Evaluation of Allelopathic and Antifungal Activity of Selected Seaweeds

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Abstract

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals, from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. Allelochemicals are a subset of secondary metabolites not required for metabolism (growth and development) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory. Most of the plants finding in nature itself new weapons with insecticidal, fungicidal, herbicidal and antiparasiticidal properties. Present investigation initiated with the aim of Allelopathic and Antifungal activity of the selected seaweeds Turbinariaornata and Halimedadiscoidea. In this study leaves of the plants were collected from natural environment, washed, shade dried pulverized and extracted with methanol. A part of Methanol extracts were subjected to Radish seed germination bioassay and the remaining extracts were subjected to antifungal assay. The main objectives of the research work were to identify the maximum inhibitory activity of the seaweeds against the allelochemicals and fungus and check the presence or absence of the phytochemical constituents of the selected seaweed leaves methanolic extract. The preliminary results of the research showed that maximum germination inhibition observed in Turbinariaornata and the antifungal activity profile of two seaweed extracts against fungus showed that methanol extract of Turbinariaornata against Cladosporium and Aspergillus.sp exhibited maximum zone of inhibition of 8.5 mm and 10.2mm respectively. And the results of these species could be used as sources of natural herbicides due to their germination inhibition activity.Turbinariaornata andHalimedadiscoidea leaves were good source for phytochemicals such as steroids, flavonoids and alkaloids. Proceeding qualitative phytochemical screening of these seaweeds may show that steroids were found to be present in the Turbinariaornata and HalimedaDiscoidea leaf methanolic extract.

Keywords - *Allelopathy, Halimedadiscoidea, Turbinariaornata, Antifungal Activity, Zone of Inhibition*

I.INTRODUCTION

Seaweeds serve as an important source of bioactive natural substances. Seaweeds are the raw material for industrial production of agar, align and carrageenan. The edible seaweeds contain a significant amount of essential vital nutrients: the reactive antioxidant molecules such as ascorbate and glutathione as well as secondary metabolites including carotenoids (α- and β - carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporineglycine) and catechins (e.g. catechin, epigallocatechin), gallate, phlorotannins (e.g. phloroglucinol), eckol and tocopherols), protein, vitamins (A, B, B12, C, D, E, riboflavin, niacin, panthothanic acid and folic acid) and minerals (Ca, P, Na, K, S, Mg, Fe etc.). Seaweeds are able to biosynthesize secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between organisms including chemical defensess. These secondary metabolites offers defence against herbivores, fouling organisms and pathogens; they also play a role in reproduction, protection from UV radiation and as allelopathic agents (Hay, 1996; Watson and Cruz-Rivera, 2003). Harder (1917) was the pioneer to observe the antimicrobial potentials of seaweeds.The components reported to be found are sterols (some are fucosterol), different molecules containing vinyl and ethyl cholesterol types, cyclohexane and some sulfated polysaccharides fucoidan, neutral glucan and guluronic and mannuronic acid residues containing alginic acid providing a medicinal value for the brown and red algae. There are several studies revealing the medicinal value of marine algae, they have antitumorals, anticoagulant, antifouling, antioxidant and antimicrobial activities. Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004). Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harriset al., 2001) In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). Several works have demonstrated in

laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers possess inhibitory properties against bacteria, fungi and insects (Davicino et al., 2007). Currently, there is little evidence on the antimicrobial properties of the medicinal plants under investigation againstphytopathogenfungi.In this line, present work dealt to assess the allelopathic and the antifungal activity of selected seaweed extracts.

II. METHODOLOGY

A. Collection of Plant Leaves

The Leaves of the plant *Turbinariaornata* and *HalimedaDiscoidea*were collected in the month of February 2014 from Mannar and Arugambay situated in Sri Lanka. Plant leaves were washed thoroughly with running tap water followed by rinsing with distilled water to remove sand particles and other debris. The leaves were shade dried at room temperature for 45 days, then pulverized into powder by using a grinder.

B. Preparation of Methanol Extract

The dried powder of plant leaves (200 g) were successively extracted with Methanol at room temperature for 24 hours by using a mechanical stirrer with 500 rpm. This process was repeated four times that afforded crude methanolic extract .The extracts were filtered through a funnel contained cotton wool and a clear filtrate was obtained. The total filtrate of methanolic extracts were evaporated by using a rotary evaporator under reduced pressure at (30-40) °C. The thick greenish black residues were obtained. It was dried and stored for further use.

C. Radish Seeds Germination

The germination rate in distilled water was examined at random before the experiment and it was found to be > 85%. Solutions of crude extracts (1000 ppm) of two seaweeds were prepared at room temperature. All the seeds were surface sterilized with 1.5% Clorox for 1 min, before being washed 3 times (3 min/wash) in sterilized deionized water. The bleach did not inhibit germination because maximum seed germination was recorded following incubation in distilled water. Four replicates each of 4 seeds were prepared for each treatment using sterile petri dishes (90 mm) while the negative control groups were treated with sterilized deionized water. Prepared plates were then placed in a growth chamber at 25ºC in dark for 5 days (120 hours). The effect of each extract was observed by calculating the percentage seed germination and by measuring radicle length to the nearest centimeter (The radicle is the embryonic root).

D. Assay of Antifungal Activity against Cladosporium

Thin Layer Chromatography (TLC) was used to check the antifungal activity of extracts against *Cladosporium*. In this method about 10 ml of Czepak Dox Broth (CDB) was added to previously prepared Cladosporium cultures and shaken well till the broth turned cloudy. The spores were filtered into the spraying apparatus through glass wool. This spore suspension was sprayed on to previously spotted TLC plate, which were air dried for about 6 hours.0.2g of each plant crude was dissolved in 10ml of methanol and 40µl of each solution was spotted on the TLC plate. The plates were incubated at room temperature in a moisture chamber for 2 days and thereafter observed for the inhibition in the growth.

E. Assay of Antifungal Activity against Aspergillus niger

Disk diffusion method, which is commonly used for bacteria and also for yeast like fungi is slightly modified in order to use for filamentous fungi like *Aspergillus*. In this method, a liquid culture of Aspergillus on CDB is prepared by inoculating 7 days old fungus were grown on Potato Dextrose Agar (PDA) and incubated for 3 days at 30oC. Sterile disk papers (Diameter 6mm) are soaked in the test samples dissolved in Methanol in order to get 200µg of the sample per a disk. Meanwhile, PDA medium is prepared, autoclaved and cooled to about 45° C and then inoculated with the liquid culture of Aspergillus (0.5ml of liquid culture for 25ml of PDA medium by checking inoculum).Then the medium with culture is poured into sterilized petri plates (20ml per each) and left until solidify. After solidification, dried disk papers with the sample are placed on the inoculated medium and the plates are transferred into an incubator (30ºC) for 3 days. Diameter of the inhibition zones is measured along the two axes at right angle to each other. Four replicates are used for each sample and sterile distilled water and methanol are used as negative controls.

F. Quantitative Approach

- One-way ANOVA Test
- Mean Comparison Test – Turkey's Pair wise Comparison

III. RESULTS AND DISCUSSION

A. Allelopathic Activity of Seaweeds

The germination of Radish seeds were observed after 5 days. *Halimedadiscoidea* showed the lowest percentage germination value (75%) in the radish seed germination assay. At the same time *Turbinariaornata* showed the highest percentage germination value (100%). Interestingly, *Turbinariaornata* showed 100% germination rate and smallest average radicle length 3.25 ± 0.395 cm compared to distilled water. Therefore, *Turbinariaornata* showed mild seed germination resistant activity. *Halimedadiscoidea* also showed seed germination resistant activity with 75% germination value and the average radicle length of

4.23 ± 0.546 cm. The allelopathic effect of aqueous extract of seaweeds on the growth of radish seeds is shown in Table1. It was clear that an aqueous leaf extract of seaweeds inhibited the germination and growth of radish seeds. The present work was carried out as a preliminary study to investigate any possible herbicidal activity of the selected species against widely spread weed.

The percentage of Radish seed germination and Average length measurement of embryonic root

Fig I:Germination of Radish Seeds after 5 days in Turbinariaornata Extract

Fig II: Germination of Radish Seeds after 5 days in Halimedadiscoidea Extract

TABLE 1.Percentage Germination and the Average Radicle Length of Seedlings under Different Treatments

Crude Seaweed (Methanol Extract)	Percentage Radish Seed Germination	Average Radicle Length (cm)
Turbinaria ornate	100	3.25 ± 0.395
Halimedadiscoidea	75	4.23 ± 0.546
Distilled water	87.5	4.70 ± 0.688

Values are mean ± S.E; n=2; p=0.01 < 0.05 vs control; one-way ANOVA and Turkeys' Pair wise comparisons. Statistically significant.

Antifungal Activity against Cladosporium

Zone of Inhibition of Methanol Extract against Cladosporium

It was observed that the methanol extracts of*Turbinariaornataagainst**Cladosporium*exhibited maximum zone of inhibition of 8.5 mm and *Halimedadiscoidea*showed minimum zone of inhibition. The control didn't show any zone of inhibition against*Cladosporium*.

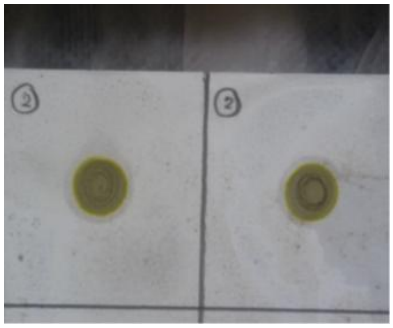

Fig III. Methanol Extract of *Turbinariaornata*against *Cladosporium*

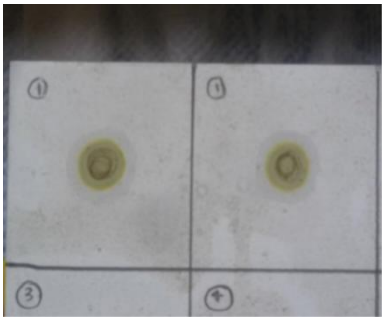

Fig IV. Methanol Extract of *Halimedadiscoidea*against *Cladosporium*

TABLE II. Interaction between Cladosporium and Seaweed Extracts

Plant Crude (Methanol Extract) and Control	Inhibition Diameter (mm)
Turbinaria ornate	8.5
Halimedadiscoidea	6.2
Methanol	0.0

Values are mean; n=2; p = 0.00 < 0.05 vs control; one-way ANOVA and Turkey's Pair wise comparisions. Statistically significant.

Antifungal Activity against Aspergillus niger

Zone of Inhibition of Methanol Extract against Aspergillus niger

It was observed that the methanol extracts of*Turbinariaornataagainst**Aspergillus niger*exhibited maximum zone of inhibition of 10.2 mm and *Halimedadiscoidea*showed minimum zone of inhibition. The control didn't show any zone of inhibition against*Aspergillus niger.*

Fig V:. Methanol Extract of*Turbinariaornata*against*Aspergillus niger*

Fig VI. Methanol Extract of *Halimedadiscoidea* against *Aspergillus niger*

TABLE III. Interaction between Aspergillus niger and Seaweed Extracts

Plant Crude (Methanol Extract) and Control	Inhibition Diameter (mm)
Turbinaria ornate	10.2
Halimedadiscoidea	8.6
Methanol	0.0

Values are mean; n=2; p = 0.00 < 0.05 vs control; one-way ANOVA and Turkey's Pair wise comparisions. Statistically significant.

IV. CONCLUSIONS

The present study concluded that these selected seaweeds have allelopathic effects.*Turbinariaornata*and *Halimedadiscoidea*has shown the significant difference in the average radical length, compared to the control distilled water. These species could be used as sources of natural herbicides due to their germination inhibition activity. Future work should target the isolation and purification of allelochemicals of all plants that used to identify the active compounds associated with the allelopathic activity.

The antifungal activity profile of two seaweed extracts against fungus, both *Cladosporium*and. *Aspergillus niger*showed that methanol extract of *Turbinariaornata*exhibited maximum zone of inhibition and *Halimedadiscoidea*showed minimum zone of inhibition. The control didn't show any zone of inhibition against*Cladosporium* and *Aspergillus niger*. *Turbinariaornata*and*Halimedadiscoidea* leaves were good source for phytochemicals such as steroids, flavonoids and alkaloids.
Future work should target the identification of Minimum Inhibitory Concentration (MIC), isolation and purification of bioactive constituents of all plants that used to identify the active compounds associated with the antifungal activity.

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