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Antimicrobial Activity of Some Plant and Algal Extracts

Moustafa A. Abbassy[1], Gehan I. Kh. Marei[1] and Selwan M. H. Rabia[1*]

[1]Department of Plant Protection, Faculty of Agriculture, Damanhour University, Al- Behera, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author MAA designed the study, wrote the protocol. Authors GIKM and SMHR performed the statistical analysis and wrote the first draft of the manuscript. Author SMHR managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The use of advanced, effective and less toxic antimicrobial agents are required for the treatment of plant pathogens. In this study, hexane and ethanol extracts of two plants (*Sesbania sesban* and *Cymbopogon citratus*) and two algae species (*Spirulina platensis* and *Scendusmus sp.*) were screened for their antifungal and antibacterial activity on eight fungi (*Rhizoctonia solani, Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Alternaria solani, Pythium debarianum, Botrytis cinerea* and *Penicillium digitatum*) and two species of bacteria (*Agrobacterium tumefaciens* and *Erwinia carotovora* var. *carotovora*). Extracts of these plants and algae showed varied levels of antifungal and antibacterial activity. It was found that, the ethanol extracts of *S. sesban* plant and *S. platensis* alga had a high activity on tested fungi. On the other hand, the ethanol extracts of *Scendesmus sp.* alga *and S. sesban* plant showed the highest antibacterial activity against tested bacteria. The ethanol extract of *S. sesban* was the most potent fungitoxic extract against both *A. niger* and *A. solani* fungi with effective concentration (EC_{50}) values of 0.135 and 0.011mg/L, respectively. On contrary, *Scendesmus sp.* ethanol extract revealed the highest inhibitory effect against *E. carotovora* var. *carotovora* bacteria.

**Corresponding author: E-mail: dr_semson@yahoo.com;*
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1. INTRODUCTION

For many years, synthetic fungicides have been used for control plant pathogenic fungi. However, the extensive use of these chemicals led to the development of resistance in many areas around the world [1]. In order to overcome this problem, higher concentrations of these chemicals were used, but these concentrations increase the risk of high-level toxic residues in the products. Thus, there has been a growing interest on the research of the possible use of plant secondary metabolites for pest and disease control in agriculture [2]. Biological control has proven to be effective against a variety of pathogenic fungi and bacteria [3,4]. A variety of biological controls are available for use, such as some plants and algae.

Sesbania sesban Linn. is used as carminative, anthelmintic, astringent, anti-inflammatory, antimicrobial, antifertility, demulcent and purgative. It is also given as a medicine against fever, ulcers etc. [5]. Previous phytopharmacological study on the leaves, flowers, and aerial parts of this plant had isolated sterols, saponins, and tannins [6]. These chemical constituents are well known for their potential health benefits and have been reported to possess valuable biological activities such as antibacterial and antifungal [7].

Lemongrass (*Cymbopogon citratus* Stapf), from the family Gramineae, is an important Asian culinary herb that has been included in a wide range of herbal products, household items and traditional medicines [8,9]. Many studies have evaluated its biological properties, such as antifungal activity against plant and human pathogens [10,11,12,13,14,15,16,17], bactericidal effects [15,18,19,20,21,22,23] and insecticidal properties [24,25].

Cyanobacteria and eukaryotic algae occur in fresh water, marine and terrestrial soil habitats. A number of cyanobacteria and microalgae produce various biologically active compounds. These include antibiotics which in laboratory tests inhibited bacteria and fungi that incite diseases of humans and plants [26]. Various strains of cyanobacteria like (*Spirulina platensis*) and green algae like (*Scenedesmus sp*.) are known to produce intracellular and extra cellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity [27].

The aim of the present work was to study the antimicrobial activity of hexane and ethanol extracts of two plants (*Sesbania sesban* and *Cymbopogon citratus*) and two algae species (*Spirulina platensis* and *Scenedesmus sp.*) *In vitro* against pathogenic bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Plant and Algae Materials

Aerial parts of *S. sesban* and *C. citratus* were collected and the authenticity of the plant was confirmed by the taxonomic characters of [28] and checked by Prof. Dr. Fath Allah Zaton, plant pathology Department, Faculty of agriculture (El-Shatby), Alexandria University. Two species of algae were used in this study, *Scenedesmus sp*. and *Spirulina platensis* (L.) and obtained from National Research Institute, Cairo, Egypt. References used for the identification of the algae species were [29,30].

2.2 Preparation of Extracts

The aerial parts of the plants were cleaned, washed, shade dried at room temperature, then in an oven at 50ºC. Plant samples were ground in an electric blender to fine powder for the phytochemical study. Each plant powder (500 g of each plant powder) was extracted in a soxhlet apparatus successively with hexane for five days and ethanol for two days till exhaustion. Each extract was dried and the solvent was evaporated under reduced pressure in rotary evaporator. Dried crude extractives were preserved in tightly colored brown bottles and stored in a refrigerator till using them in bioassay tests. The powder of the Two species of algae (100 g) were extracted successively with hexan for 7days then filtrated and the powder was left over night to make sure that the solvent was evaporated then it was extracted with ethanol for 4 days and the same filtration procedure was repeted. The solvents were filtered and evaporated to be separated from the crude extracts by rotary evaporator then the crude extract preserved in tightly colored brown bottles and stored in a refrigerator till using them in bioassay tests.

2.3 Qualitative Phytochemical Analysis

According to the methods adopted by [31,32]. The extracts of each powder were subjected to different tests for the identification of various phytochemical constituents.

2.4 Tested Fungi

Eight plant pathogenic fungi species used, *Rhizoctonia solani* (Kuhn, isolated from *Phaseolus vulgaris*), *Fusarium oxysporum* (Schltdl., isolated from Zea mays seeds), *Penicillium digitatum* (Pers., isolated from *Citrus sinensis*), *Aspergillus niger* (Tiegh, isolated from *Solanum melogena*), *Rhizopus stolonifer* (Ehrenberg, isolated from *Cucumis sativus*), *Alternaria solani* (Sorauer , isolated from *Solanum lycopersicum*), *Pythium debarianum* (R. Hesse, isolated from *Citrus sinensis*) *and Botrytis cinerea* (Sardiña, isolated from *Capsicum annuum*) were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University and the fungi were determined by mycological key literature . The fungi were maintained during the course the experiments on potato dextrose agar medium (PDA: potato 200, dextrose 20 and agar 15 gl^{-1} in distilled water) at 25ºC.

2.5 Antifungal Activity

The antifungal activity was performed by using radial growth technique. Appropriate volumes of the stock solutions in dimethyl sulfoxide (DMSO) were added to PDA medium immediately before it was poured into the Petri dishes (9.0 cm diameter) at 40–45ºC and prepared a stock solution to obtain a series of concentrations (100, 500, 1000, 2500, 5000 mg/L). Each concentration was tested in triplicate. Parallel controls were maintained with DMSO (1 mL) mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of Petri dishes. The treatments were incubated at 27ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition = [(DC-DT)/DC] ×100 [33]. Where DC and DT are average diameters of fungal colony of control and treatment, respectively. The EC_{50} values with significance 95% were calculated by using the LDP line programe.

2.6 Tested Bacteria

Bacteria of crown gall disease *A. tumefaciens* (E. F. Smith and Town.) (Family: Rhizobiaceae; Class: Alpha Proteobacteria) and soft mold disease *E. carotovora* var. *carotovora* (Family: Enterobacteriaceae; Class: Gamma Proteobacteria) were provided by Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were maintained on nutrient agar medium (NA: peptone 10, meat extract 5, sodium chloride 2.5 and agar 10 g litre−1 in distilled water) at 37ºC.

2.7 Minimum Inhibitory Concentration (MIC) Assay

Agar dilution method assay was used as recommended by European Society of Clinical Microbiology and Infectious Diseases [34] for determination of MIC. Appropriate volumes of the stock solutions were added to molten NA to obtain a series of concentrations (100, 500, 1000, 2500, 5000, 7000 and 10000 mg/L) before pouring to Petri dishes. After solidifications, 6 µL of bacterial cultures (approximately 10^8 CFU/mL) was spotted (three spots per each plate) using 2 µL standard loop on the surface of agar. The inoculum spots were allowed to dry before inverting the plates for incubation at 37ºC for 24 h. The growth of the inoculum spots on petri plates of each concentration was determined.

3. RESULTS AND DISCUSSION

Results of the antifungal activity of different extracts of *C. citratus* and *S. sesban* against tested fungi were summarized in (Table 1). It was found that ethanol extract of *S. sesban* plant showed maximum antifungal activity against *A. solani* and *A. niger* fungi with EC_{50} values 0.011 mg/L and 0.135 mg/L, respectively. Also, higher activity was recorded against tested fungi by using hexane extract of *S. sesban* especially against *P. debaryanum* and *F. oxysporum* fungi with EC_{50} values 0.0031 and 2.008 mg/L, respectively [35]. While hexane extract of *C. citratus* showed less inhibitory effect against tested fungi.

Table 1. Antifungal Activity of *Cymbopogon citratus* and *Sesbania sesban* plant extracts against tested fungi

Tested fungi	EC_{50} values (µg/mL)			
	Cymbopogen citratus		***Sesbania sesban***	
	Hexane	**Ethanol**	**Hexane**	**Ethanol**
F. oxysporum	14.42	357.9	2.008	84.36
A. niger	146.93	54.42	16.913	0.135
A. solani	191.07	4.822	13.88	0.011
P. debarianum	548.4	1145	0.0031	23.32
R. solani	≥5000	≥5000	≥5000	≥5000
R. stolonifer	≥5000	2073.7	≥5000	1101
B. cinerea	1184	1930	2809	856.54
P. digitatum	278.5	220.55	72.39	69.31

On the other hand, the activity of extracts of *Scendesmus sp.* and *S. platensis* against tested fungi were illustrated in Table 2. It was found that ethanol extract of *S. platensis* recorded the highest inhibitory effect against *P. digitatum* and *P. debarianum* with EC_{50} 10.41 mg/L and 10.59 mg/L, respectively.

Spirulina platensis extract has been reported that possess antifungal potential against many plant pathogenic fungi [36]. Moderate activity was reported against tested fungi by using ethanol extract of *Scendesmus sp.* Among all the algae extracts tested, the hexane extract of *Scendesmus sp.* showed the lowest antifungal activity against the tested fungi (Table 2).

Results in Tables (1, 2) revealed that ethanol extract of *S. sesban* plant was found to be the most effective extract against tested fungi followed by ethanol extract of *S. platensis* alga. The ethanol extracts of tested plants and algae showed the highest antifungal activity compared with hexane extract, so the ethanol extract of plants and algae were used in antibacterial assay.

Table 2. Antifungal Activity of *Scendesmus sp.* and *Spirulina platensis* algae extracts against tested fungi

Tested fungi	EC_{50} values (µg/mL)			
	Scendesmus sp.		***Spirulina platensis***	
	Hexane	**Ethanol**	**Hexane**	**Ethanol**
F. oxysporum	120.43	2.801	≥5000	16.75
A. niger	441.5	≥5000	≥5000	≥5000
A. solani	137.69	116	94.18	71.46
P. debarianum	1844	124.3	10.17	10.591
R. solani	1157	625	348	28.38
R. stolonifer	≥5000	≥5000	2031	816
B. cinerea	≥5000	770.17	≥5000	≥5000
P. digitatum	1231.02	375.99	431.93	10.41

Result of the antibacterial activity as minimum inhibitory concentration (MIC) of the ethanol extracts of the two plants, *Cymbopogen citratus* and *Sesbania sesban* and the two algae, *Scendesmus sp. and Spirulina platensis* against *Agrobacterium tumefaciens and Erwinia carotovora* were recorded in (Table 3). In general, *E. carotovora* was more sensitive than *A. tumefaciens* to all of the tested extracts. It was found that the ethanol extract of *Scendesmus sp.* alga showed the highest inhibitory effect against *Erwinia carotovora* with MIC value of 2500 mg/L. In contrast, *Spirulina platensis* extract was the least effective extract followed by *Cymbopogen citratus and Sesbania sesban*. Thus, the chlorophycean alga *Scenedesmus sp.* can be used as a source for natural antibacterial agents.

Table 3. Minimum inhibitory concentration (MIC) in mg/L **of some ethanol extracts of plants and algae against some *Agrobacterium tumefaciens and Erwinia carotovora***

	Cymbopogen citratus	***Sesbania sesban***	***Scendesmus sp.***	***Spirulina platensis***
A. tumefaciens	7000	7000	5000	7000
E. carotovora	5000	5000	2500	7000

3.1 Phytochemical Analysis

The result of phytochemical screening on *C. citratus, S. sesban, S. platensis* and *Scendesmus sp*. showed that *C. citratus, S. sesban* plants and *S. platensis* and *Scendesmus sp.* algae revealed the presence of triterpenes and flavonoids (Table 4). In addition, *S. sesban* plant and *S. platensis* alga contain glycosides in contrast to *C. citratus*

plant and *Scendesmus sp*. which were free of glycosides. However, plants and algae contain alkaloids except *S. platensis*. Along with other water soluble components which are naturally occurring in most plant materials, these bioactive components are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants [38,39,40]. This result agrees with those of [4,35,41,42] who investigated the phytochemical studies of *C. Citratus, S. sesban* plants.

Table 4. Preliminary phytochemical investigation of the tested plants and algae

Constituents	Tested plant			
	C. citratus	***S. sesban***	***S. platensis***	***Scendesmus sp.***
Alkaloids	+	+	-	+
Triterpenes	+	+	+	+
Glycosides	-	+	+	-
Flavonoids	+	+	+	+

+ = Present, - = Absent

4. CONCLUSION

In conclusion, the results of the present study demonstrated that ethanol extract of S. *sesban* plant was found to be the most active as fungicide against tested fungi and the ethanol extract of *Scendesmus sp.* alga showed the highest inhibitory effect against tested bacteria. So ethanol extract of *S. sesban* plant and ethanol extract of *Scendesmus sp.* alga can be used as a natural antimicrobial agents against plant fungal and bacterial diseases, respectively. Future research will be aimed to testing the effect of this extracts under field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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