

Preliminary Phytochemical and Antifungal Studies of Sea Grass, *Posidonia oceanica* obtained from Mediterranean Sea of Libya

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Abstract: *The aim of the present study was to assess the antifungal activity of Mediterranean tape weed or Neptune grass, Posidonia oceanica, most common plant found in the Mediterranean Sea. The aqueous extract of sea grasses powder was tested for antifungal activity against two fungal strains Pythium spp. And Aspergillus flavus using fungal growth inhibition assay method Results obtained showed that all the extracts concentrations (5, 10, 15%, w/v) reduced fungal colony growth by 33.33 - 50 %. It was found that all tested extract's concentrations inhibited growth of Aspergillus flavus by 33.33%, however, among all tested extracts concentrations; maximum inhibition was noted against Pythium spp. (50%) at 5%, w/v extract's concentration. These findings indicated that Posidonia oceanica can be used as antifungal agent against the above two tested fungal strains and this plant seems to be considered for further study in an attempt to investigate the active chemical entities combating against pathogenic fungal strains.*

Keywords: Mediterranean tape weed, Neptune grass, Phytochemicals screening, antifungal activity, fungal growth inhibition assay method.

1. Introduction

Marine natural products have been known as rich source of biologically active compounds [1]. Microbial infections constitute a major cause of morbidity and mortality in humans and aquaculture organisms and interest in plant derived antimicrobial agents including seaweeds has been increasing, mainly due to the current widespread belief that “green medicine” is safer and more dependable than costly synthetic medicine, many of them have adverse side effects. [2-7].

Mediterranean tape weed or Neptune grass, *Posidonia oceanica* (L.), Delile (family: Posidoniaceae) is widely distributed phanerogam and common plant endemic to the Mediterranean Sea, able to grow in the clean water with a depth reaching about 45 m, and the grass is highly sensitive to marine pollution and makes sea-water of good quality. It is one of the largest, slowest growing, and longest-lived plants. Like other sea grasses, it forms large underwater meadows in the submerged photic zone of sheltered coastal waters. The plant also plays a dominant role in coastal ecosystem dynamics such as stabilizing the sea floor with its roots. The plant consists of compounds such as amino acids [8], carbohydrates [9], fatty acids [10], and sterols [11]. Phenolic compounds, one of the chemical entities of *Posidonia oceanica* have been reported as biotic and abiotic resistant agents [12-14]. According to our knowledge, there are very few reports available about the antimicrobial properties of crude extract of *P. oceanica*. Therefore, in this present investigation, the preliminary phytochemical testing's and antifungal activity of aqueous extract of *P. oceanica* sea grasses grown in Libyan Mediterranean Sea was carried out to check the phytochemicals present in samples which are responsible for antifungal activity.

2. Materials and Methods

2.1. Collection of Plants materials:

Posidonia oceanica grasses were collected by scuba diving from 5 meters depth in July-August 2013, from the White Mediterranean Sea coast of Al-Khums, Libya. Identification of sea grasses was done by Plant Taxonomist of the Department of Biology, Faculty of Sciences El-Mergeb University Al-Khums Libya. The sea grass was washed by tap water to remove the salts followed by distilled water and kept for two days away from sunlight. Then the grasses were dried by oven at 60 °C for 48 hours. The grasses were ground with electric grinder till having very fine powder.

2.2. Preparation of Extract

10g crude powder of sea grasses was extracted in 200 ml sterile distilled water concentration by using Soxhlet method for 8 h and filtered. The extracts were stored in refrigerator at 4°C until further use.

2.3. Phytochemicals Screening

Chemical tests were performed for the aqueous extracts of sea grasses using standard procedures to identify the presence of various phytochemicals as described by Sofowora, 1993[7] and Raman, 2006 [15].

2.3.1. Tannins

A small quantity of each extracts was heated on water bath and filtered. A few drops of ferric chloride were added to the filtrate. A dark green solution indicated the presence of tannins.

2.3.2 Saponins

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Small amount of filtered plant extract was shaken and heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

2.3.3 Phlobatanins

Small amount of filtered extract was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

2.3.4. Flavonoids

Filtered extract of each plant was mixed with diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

2.3.5. Terpenoids

Small amount of each extract was filtered and mixed with 2 ml of chloroform (CHCl₃) and concentrated H₂SO₄(3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

2.3.6. Cardiac glycosides

Small amount of each extract was filtered and shaken with 1ml of glacial acetic acid. A drop of ferric chloride and a drop of concentrated sulfuric acid were added. Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides.

2.4. Antifungal Screening

2.4.1. Test Organisms

Two fungal strains *Aspergillus flavus* and *Pythium spp.* were chosen for antifungal screening study.

2.4.2. Determination of Antifungal activity by 'Fungal Growth inhibition Assay' method:

Antifungal activity of plant extracts against *Pythium spp.* and *Aspergillus flavus* was determined by fungal growth inhibition assay as described by Fiori *et al.*,(2000) [16] with some modification. The filtered sterilized sea grasses extract was mixed with molten Potato dextrose medium (PDA) to provide desired concentration. An 8mm diameter disc or 18x104 spore/ml in performed well (8 mm) were added into the pre-sterilized PDA medium and incubated at 28±2oC. For the control treatment only PDA medium was used without plant extract. The colony diameter was measured after 72hrs and inhibition percentage of the fungal growth in relation to control treatment was calculated according to the given formula:

$$I = C - T / C \times 100$$

Where, I = percentage inhibition

C = radial growth in control

T = radial growth in treatment (Test)

3. Results

3.1. Phytochemicals Screening

Table 1 shows the results of phytochemicals screening of aqueous extracts of *Posidonia oceanica sea grasses*. Test observations revealed the presence of tannins, saponins,

phlobatanins and terpenoids and absence of flavonoids and cardiac glycosides in aqueous extract of sea grasses.

Table 1 Phytochemical screening of aqueous extracts of *Posidonia oceanica sea grass*.

Chemical Component	Observations
Tannins	+
Saponins	+
Phlobatanins	+
Flavonoids	-
Terpenoids	+
Cardiac Glycosides	-

Key: + =present; - = absent

3.2. Antifungal Activity

Table 2 shows the results of antifungal activity of aqueous extract of *Posidonia oceanica* against *Pythium spp.* and *Aspergillus* fungal strains. Results exhibited good antifungal activity against both tested fungal strains, however, most potent antifungal action (50%) of the extract was seen against *Pythium spp.* in lower concentration (5% w/v). 33.33% antifungal action of the extract was also observed against *Aspergillus flavus fungal strain* in all its tested concentrations (5, 10, 15 % w/v).

Table 2: Antifungal activity of aqueous extract of *Posidonia oceanica*

Fungal strains	Extract concentration (w/v)		
	5%	10%	15%
<i>Pythium spp.</i>	50	38.88	33.33
<i>Aspergillus flavus</i>	33.33	33.33	33.33

4. Discussion

The results of the antifungal screening of aqueous extract of *Posidonia oceanica* have demonstrated good antifungal activity against tested *Pythium spp.* and *Aspergillus flavus* fungal strains, however, the presence of tannins, saponins, phlobatanins and terpenoids, was noted among the tested phytochemicals *viz.* tannins, saponins, phlobatanins, flavonoids, terpenoids, cardiac glycosides in aqueous extract of *Posidonia oceanica sea grasses*. The broad antimicrobial activity of the plant species was shown to be related to the presence of saponins, alkaloids and tannins [17].

The antifungal activity of *Posidonia oceanica extract* probably may be due to presence of saponins and tannins in its content [18-22].

It is reported in literature that *Aspergillus flavus* play a crucial role as signals for regulating the biosynthesis of aflatoxins, responsible for pathogenic phase. Tannins can be hydrolyzed by a fungal tannase present in *A. Flavus*, yielding Gallic acid and Ellagic acid, testing of which showed that Gallic acid had potent inhibitory activity toward aflatoxin biosynthesis [18]. Thus, the presence of tannins in *Posidonia oceanica* might be responsible for the antifungal action by inhibiting aflatoxigenesis in *Aspergillus flavus*. Plant diseases caused great loss estimated to be about 14%

worldwide [23] and 20% for major foods and cash crops [24].

The current status of research suggests that there are indeed alternatives to replace the synthetic fungicides as antifungal agents because of fungal pathogens have developed resistant against synthetic fungicides. Hence there is need to replace the chemical fungicides by bio-fungicides, prepared from plant extracts as an economically viable alternative can be implemented at the farm level. For crop production system can be developed. So the use of bio-fungicides proved to be economical alternative that can be implemented at the farm level for effective production of crops. Besides this the use of bio fungicides will not leave any ill effect in the soil, water as well as in the environment.

Our findings encouraged us to continue screening more plant species for antifungal agents. The results of this study may form the basis of further investigation on fractionation for finding active fractions, the effect of origin of growth on the quality and quantity of active compounds, the amount of bioactive compounds in different plant parts and finally *in vivo* application of the extracts.

5. Conclusion

Phytochemical screening carried out with aqueous extract of *Posidonia oceanica* sea grasses has revealed the presence of tannins, saponins. Terpenoids phytochemicals and extract has demonstrated good antifungal activity. The present study portrays that the phytochemical constituents present in the plant may contribute to exert antifungal activity. In future study it is required to characterise the compound(s) by applying fractionation, isolation purification and identification of the active bioactive ingredient(s) of the plant's extract responsible for antifungal / fungicidal action.

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