positive control used was 20 microgram/mL.

Phycochemical Screening

The extracts were then subjected to preliminary phychochemical screening. The extracts were screened for the presence of alkaloids, carbohydrates, saponins, phytosterol, phenols, flavonoids, terpenoids, and steroids.

RESULTS AND DISCUSSION

Table 1 shows that the Crude Methanol extract had the highest percent yield. This could be attributed to the fact that methanol was the total extracting solvent for the extraction with its ability to penetrate cell wall, thus all components were found in this solvent. It is followed in percent by the Aqueous, DCM, Hexane, and Ethyl acetate extract.

Table 1: Description and Percent Yield of the Different Extracts

Extract	Appearance of residue	Percent Yield
Hexane	Dark green	0.20832
Dichloromethane	Dark red-yellow	0.50950
Ethyl Acetate	Dark gold-yellow	0.087628
Aqueous	Dark red	1.6566
Crude Methanol	Red-brown	2.4146

Brine shrimp lethality assay

At 6 hours all of the extracts including the positive control Etoposide have an LC_{50} > 1000 ppm while only Vinblastine exhibited an LC_{50} < 1000 ppm. It was observed in the case of Etoposide, the brine shrimp moved in slow, zigzag manner at 6 hours after treatment. Ethyl acteate was also observed to exhibit the same adverse effect on brine shrimp although not as pronounced compared to Etoposide.

For 12 hours exposure, DCM, Ethyl acetate, Methanol and Aqueous extract have LC50 > 1000 ppm while Hexane, Vinblastine, and Etoposide have lower values. The movement of brine shrimp in the different treatments was greatly reduced compared to 6 hours exposure. At 12 hours exposure, the order of noticeable effect is: Vinblastine > Etoposide > Ethyl acetate > Hexane > DCM.

Brine shrimp deformations were also observed in the positive controls, Hexane, and Ethyl acetate extracts.

Only Aqueous extract had LC_{50} > 1000 ppm after 24 hours exposure. The decreasing order of cytotoxicity is Vinblastine > Etoposide > DCM extract> Methanol extract > Ethyl acetate > Hexane extract and lastly by Aqueous Extract. Although the Aqueous extract has an LC50 > 1000 ppm, there was a noticeable slowing and deformation of the brine shrimp treated with the said extract. All treatments showed deformation on brine shrimp nauplii.

At 48 hours of exposure the order of cytotoxicity was Vinblastine > Methanol extract> Etoposide> Ethyl acetate > Hexane extract> DCM extract> Aqueous Extract. The Methanol extract has the highest LC_{50} of all the extracts for the said assay. This is followed by the Hexane extract, and Ethyl acetate extract which had LC_{50} near 30 ppm. The said extracts exhibited antitumor potential in accordance with the National Cancer Institute standard of LC_{50} near 30 ppm during 48 hour observation.

It is noted that the extracts have shown dose-response relationship in which an increase in the length of exposure is proportional to the number of dead brine shrimp.

Anticancer Property

Hexane extract was found to inhibit the growth of strain 1140 which is known to be sensitive to Topoisomerase I inhibitors. The Ethyl acetate extract also showed inhibition of strain 1353 which is sensitive to Topoisomerase II inhibitors. The activity for both cases was considered to be selective or specific as well as differential since they did not show activity against other strains. IC_{50} for Hexane extract shows a value of >1000 ppm. On the other hand, Ethyl acetate IC_{50} is 665.56 $\pm$ 319.62 ppm. (table 2).

Table 2: Inhibition of different extracts against yeast strains

Sample	1138	1140	1353
Hexane Extract	-	+	-
DCM Extract	-	-	-
Ethyl Acetate	-	-	+
Crude Methanol Extract	-	-	-
Aqueous Extract	-	-	-
Nystatin	+	+	+

The Sc7 yeast bioassay (table 3) was conducted for possible cytotoxic, antitumor, or antifungal activity of the different extracts. Table showed that the Hexane, Ethyl acetate, and the Methanol extracts exhibited positive inhibition. The IC50 values for the extracts are: Hexane, 142.19 $\pm$ 58.69 ppm; for Ethyl acetate 18.99 $\pm$ 5.162 ppm, and Methanol extracts 23.473 $\pm$ 8.6583 ppm. ANOVA showed that the inhibition of the Sc7 yeast strain due to the three extracts was significantly different compared to the positive control Nystatin. Coupled with absence of any antifungal activity of extracts from *P. minor*, this might suggest that inhibition is due to cytotoxic bioactive components which can be possible antitumor agents.

Table 3: Inhibition of different extracts against Sc7 yeast

Sample	Result
Hexane	+
DCM	-
Ethyl acetate	+
Methanol	+
Aqueous	-
Nystatin	+

Phycochemical Analysis

Table 4 showed the nature of the natural products in the different extracts. Ethyl acetate and methanol, both polar solvents, were found to contain the most bioactive component in the extraction. The Methanol extract showed positive inhibition against both Sc7 and 1353. It showed that the bioactive component contain polar functional group or by nature polar as given by the polarity of their extracting medium. The Hexane extract showed positive inhibition against Sc7 and 1140. The bioactive component in this extract contain nonpolar functional groups or by nature nonpolar.

Table 4: Phycochemical screening of P. minor Yamada extract

Test	Hexane	DCM	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	+	+	-
Carbohydrates					
Barfoed's	+	+	+	+	+
Benedict's	+	+	+	+	+
Saponin					
Foam Test	+	+	+	+	-
Protein					
Millon's	-	-	+	+	-
Terpenoids	+	+	+	+	-
Steroids	-	+	+	+	-
Phenols and Tannins					
Feric chloride	+	+	+	+	+
Lead acetate	+	+	+	+	+
Flavonoids	-	+	+	+	+

The preliminary phycochemical screening showed the possible nature of the bioactive components; however, more intensive phycochemical screening and further biofractionation are needed to be done to show the real and exact nature of the most bioactive component in each solvent extracts in which inhibiting property can be attributed.

CONCLUSION

P. minor Yamada shows potential source of antitumor and anticancer drug sources. The Methanol extract, Hexane extract, and the Ethyl acetate extract have shown antitumor potential as set by the NCi standard using brine shrimp lethality assay. The three said extracts also showed significant inhibition against the Sc7 yeast strain for the mecyhanism-based anticancer property, the Hexane extract showed topoisomerase I inhibiting property while the Ethyl acetate showed topoisomerase II inhibiting property. The abundance of the metabolites is preliminarily polar by weight. It is suggested that further biofractionation should be done to identify, isolate, and characterize the bioactive substance responsible for the effect.

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