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Investigation of Antimicrobial Effect by Improving Various Compounds in Padina pavonica (Aydın, Turkey)

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Abstract
Padina pavonica is a member of the brown algae family and is a food source for many organisms in terms of the vitamins, minerals, amino acids and lipids it contains. For other living things, habitat maintenance also gives importance to biodiversity. *Padina pavonica*, which is mainly used in food, cosmetic, feed industry and drug industry, has been reported to have antibacterial and antifungal effect in antimicrobial activity studies. Silver nitrate is the most important silver salt known; colorless, crystalline structure. It can be used as antihemorrhagicand is known to have antibacterial properties. Vinegar is a sour juice that is used as a sweetener in meals, as a sweetener in salads or as a preservative such as brine. Antioxidant properties as well as antimicrobial effects are known for humans. In this study, the antimicrobial different effects of extracts from *Padina pavonica* against various microorganisms were examined by adding vinegar, cider and silver nitrate.

Keywords: Brown algae, *Padina pavonica*, vinegar, cider, silver nitrate

INTRODUCTION

Padina pavonica belongs to the family of brown algae and has a dark color due to the chlorophyll dominance of the phycocyanin in its contents. It is therefore called brown algae [1].It is rich in vitamins, various minerals, amino acids and proteins; polysaccharides, sterizers, and lipids. Its use is preferred in many areas (food, cosmetics and pharmaceutical industry). In addition, antibacterial activities of brominated compounds and etheric oils were determined; some brown algae in the same family have different protein fractions, antitumoral, anticoagulant and antiulcerative activities [2, 3, 4, 5]. In recent years, irregular and widespread uses of drugs and antibiotics that suppress the immune system have resulted in an increase in fungal infections [6]. The use of antibiotics and antifungals are causing microorganism resistance. In this study, we investigated the antimicrobial effect of *Padina pavonica* collected from Aydın-Akbük coastline by adding 5 different extracts, cider, vinegar, silver nitrate, Amphotericin B and Fluconazole.

MATERIALS and METHODS

Plant material

Padina pavonica was collected from the Saplı Island inAydın-Akbük(37 ° 24’34.4 “N 27 ° 24’32.7” S) on April 2016.

Preparation of extracts

The samples brought to the laboratory were washed with tap water and then with 5% low hypochlorite solution. The necrotic parts and the epiphytes were removed and washed with distilled water. *Padina pavonica* weighed 630 grams with the wet weight. They were lyophilized after waiting for 3 days at -80°C. Five different solvents (ethanol, methanol, hexane, acetone and di-ethyl ether) were used to extract in different fractions from *Padina pavonica*. For this, 50 grams of *Padina pavonica*were weighed and placed in 10 pre-sterilized bottles separately and solubilized. At the end of the 26th day, the color in the bottles became darkest and the process of extracting with the evaporator was completed [7]. The obtained extracts were stored at a temperature of + 4°C in the dark light. Five different extracts from *P. pavonica* were all diluted with sterile distilled water to give 500 mg at 150 mL.

Cider and vinegar were bought from the market. Silver nitrate was weighed on a precision scale and transferred into sterile conical. Sterile distilled water was added to obtain a ratio of 1%. The antifungals in the vial form (Lumen 100mL (2mg/mL) Fluconazole vial and Amphotericin B (Fungizone 100mL/50mg) are commercially available.

Microorganisms and condition for cultivation

The microorganisms used in the study were obtained from Adnan Menderes University Faculty of Science and Literature Microbiology laboratory culture collection.*Escherichiacoli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 35032, *Proteus vulgaris* ATCC 33420, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Enterococcus*ATCC29212, *Listeria monocytogenes* ATCC 19112, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13882, *Serratia marcescens* ATCC 13880, *Mycobacterium smegmatis* ATCC 607, *Bacillus subtilis* ATCC 6633, and*Micrococcus luteus* ATCC 9341,were incubated for 24 h at30-37˚C on Nutrient Agar. *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950 and*Saccharomyces cerevisiae* ATCC 9763 incubatedfor 24 h. at30˚C on Saboraud Dextrose Agar.

Antimicrobial assays

Screenings for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms [8, 9, 10, 11]. The inoculum size of each group of bacteria and yeast were prepared by using a no.

0.5 McFarland tube to give a concentration of $1x10^8$ bacteria and $1x10^6$ yeast per milliliter. Mueller Hinton Agar (MHA) was used to test antimicrobial activity. 0.1 ml from cell culture media was inoculated to each plate. It was kept to solidify at room temperature for a while and then holes were made on top with a sterile stick. These holes were filled with 30µL of plant extracts. It is known that the well diffusion method provides stabilization of the active ingredients, especially when investigating the antimicrobial activities of herbal medicines and because they are inoculated into the medium as a liquid. Then, bacterial cultures were incubated at 30-37°C and yeast cultures were incubated at 27-30°C for 18-24 h. After incubation the diameters of the inhibition zones were evaluated in millimeters. The synergistic effect was determined by the checker-board method for mixtures with strong affinity and MIC values[12, 13, 14].

RESULTS and DISCUSSION

In this study, which was investigated to increase the antimicrobial effect of the extracts of *Padina pavonica* by means of well diffusion method by adding apple vinegar, grape vinegar, silver nitrate, Amphotericin B and Fluconazole to 5 different solvent extracts (Table 1)? The findings were given in Tables 2, 3, 4, 5 and Figures 1,2, 3.

The value is determined by the Checker-board method, which is made with the mixtures with which we determined strong activity, and it is given in Table 7, 8, 9.

Defense molecules in plants are known as secondary metabolites. Therefore, the season in which the plant is collected, the temperature of the aquatic environment, the rate of salinity, the rate of heavy metal accumulating in the soil and the aquatic environment, and the rate of pollution change the metabolites of the plant[15].Antifungal and antibacterial efficacy studies also show monthly changes. We think that it may be an antimicrobial agent that can be used in the future by carrying out more detailed studies with molecular techniques, together with finding meaningful effects in our research.

Ethyl ether and hexane, which are most effective from the solvents used, are characterized by the fact that have strong effect on *M. smegmatis* ATCC 607 and *C. albicans* ATCC 10231 by adding 1% $AgNO_3$ (1:1) to the extracts obtained from *Padina pavonica*. Minimal inhibitor concentration (MIC) values of these effects were determined by microdilution of the synergistic effect with the checkerboard method.

Experiments conducted by adding cider, it showed a low effect against various bacteria, but in yeast group the effect of *P. pavonica* hexane isolate on *S.cerevisiae* ATCC 9763 was observed.

The addition of vinegar (1:1) to the extracts from *Padina pavonica* showed a weak effect on Gram-positive (+) and Gram-negative (-) bacteria and a moderate effect against *M. luteus* ATCC 9341 no effect was observed against yeasts.

Amphotericin B and Fluconazole, which are known to have antifungal activity and are used as drugs, have been found to increase the existing effects of extracts obtained from *P. pavonica* on *C.albicans* ATCC 10231, *C.utilis* ATCC 9950, and *S. cerevisiae* ATCC 9763 after addition to the extracts.

Gonzalez del Val (2001) that methanol extracts of *P. pavonica* show antibacterial activity only against *Bacillus subtilis*.

Tüney et al. (2006) researched antimicrobial activity of *P. pavonica* extracts against some pathogenic microorganisms.The ethanol extracts of *P. pavonica* showed low antimicrobial effect against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida* sp. However, the acetone, methanol, and diethyl ether extracts of P. pavonica had no antibacterial or antifungal activities.

Ben-Ali et al. (2010) observed antimicrobial activity of *P. pavonica* in different seasons and it has higher antibacterial effect in summer. They show that *P. pavonica* extracts had large antibacterial activity against Gram (+) pathogens and less important against Gram (-), but all extracts of *P. pavonica* indicate any effect against *E. coli* O126:B16.

Christobel et al. (2011) investigated antimicrobial activity of *Padina tetrastromatica* extracts against some pathogenic bacteria. The brown alga *P. tetrastromatica*showed against the pathogens *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Vibrio harveyi*.

Taherpour et al. (2016) screened antibacterial and antifungal activity of *Padina* sp. as marine alg They indicated that ethanol, ethyl acetate, chloroform extracts of *Padina* sp. did not have effect, but hexane extract of *Padina* sp. had activity against *Staphylococcus aureus*. However, all *Padina* sp. extracts did not infer any antifungal activities against fungi.

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Table 1. Extract amounts from *Padina pavonica*

Solvents	Boiling Point	Drying Method	Extract amount	One dosage (30µL/ mg)
Diethyl ether	34.6	Lyophilised	1.5 mL	1000 mg
Asetone	56	Lyophilised	10.5 mL	143 mg
Methanol	64.7	Lyophilised	1.8 mL	833 mg
Hexane	68	Lyophilised	0.7 mL	2143 mg
Ethanol	78.37	Lyophilised	4 mL	375 mg

Table 2. Antimicrobial effects of $AgNO_3$added*P.pavonica* extracts

Test Microorganisms	Inhibition Diameter (mm)						
	AgNO3 Added Extracts						
	1	**2**	**3**	**4**	**5**	**6**	**7**
*Escherichia coli*ATCC 35218	10	10	9	9	9	10	9
*Pseudomonas aeruginosa*ATCC 35032	14	15	14	13	13	15	14
*Proteus vulgaris*ATCC 33420	10	11	10	10	11	14	12
*Stapylococcus aureus*ATCC 25923	13	13	12	10	14	15	13
Stapylococcus.epidermidis ATCC 12228	10	12	10	9	10	10	10
*Salmonella typhimurium*ATCC 14028	13	14	12	12	12	13	13
*Enterococcus faecalis*ATCC29212	12	13	12	12	13	11	11
Listeria monocytogenes ATCC 19112	-	-	-	-	-	-	-
*Enterobacter aerogenes*ATCC 13048	9	9	9	-	8	9	9
*Klebsiella pneumoniae*ATCC 13882	10	11	11	10	11	10	10
*Serratia marcescens*ATCC 13880	10	10	10	9	10	11	11
*Mycobacterium smegmatis*ATCC 607	17	18	16	14	17	15	15
*Bacillus subtilis*ATCC 6633	14	15	14	14	15	15	15
*Micrococcus luteus*ATCC 9341	15	15	14	13	14	15	15
*Candida albicans*ATCC 10231	19	21	15	14	-	18	19
*Candida utilis*ATCC 9950	19	18	16	15	19	19	19
*Saccharomyces cerevisiae*ATCC 9763	18	19	18	17	20	19	19

1) *P.pavonica* extract obtained from di-ethyl ether + $AgNO_3$(1:1)
2)*P.pavonica* extract obtained from hexan+ $AgNO_3$(1:1)
3)*P.pavonica* extract obtained from aceton+ $AgNO_3$(1:1)
4)*P.pavonica* extract obtained from methanol+ $AgNO_3$(1:1)
5)*P.pavonica* extract obtained from ethanol+ $AgNO_3$(1:1)
6)$AgNO_3$
7) $AgNO_3$+ dH_2O(1:1)

Table 3. Antimicrobial effects of vinegar added *P. pavonica* extracts

Test Microorganisms	Inhibition diameter (mm) Vinegar Added Extracts						
	1	2	3	4	5	6	7
*Escherichia coli*ATCC 35218	9*	10*	10*	-	9*	14	10*
*Pseudomonas aeruginosa*ATCC 35032	-	-	-	-	-	12	-
*Proteus vulgaris*ATCC 33420	-	-	-	-	-	-	-
*Stapylococcus aureus*ATCC 25923	9	-	-	-	-	15	-
Stapylococcus epidermidis ATCC 12228	11*	9*	10	-	-	14*	-
*Salmonella typhimurium*ATCC 14028	10*	11*	11*	10*	9*	15	10
*Enterococcus faecalis*ATCC29212	-	-	-	-	-	-	-
Listeria monocytogenes ATCC 19112	-	-	-	-	-	-	-
*Enterobacter aerogenes*ATCC 13048	-	-	-	-	9*	13	-
*Klebsiella pneumoniae*ATCC 13882	-	-	-	-	-	-	-
*Serratia marcescens*ATCC 13880	11	10*	10*	10*	13*	17	12
*Mycobacterium smegmatis*ATCC 607	-	12	12	13	-	17	-
*Bacillus subtilis*ATCC 6633	10	12	14	14	14	17	10
*Micrococcus luteus*ATCC 9341	12	11	14	14	17	10	-
*Candida albicans*ATCC 10231	-	-	-	-	-	-	-
*Candida utilis*ATCC 9950	-	-	-	-	-	-	-
*Saccharomycescerevisiae*ATCC 9763	-	-	-	-	-	-	-

*** Static effect**
1) *P.pavonica* extract obtained from di-ethyl ether + Vinegar (1:1)
2)*P.pavonica* extract obtained from hexan+ Vinegar(1:1)
3)*P.pavonica* extract obtained from aceton+ Vinegar(1:1)
4)*P.pavonica* extract obtained from methanol+ Vinegar(1:1)
5)*P.pavonica* extract obtained from ethanol+ Vinegar(1:1)
6)Vinegar
7) Vinegar + dH_2O(1:1)

Table 4: Antimicrobial effects of cider added *P. pavonica* extracts

Test Microorganisms	Inhibition zone (mm) Cider Added Extracts						
	1	2	3	4	5	6	7
*Escherichia coli*ATCC 35218	14	13	12	12	14	18	14
*Pseudomonas aeruginosa*ATCC 35032	-	-	-	-	9	16	-
*Proteus vulgaris*ATCC 33420	-	-	-	-	-	16	-
*Stapylococcusaureus*ATCC 25923	-	-	-	-	-	-	-
Stapylococcusepidermidis ATCC 12228	-	-	-	-	-	-	-
*Salmonella typhimurium*ATCC 14028	-	-	-	-	-	-	-
*Enterococcus faecalis*ATCC29212	-	-	-	-	-	-	-
Listeria monocytogenes ATCC 19112	-	-	-	-	-	-	-
*Enterobacter aerogenes*ATCC 13048	-	-	-	-	-	-	-
*Klebsiella pneumoniae*ATCC 13882	15	15	13	16	14	19	14
*Serratia marcescens*ATCC 13880	12	14	13	12	14	18	14
*Mycobacterium smegmatis*ATCC 607	-	13	15	14	12	15	12
*Bacillus subtilis*ATCC 6633	11	12	12	14	13	18	12
*Micrococcus luteus*ATCC 9341	-	-	12	-	16	22	12
*Candida albicans*ATCC 10231	-	-	-	-	-	-	-
*Candida utilis*ATCC 9950	-	-	-	-	-	-	-
*Saccharomyces cerevisiae*ATCC 9763	-	11	-	-	-	-	-

1) *P.pavonica* extract obtained from di-ethyl ether + Cider (1:1)
2)*P.pavonica* extract obtained from hexan+ Cider (1:1)
3)*P.pavonica* extract obtained from aceton+ Cider (1:1)
4)*P.pavonica* extract obtained from methanol+ Cider (1:1)
5)*P.pavonica* extract obtained from ethanol+Cider (1:1)
6)Cider
7)Cider + dH_2O

Table 5: Antimicrobial effects of Flukanazoladded *P.pavonica* extracts

Test Microorganisms	Inhibition Zone (mm)						
	Flukanazol	FlukanazolAdded Extracts					
		1	2	3	4	5	6
*Candida albicans*ATCC 10231	30	35	33	34	33	36	36
*Candida utilis*ATCC 9950	28	27	26	28	26	32	26
*Saccharomyces cerevisiae*ATCC 9763	-	16	15	17	15	-	14

1) *P.pavonica* extract obtained from di-ethyl ether + flucanazole (1:1)
2) *P.pavonica extract* obtained from the hexan + flucanazole (1:1)
3) *P.pavonica* extract obtained from aceton+ flucanazole(1:1)
4) *P.pavonica* extract obtained from methanol+ flucanazole (1:1)
5) *P.pavonica* extract obtained from ethanol+ flucanazole (1:1)
6) AgNO3 + flucanazole(1:1)

Table 6: Antimicrobial effects of Amphotericin B added*P. pavonica* extracts

Test Microorganisms	Inhibition zone (mm)						
	Amfoterisin B	Amphotericin B Added Extracts					
		1	2	3	4	5	6
*Candida albicans*ATCC 10231	22	29	25	26	25	24	26
*Candida utilis*ATCC 9950	25	22	24	27	25	28	27
*Saccharomycescerevisiae*ATCC 9763	15*	16	15	17	15	-	14

*** Static effect**
1) *P.pavonica* extract obtained from di-ethyl ether + Amphotericin B(1:1)
2) *P.pavonica* extract obtained from the hexan + Amphotericin B(1:1)
3) *P.pavonica* extract obtained from aceton+ Amphotericin B(1:1)
4) *P.pavonica* extract obtained from methanol + Amphotericin B(1:1)
5) *P.pavonica* extract obtained from ethanol+ Amphotericin B(1:1)
6) AgNO3 + Amphotericin B(1:1)

Table 7. MIC values determined with Cheker-Board method

P. pavonica extract obtained from hexan+ $AgNO_3$	Test Microorganisms	
	Mycobacterium smegmatis ATCC 607	*Candida albicans* ATCC 10231
10 µL extract+ 90 µL AgNO3	+	+
20 µL extract+ 80 µL AgNO3	+	+
30 µL extract+ 70 µL AgNO3	+	+
40 µL extract+ 60 µL AgNO3	+	-
50 µL extract+ 50 µL AgNO3	-	-
60 µL extract+ 40 µL AgNO3	-	-
70 µL extract+ 30 µL AgNO3	-	-
80 µL extractt+ 20 µL AgNO3	-	-
90 µL extract+ 10 µL AgNO3	-	-

Table 8. MIC values determined with Cheker-Board method

P.pavonica extract obtained from ethanol + vinegar	Test Microorganism *Micrococcus luteus* ATCC 9341
10 µL extract+ 90 µLvinegar	+
20 µL extract+ 80 µLvinegar	+
30 µL extract+ 70 µLvinegar	+
40 µL extract+ 60 µLvinegar	+
50 µL extract+ 50 µLvinegar	+
60 µL extract+ 40 µLvinegar	+
70 µL extract+ 30 µLvinegar	-
80 µL extract+ 20 µLvinegar	-
90 µL extract+ 10 µLvinegar	-

Table 9. MIC values determined with Cheker-Board method

P. pavonica extract obtained from di-ethyl ether + Cider	Test Microorganism
	Klebsiella pneumoniae ATCC 13882
10 µL extract+ 90 µLcider	+
20 µL extract+ 80 µL cider	+
30 µL extract+ 70 µLcider	+
40 µL extract+ 60 µLcider	+
50 µL extract+ 50 µLcider	+
60 µL extract+ 40 µLcider	-
70 µL extract+ 30 µLcider	-
80 µL extract+ 20 µLcider	-
90 µL extract+ 10 µL cider	-

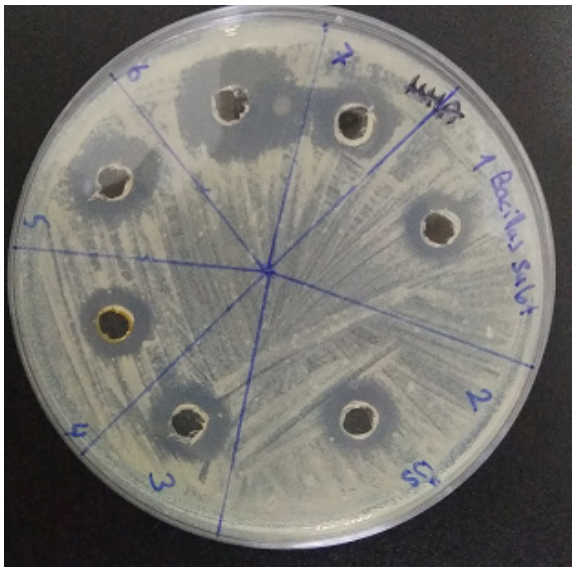

Figure 1. Antibacterial zones formed (**1)** 30µL di-ethyl ether + 30 µL vinegar, **2)** 30 µL hexane + 30 µL vinegar, **3)** 30 µL asetone + 30 µL vinegar, **4)** 30 µL methanol + 30 µL vinegar, **5)** 30 µL ethanol + 30 µL vinegar, **6)** 60 µL vinegar, **7)** 30 µL vinegar + 30 µl dH_2O

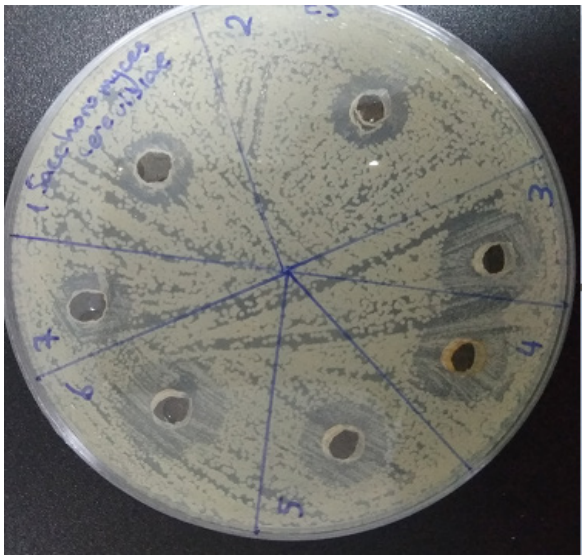

Figure 2. Antibacterial zones formed (**1)** 30µL di-ethyl ether + 30 µLcider, **2)** 30 µL hexane + 30 µLcider, **3)** 30 µL asetone + 30 µLcider, **4)** 30 µL methanol + 30 µLcider, **5)** 30 µL ethanol + 30 µLcider, **6)** 60 µLcider, **7)** 30 µL cider + 30 µl dH_2O

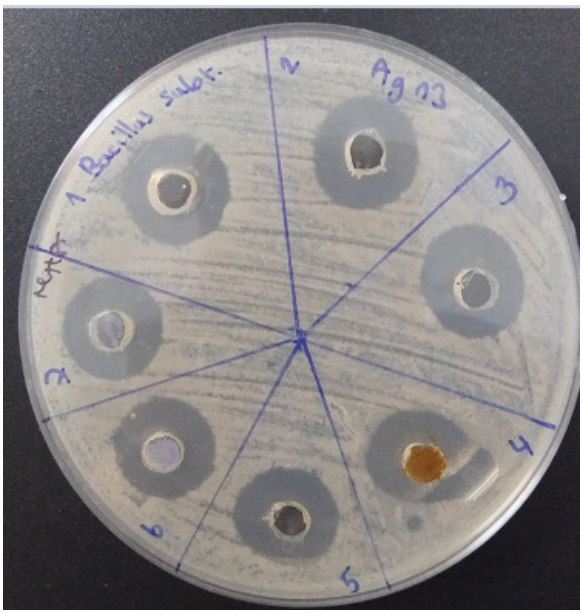

Figure 3. Antibacterial zones formed (**1)** 30µL di-ethyl ether + 30 µL %1 $AgNO_3$, **2)** 30 µL hexane + 30 µL %1 $AgNO_3$, **3)** 30 µL asetone + 30 µL %1 $AgNO_3$, **4)** 30 µL methanol + 30 µL %1 $AgNO_3$, **5)** 30 µL ethanol + 30 µL %1 $AgNO_3$, **6)** 60 µL %1 $AgNO_3$, **7)** 30 µL %1 $AgNO_3$+ 30 µL dH_2O