GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Determination of antimicrobial activities and DNA protection properties of *Scytosiphon lomentaria* extracts

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GSC Biological and Pharmaceutical Sciences, 2024, 29(02), 008–014

Publication history: Received on 19 September 2024; revised on 28 October 2024; accepted on 31 October 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.2.0399

Abstract

Scytosiphon lomentaria is a brown algae that has many promising biological activities in medicine as well as being consumed as food in many countries. This study aims to investigate the antimicrobial, antimutagenic and DNA damage protective activity of *S. lomentaria* by obtaining hexane, methanol and water extracts. To evaluate the antimicrobial effect of the extracts, minimum inhibitory concentration (MIC) tests were performed on both yeasts and bacteria. The antimutagenic activities of the extracts were investigated by the Ames test, and their protective effects against oxidative DNA damage were investigated by agarose gel electrophoresis using pBR322 plasmid DNA. Antimicrobial results showed that hexane methanol and aqueous extract were more effective on *Candia albicans* (5, 2.5 and 2.5 mg/mL, respectively) compared to all strains used. All concentrations of the methanol extract exhibited strong antimutagenicity (more than 40% inhibition) on both TA98 and TA100 mutant strains. It was determined that the extracts protected DNA against oxidative DNA damage. The results show that *S. lomentaria* extracts have antimicrobial activity and DNA protection properties, making them have the potential to be evaluated as new natural resources that can be used in antimicrobial and anticancer formulations

Keywords: *Scytosiphon lomentaria*; Antimicrobial; Antimutagenic; Oxidative DNA Damage

1. Introduction

Algae are a wide variety of eukaryotic photosynthetic organisms commonly found in marine environments. These organisms produce many metabolites to help them survive in harsh marine environments [1]. These metabolites are various bioactive substances such as peptides, proteins, vitamins, minerals, polysaccharides, and polyphenols and have the potential to be used as dietary supplements and functional foods [2]. In addition, these metabolites have a wide variety of pharmacological properties [3]. In recent years, studies supporting alternative therapy using algae metabolites have attracted great interest. Metabolites, which are especially effective in preventing, suppressing, or correcting carcinogenic progression, attract attention as newly proposed chemopreventive agents [4]. It is also known that these metabolites have various bioactivities such as strong antioxidant, antimutagenic and anticarcinogenic effects. Due to their antiproliferative effects, they inhibit the proliferation of tumor cells. Some of these have been seen as antimutagenic agents that prevent the carcinogenic progression of DNA damage (e.g., inhibition of mutagens, protect the cell from mutagen-induced oxidative stress, block carcinogenic biological activation) or are effective in DNA damage repair [5]. In addition, the strong antioxidant activities of metabolites make them good antimicrobial agents [5].

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Oxidative changes of lipid proteins and small particle molecules in DNA by reactive oxygen species (ROS) play a role in common diseases and age-related degenerative diseases [6, 7]. These include, in particular, inflammatory conditions, cardiovascular diseases and neurodegenerative diseases such as dementia [8], mutations, and cancer diseases [9]. Although new anti-proliferative drugs are used in the treatment of cancer, their use is limited due to their serious side effects, toxicity and high costs. In this context, the use of natural products in the treatment of diseases is promising, mainly because they are safe and inexpensive [10-12].

Brown algae are a large group of photoautotrophic marine macroalgae that includes more than 1500 species. Recent research has demonstrated that the secondary metabolites in brown seaweed have medicinal benefits [13]. Brown alga called *S. lomentaria* is a common food in several Asian nations, including China and Japan [14]. Secondary metabolites of *S. lomentaria* are known to have promising therapeutic activities such as antioxidant, anti-inflammatory, anti-inflammatory, anti-cancer and anti-obesity [15], and antimicrobial activity [16].

In this study, hexane, methanol, and aqueous extracts of *S. lomentaria* were obtained by soxhlet extraction method. The effect of the obtained extracts on microorganisms was determined by minimum inhibition concentration (MIC) assay. The DNA protective effect of the extracts was tested in the Ames/*Salmonella* test against TA98 and TA100 mutant strains, and *in vitro* analyzes were performed using the agarose gel electrophoresis method with pBR322 plasmid DNA

2. Material and methods

2.1. Chemicals and reagents

Methanol, hexane, D-biotin, L-histidine, sodium azide (NaN3), Dimethyl sulfoxide (DMSO), 4-nitro-o-phenylenediamine (NPD), Agarose, Tris Base, Ethidium Bromide, Ethylenediaminetetraacetic acid (EDTA), Mueller Hinton Broth, RPMI 1640, glucose, agar (Difco) and H_2O_2 were from Sigma Aldrich; pBR322 plasmid DNA was purchased from Thermo Fisher Scientific. Magnesium sulfate ($MgSO_4.7H_2O$), potassium phosphate (K_2HPO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), citric acid monohydrate ($C_6H_8O_7$. H_2O) and sodium dihydrogen phosphate monohydrate ($Na_2HPO_4.H_2O$) were purchased from Merck Millipore.

2.2. Plant materials

S. lomentaria was collected from the Dardanelles Sarısığlık location at a depth of 0-1 m. The sample was identified by Prof. Dr. Huseyin ERDUGAN and kept in his personal herbarium.

2.3. Preparation of alg extracts

After the samples were collected, they were cleaned from epiphytes in the laboratory and left to dry in the shade. Then it was completely dried in an oven at 105 ºC and turned into powder. 10 g of dried algae was extracted using hexane, methanol, aqueous in soxhlet for 18 hours. These three types of extracts with different polarities were evaporated in a double boiler to reach the raw extract. Each extract was then resuspended in sufficient solvent.

2.4. MIC Test

Antimicrobial activity of *S. lomentaria* extracts was evaluated by MIC test. The antimicrobial activity of the extracts was carried out on four Gram-negative bacteria (*E. coli* (NRRL B-3704), *E. coli* (ATCC 25922), *P. aerouginosa* (ATCC 27853) and *P. vulgaris* (ATCC 13315)), three Gram-positive bacteria (*S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *B. subtilis* (ATCC 6633)) and two strains of yeast *C. albicans* (ATCC 60193) and *C. tropicalis* (ATCC 13803). Serial dilutions of the extracts were made in 96-well sterile microplates. The bacteria and yeasts used in the experiment were adjusted to equal 0.5 McFarland. The plates were incubated at 37°C for 24 hours for bacterial strains and 48 hours for fungal strains. After incubation, the MIC values of the extracts against the tested bacteria and yeasts were determined.

2.5. Antimutagenic Activity

Antimutagenic activity of *S. lomentaria* extracts was determined by the Ames test using *S. typhimurium* TA98 (frameshift mutation) and TA100 (base substituted) strains [17]. The antimutagenicity of all extracts was evaluated against NPD for the TA98 mutant strain and NaN3 mutagen for the TA100 mutant strain. The percent inhibition of mutagen-induced revertant colonies was calculated as described by Hong and Lyu [18]. According to Hong and Lyu, less than 25% inhibition indicates weak or no antimutagenicity; Inhibition in the range of 25-40 % is moderate and inhibition greater than 40 % is strong antimutagenicity.

2.6. DNA Agarose Gel Electrophoresis

The protective effect of *S. lomentaria* extracts on oxidative DNA damage was examined by agarose gel electrophoresis method. Briefly, the experiment was mixed with Tris-HCl buffer (10 mM, pH:7.2), pBR322 DNA (0.1 μg/μL^{-1}), sample and H_2O_2 and incubated at 37 ^{0}C for 3 hours. After incubation, samples were electrophoresed on a 1 % agarose gel prepared with tris-acetic acid-EDTA (TAE) buffer for 60 min at 60 V. The tapes were then examined under a UV lamp and photographs were taken.

3. Results and discussion

3.1. Antimicrobial Activities

The MIC assay is widely used to evaluate antibacterial and antifungal activity in pharmaceutical testing [19, 20]. In our study, we used the MIC test to assess the antimicrobial effects of extracts and found that all extracts had a high antifungal effect against *C. albicans* and *C. tropicalis*, but a low antimicrobial effect on bacteria (Table 1).

Table 1 MIC values of *S. lomentaria* extracts

Microorganisms	**Extracts (mg/mL)**		
	Hexane	**Methanol**	**Aqueous**
S. aureus ATCC 25923	2.5	5	20
E. faecalis ATCC 29212	10	20	20
B. subtilis ATCC 6633	10	20	20
E. coli ATCC 25922	20	20	20
E. coli NRLL B-3704	20	20	20
P. aeruginosa ATCC 27853	20	20	20
P. vulgaris ATCC 13315	20	20	20
C. albicans ATCC 60193	5	2.5	2.5
C. tropicalis ATCC 13803	5	5	10

The hexane extract and methanol extract exhibited significant effect against *S. aureus* ATCC 25923, outperforming other bacterial strains. The effectiveness of the extract can be attributed to differences in the cell wall structure of microorganisms (Gram-negative and Gram-positive bacteria). Since Gram-positive bacteria have a single-layered cell wall, they may be more susceptible to the extract's activity [21]. Additionally, the extract showed greater efficacy against yeasts than bacteria, likely due to the differences in cellular structures between prokaryotic and eukaryotic cells.

Studies have shown that extracts of brown algae, such as *Eisenia bicyclis* and *Asparagopsis armata*, have antifungal properties, particularly against *C. albicans* strains [22]. In a study conducted, the antimicrobial effects of extracts from nine species of brown algae were determined. The results showed that the antimicrobial activity varied according to the solvent and species of algae tested. Among the extracts tested, *B. bifurcata* showed the highest antimicrobial activity [23]. These findings suggest that brown algae have high antimicrobial potential, although the specific activity may vary depending on the species and extraction method used.

3.2. Ames/*Salmonella* Test

In the study, antimutagenicity of the *S. lomentaria* extracts was investigated using *S. typhimurium* TA98 and TA100 mutant strains. The results revealed that both methanol and aqueous extracts had a strong antimutagenic effect (>40 %) at all concentrations tested on *S. typhimurium* TA98 strain. The hexane extract exhibited a moderate antimutagenic effect (25-40 %) at 4 mg/mL and a strong antimutagenic effect (>40 %) at all other concentrations on *S. typhimurium* TA98 strains (Table 2). It was observed that *S. lomentaria* extracts had a greater effect on TA100 mutant strains than on *S. typhimurium* TA98 strains (Figure 1). Additionally, *S. lomentaria* extracts have been shown to strongly inhibit base substituted mutation. These findings suggest that *S. lomentaria* extracts may have potential as an antimutagenic agent and could be further studied for their applications in preventing mutations and reducing the risk of cancer.

Table 2 Antimutagenicity test results of the extracts of *S. lomentaria*

Treatment		Concentration (mg/plate)	His⁺Revertant Number of Colony /Plate			
			TA98	% Inhibition	TA100	% Inhibition
			Means±SE		Means±SE	
Positive Control	NPD	10^{-2}	546.33±06.65			
	NaN_3	10^{-3}			576.00±06.68	
Hexane		0.5	273.00±38.04	52.13	274.00±13.53	73.17
		1	262.33±45.35	54.16	296.00±05.29	67.85
		2	252.33±20.60	56.07	292.33±35.16	68.73
		4	348.33±27.54	37.76	386.66±32.15	45.93
Methanol		0.5	278.66±11.53	51.54	351.00±23.52	54.55
		1	268.33±28.43	53.02	323.33±25.17	61.24
		2	216.33±35.64	62.94	311.33±12.06	64.14
		4	180.33±55.19	69.80	303.66±44.61	66.00
Aqueous		0.5	303.33±11.50	46.34	290.66±17.21	69.14
		1	276.66±27.54	51.43	345.14±25.00	56.09
		2	263.33±30.14	53.97	348.33±23.63	55.02
		4	252.33±12.50	56.07	340.00±20.00	57.21
Negative Control (DMSO)			27.00±03.00		175.00±5.03	
Spontaneous Control			22.00±04.00		163.00±02.52	

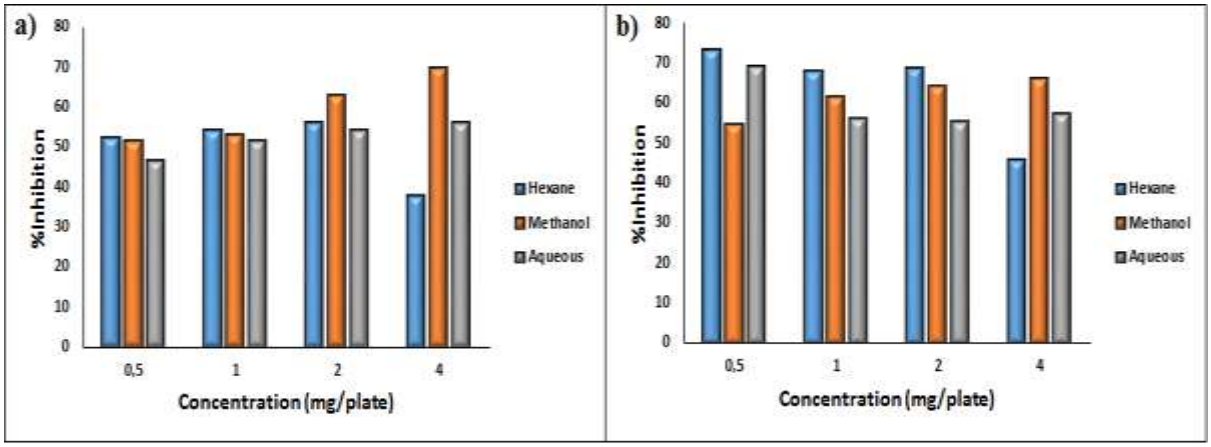

Figure 1 Antimutagenic activity of *S. lomentaria* extracts a) TA98 mutant strains and b) TA100 mutant strains

Sterols are a type of bioactive compound that exhibit various health benefits such as hypocholesterolemic effects, anti-inflammatory, anti-cancer, anti-diabetic, anti-atherosclerotic, and osteoarthritic effects [24]. These effects are attributed to the excellent antioxidant properties of sterols [25, 26]. Some studies have reported that sterols play a significant role in the antimutagenic activity of certain plant extracts [27]. *S. lomentaria* is known to contain bioactive sterol components [28]. Therefore, the antimutagenic activity of *S. lomentaria* extracts on *S. typhimurium* TA98 and TA100 strains may be attributed to the presence of sterols.

Cancer is characterized by uncontrolled cell invasion and proliferation. The use of natural antimutagens is considered one of the best strategies to reduce the negative effects of mutagens [29]. In this context, the search for new chemopreventive antimutagens is a promising area of research. Given the potential health benefits of sterols found in *S. lomentaria*, further investigation into the use of these bioactive compounds as a natural antimutagen for cancer prevention may be warranted.

3.3. Prevention of Oxidative DNA Damage by *S. lomentaria* Extracts

Reactive oxygen species and free radicals can damage DNA, causing oxidative damage and affecting DNA integrity, leading to cell changes, denaturation, and potentially fatal consequences [30]. The protective effect of *S. lomentaria* extracts on oxidative DNA damage was performed by gel electrophoresis using plasmid pBR322 DNA. With the agarose gel electrophoresis method, three different forms of pBR322 DNA can be observed in three different bands in the gel. The original supercoiled form (Form I) of the pBR322 plasmid is opened by DNA damage, forming an open circular form (Form II) and a linear form (Form III) with further cleavage. When DNA is run in gel electrophoresis, Form I moves faster, Form II moves more slowly in the gel, and Form III moves between Form I and Form II [31].

The protective effect of *S. lomentaria* extracts on oxidative DNA damage is shown in Figure 2. As shown in Figure II, DNA completely denatured with H_2O_2 was preserved in the supercoiled (SC) form and notched circular (NC) form in the presence of the extracts. Polyphenols are considered the most active antioxidants in plants and neutralize free radicals formed in cells, therefore they constitute an important group of pharmacologically active compounds [32, 33]. It has been reported in the literature that polyphenols with antioxidant properties have DNA protection activity [34]. Methanol and water extracts of *S. lomentaria* also contain polyphenols [28). The DNA protection activity of *S. lomentaria* extracts may be due to their polyphenol content. It has been determined that the polyphenol extract of *Ascophyllum nodosum* has a protective effect against DNA damage[35]. In addition, studies have shown that red algae extracts have a protective effect in the prevention of DNA damage [36, 37].

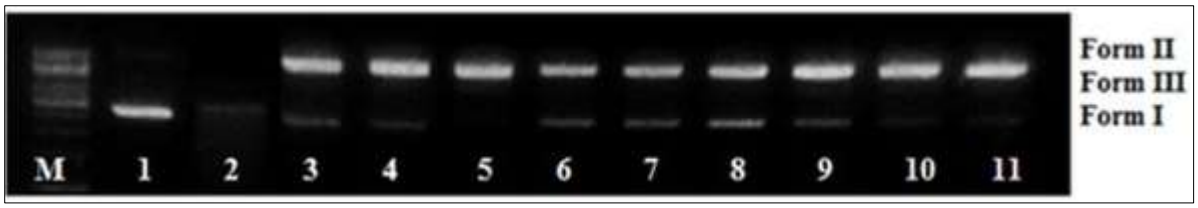

Figure 2 Protective effect of *S. lomentaria* extracts on oxidative DNA damage, M: marker (1 kb), 1.DNA+ H_2O_2, 2. DNA+Hex extract (0.25 mg/mL)+ H_2O_2, 3.DNA+Hex extract (0,5 mg/mL)+ H_2O_2, 4. DNA+Hex extract (0.1 mg/mL)+ H_2O_2, 5. DNA+ aqueous extract (0.25 mg/mL)+ H_2O_2, 6. DNA+ aqueous extract (0.5 mg/mL)+ H_2O_2, 7. DNA+ aqueous extract (1 mg/mL)+ H_2O_2, 8.DNA+MeOH extract (0.25 mg/mL)+ H_2O_2, 9. DNA+ MeOH extract (0.5 mg/mL)+ H_2O_2, 10. DNA+ MeOH extract (1 mg/mL)+ H_2O_2

4. Conclusion

Algae, a natural source, contains secondary metabolites with microbiological and DNA protective properties. With these features, their potential in various medical applications has been frequently emphasized from past to present. In this study, hexane-methanol and aqueous extracts of *S. lomentaria* were obtained and antimicrobial, antimutagenicity and DNA protection activities were reported. The antimicrobial test results of *S. lomentaria* extracts showed that they were more effective against yeast, suggesting potential use in treating various yeast-related diseases. The Ames test results indicated that the extracts did not cause frameshift or base-pair changes and had a high protective effect (>40 %) on DNA. Furthermore, the hexane, methanol, and aqueous extracts of *S. lomentaria* were found to have a protective effect against oxidative DNA damage, suggesting that they may serve as protective agents against chemical-induced DNA damage. However, the antimicrobial and chemopreventive activities of *S. lomentaria* extracts need to be supported by *in vivo* studies and the underlying mechanisms need to be elucidated.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflicts of interest to declare.

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