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Annals of Phytomedicine 7(2): 70-75, 2018
DOI: 10.21276/ap.2018.7.2.10; Print ISSN : 2278-9839 and Online ISSN : 2393-9885

Ann. Phytomed.,
7(2):70-75 (2018)

Original article

Antimicrobial effects of the stem extracts of *Apium graveolens* Mill.

Esin Poyrazoglu Coban, Zeynep Ercin, Bahadir Torun and H. Halil Biyik
Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey

Received September 14, 2018: Revised October 30, 2018: Accepted November 6, 2018: Published online December 30, 2018

Abstract

Apium graveolens Mill. is a vegetable that is abundantly found and consumed in the markets of Aydýn province. The aim of this study was to test the antimicrobial activity of *A. graveolens* stem. Plant stems were dried and extracted with methanol, n-propanol, ethanol, acetone, n-hexane, and ethyl acetate. Antimicrobial activity of the extracts was determined by the disc diffusion method. Test microorganisms were 18 bacteria and 4 yeasts. Also standard antibiotic discs were used for comparison of inhibition zones. Results showed that n-propanol and acetone extracts of *A. glaveolens* stem were most effective against used microorganisms. n-propanol extract of *A. glaveolens* stem inhibited the growth of twenty microorganisms and the inhibition zones ranged between 9-16 mm. The acetone extract of *A. glaveolens* stem inhibited the growth of eighteen microorganisms and the inhibition zones ranged between 8-15 mm. The ethanol extract of *A. glaveolens* stem inhibited the growth of ten microorganisms and the inhibition zones ranged between 9-13 mm. However, methanol, hexane and ethyl acetate extracts did not show any antimicrobial effects against used microorganisms. The inhibition zones of standard antibiotics used as positive control (chloramphenicol, gentamycin, tetracycline, erythromycin and ampicillin) were between 11-30 mm. against bacteria while the inhibition zones of standard antibiotic (nystatin) were between 20-22 mm. against yeasts.

Key words: *Apium graveolens* Mill., antimicrobial effect, folkloric medicine

1. Introduction

Plants synthesize natural active products depending on their mechanism and biological properties (Akhtar *et al*., 2014; Babu *et al*., 2015; Mahendra *et al*., 2016; Kooti and Daracei, 2017). One of these plants is celery, *A. graveolens* (Celery) is a marshland plant in the family Apiaceae that has been cultivated as a vegetable since antiquity. Celery leaves are pinnate to bipinnate with rhombic leaf 3 - 6 cm (1.2 - 2.4 in) long and 2 - 4 cm (0.79 -1.57 in) broad. Wild celery, *Apium graveolens* var. *graveolens*, grows to 1 m (3.3 ft) tall. It occurs around the globe (Lim, 2015). The first cultivation is thought to have happened in the Mediterranean region where the natural habitats were salty and wet, or marshy soils near the coast. *AG* has wide commercial significance all over the World, in Europe, North America, India, Iran and Pakistan (Vimorin, 1950).

Celery seed is also used as a spice and its extracts have been used in herbal medicine. Seed decoction were used for bronchitis, rheumatism and arthritis and as a sedative, blood purifier. Juices extracted from the stem were used to treat edema, rheumatic tendencies, gout, flatulence, overweight, lack of appetite and as a strong diuretic and an antiseptic (Shanmugapriya and Ushadevi, 2014).

Author for correspondence: Dr. Esin Poyrazoglu Coban
Associate Professor, Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey
E-mail: epoyrazoglu@adu.edu.tr
Tel.: +90-2562182000

Chemically, *A. graveolens* contains a class of phenolic compounds called caffeic acid, caffeoylquinic acid, cinnamic acid, coumaric acid, ferulic acid, falvones such as apigenin and luteolin, flavonols such as quercetin and kaempferol, lunularin, dihydrostilbenoids, phytosterols, beta-sitosterol, furanocoumarins like psoralen, xanthotoxin and bergapten and phthalide derivatives such as sedanolide and senkyunolides (Patel *et al.*, 2013). Celery contains vitamin A, B1, B2, B6, C, E, K and minerals such as iron, calcium, phosphorus, magnesium, molybdenum and zinc. Thus, celery has the ideal quantities of iron and magnesium to stop oncological diseases (Uddin *et al.*,, 2015).

The aim of this study was to find out the antimicrobial effects of six different extracts of *AG*. Mill.

2. Material and Methods

2.1 Plant materials

AG. Mill. was bought from local market in Aydin province in Turkey and was used stem of plant.

2.2 Preparation of plant extracts

Stem of the plant samples were washed with distilled water and reduced to powder with liquid nitrogen. Ten gram of this material was added to separately in 100 ml of n-propanol, methanol, acetone, n-hexane, ethyl acetate and ethanol. Then the mixtures were agitated for a period of 72 h. They were filtered with Whatman No. 389 filter paper. Under aseptic conditions, the extracts were filtered through 0.45 micrometer-pore size diameter filters and stored at 4°C (Coban *et al*., 2017a).

2.3 Microorganisms and condition for cultivation

The eighteen bacteria and four yeasts were used to test the antimicrobial effect. They were: *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans*, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida tropicalis*, *Candida glabrata*. The bacteria and yeasts were cultured in Tryptic Soy Agar (Merck) at 30-37°C, Malt Extract Agar (Merck) at 27-30°C for 24 h, respectively (Coban *et al.*, 2017b).

2.4 Antimicrobial assays

2.4.1 Disc diffusion method

Screening for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms (Collins *et al.*, 1995; CLSI, 2015). 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 $1x10^6$ yeasts per milliliter. In order to test the antimicrobial activity of plants, 20 ml of Mueller Hinton Agar (MHA) were poured in petridishes and kept to solidify at room temperature. Then, it was inoculated with strains of bacteria and yeasts by taking 0.1 ml from cell culture media. Afterwards, a hole of 6 mm in diameter and depth were made on top with a sterile stick and was filled with 50 µl of plant extracts. Then, bacterial cultures were incubated at 30-37°C and yeast cultures were incubated at 27-30°C for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimeters. Discs of chloramphenicol (C30), gentamycin (CN10), tetracycline (TE30), erythromycin (E15), ampicillin (AM10), and nystatin (NS100) were used as positive controls (Coban *et al.*, 2017c).

2.4.2 Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was carried out according to reported method (Jones *et al*., 1985; CLSI, 2009). The used bacteria were inoculated in Tryptic Soy Broth (TSB) and Brain Heart Infusion Broth (BHIB) and incubated at 30-37°C for 24 h while the yeasts were inoculated in Malt Extract Broth (MEB) and incubated at 27°C for 24 h. The inoculums were adjusted according to 0.5 McFarland standard tubes. Firstly, 100 µl of Mueller Hinton Broth (MHB) was placed in each well. Afterwards, the extracts were added into the first well. Two fold serial dilutions of the compounds were carried out to determine the MIC, within the concentration range 256 to 0.25 μgml^{-1}. Bacteria cultures were grown at 30-37°C (18-20 h) and the final inoculum was approximately 10^6 μgml^{-1} while yeasts cultures were grown at 27°C (18-20 h) and the final inoculum was approximately 10^5 μgml^{-1}.

The lowest concentration of antimicrobial agent that resulted in complete inhibition of the microorganisms was represented as MIC (μgml^{-1}). As positive controls, streptomycin (I.E. Ulagay) for bacteria and nystatin (NS100, Oxoid) for yeast were used in the dilution method. In each case, the test was performed in triplicate and the results were expressed as means.

2.5. Statistical analysis

Mean values and standard deviation calculations were made by SPSS v22 (Statistical Package for Social Sciences).

3. Results and Discussion

3.1 Antimicrobial activity

The antimicrobial activity of methanol, n-propanol, ethanol, acetone, n-hexane and ethyl acetate of *A. graveolens* plant were investigated and the results were given in Tables 1, 3.

From the results presented in Table 1, n-propanol extract of *A. graveolens* showed strong effect against *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Bacillus cereus.* ATCC 11778, *Candida utilis* ATCC 9950 and the inhibition zones ranged between 13-16 mm while the extract demostrated moderate effect against *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Salmonella typhimirium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 35032, *Micrococcus luteus* ATCC 9341 and the inhibition zones ranged between 11-12 mm. However, the extract indicated slight effect against *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Proteus vulgaris* ATCC 33420, *Bacillus subtilis* ATCC 6633, *Candida tropicalis*, *Candida glabrata* while it did not show any effect against *Streptococcus mutans* and *Candida albicans* ATCC 10231.

The acetone extract of *A. graveolens* showed high effect against *Serratia marcescens* ATCC 13880 and *Bacillus subtilis* ATCC 6633 and the inhibition zones ranged between 13-15 mm while the extract displayed moderate effect against *Corynebacterium xerosis* ATCC 373, *Bacillus cereus* ATCC 11778, respectively, 12-11 mm. Besides, the extract demostrated slight effect agaist *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimirium* ATCC 14028, *Mycobacterium smegmatis* ATCC 607, *Enterococcus faecalis* ATCC 29212, *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Pseudomonas aeruginosa* ATCC 35032, *Candida albicans* ATCC 10231, *Candida tropicalis*, *Candida glabrata* and the inhibition zones ranged between 8-10 mm while the extract did not indicate any effect against *Micrococcus luteus* ATCC 9341, *Streptococcus mutans*, *Streptococcus pneumoniae* ATCC 27336, *Klebsiella pneumoniae* ATCC 13882.

The ethanol extract of *A. graveolens* showed high effect (13 mm) against *Serratia marcescens* ATCC 13880 and *Micrococcus luteus* ATCC 9341. However, the extract indicated very low effect (9-10 mm) against *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19112, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Candida utilis* ATCC 9950, *Candida tropicalis* while the extract did not reveal any effect against *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimirium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Streptococcus pneumoniae* ATCC 27336, *Proteus vulgaris* ATCC 33420, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans*, *Candida albicans* ATCC 10231, *Candida glabrata*.

Table 1: Antimicrobial activities of the extracts of *A. graveolens* on some bacteria and yeasts

Test microorganisms	Inhibition zones (mm)											
	Stem extracts						Reference antibiotics					
	1	2	3	4	5	6	C 30	CN 10	TE 30	E 15	AMP 10	NS 100
Escherichia coli ATCC 35218	-	9	-	10	-	-	24	21	15	11	-	NT
Stapylococcus aureus ATCC 25923	-	11	10	10	-	-	23	20	22	23	20	NT
Stapylococcus epidermidis ATCC 12228	-	12	10	10	-	-	22	17	19	11	17	NT
Enterobacter aerogenes ATCC 13048	-	9	-	8	-	-	19	20	14	-	-	NT
Salmonella typhimirium ATCC 14028	-	11	-	9	-	-	17	16	15	8	8	NT
Klebsiella pneumoniae ATCC 13882	-	10	-	-	-	-	21	19	20	14	-	NT
Mycobacterium smegmatis ATCC 607	-	10	-	10	-	-	23	18	26	25	19	NT
Corynebacterium xerosis ATCC 373	-	10	-	12	-	-	20	17	25	26	27	NT
Enterococcus faecalis ATCC 29212	-	11	9	9	-	-	16	11	19	-	14	NT
Streptococcus pneumoniae ATCC 27336	-	13	-	-	-	-	24	20	25	15	14	NT
Serratia marcescens ATCC 13880	-	15	13	15	-	-	23	19	13	-	19	NT
Proteus vulgaris ATCC 33420	-	9	-	10	-	-	17	24	17	20	-	NT
Listeria monocytogenes ATCC 19112	-	14	10	10	-	-	19	14	12	-	12	NT
Pseudomonas aeruginosa ATCC 35032	-	11	-	10	-	-	22	20	20	21	-	NT
*Streptococcus mutans***	-	-	-	-	-	-	28	22	19	-	-	NT
Micrococcus luteus ATCC 9341	-	11	13		-	-	25	15	26	30	28	NT
Bacillus cereus ATCC 11778	-	16	10	11	-	-	-	23	24	25	26	NT
Bacillus subtilis ATCC 6633	-	10	9	13	-	-	22	20	12	25	-	NT
Candida albicans ATCC 10231	-	-	-	10	-	-	NT	NT	NT	NT	NT	22
Candida utilis ATCC 9950	-	14	10	12	-	-	NT	NT	NT	NT	NT	21
*Candida tropicalis**	-	10	9	10	-	-	NT	NT	NT	NT	NT	20
*Candida glabrata**	-	9	-	10	-	-	NT	NT	NT	NT	NT	21

1: Methanol, 2: n-Propanol, 3: Ethanol, 4: Acetone, 5: Hexane and 6: Ethyl Acetate

C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erytromycin (15 mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid)

(-): No zone; NT: Not tested,

(*): Special gift from Faculty of Medicine, Adnan Menderes University, Aydin, Turkey

(**): Special gift from Department of Microbiology, Ege University Faculty, Aydin, Turkey

Table 2: Mean values of each extract with standart deviation

Solvents	Mean	StdDev
n-propanol	11,25	4,96
Methanol	0,41	5,4
Acetone	8,6	4,92
n-hexane	0	0
Ethyl acetate	0	3,85
Ethanol	4,68	5,63

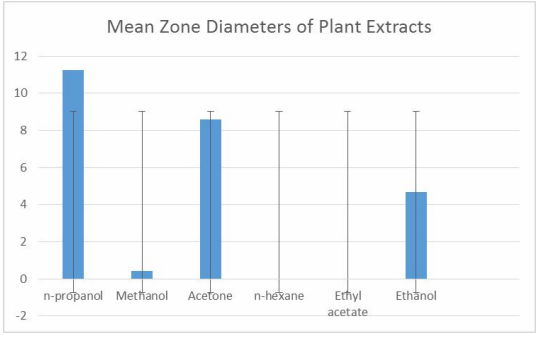

Figure 1: Zone range of each extract with standart deviation.

Table 3: Antimicrobial activities of the extracts of *A. graveolens*. (MIC, µg ml^{-1})

Test microorganisms	2	3	4	Str	NS 100
Escherichia coli ATCC 35218	256	-	128	64	NT
Stapylococcus aureus ATCC 25923	128	128	128	32	NT
Stapylococcus epidermidis ATCC 12228	64	128	128	32	NT
Enterobacter aerogenes ATCC 13048	256	-	256	32	NT
Salmonella typhimirium ATCC 14028	128	-	256	64	NT
Klebsiella pneumoniae ATCC 13882	128	-	-	64	NT
Mycobacterium smegmatis ATCC 607	128	-	128	128	NT
Corynebacterium xerosis ATCC 373	128	-	64	64	NT
Enterococcus faecalis ATCC 29212	128	256	256	64	NT
Streptococcus pneumoniae ATCC 27336	64	-	-	128	NT
Serratia marcescens ATCC 13880	32	64	32	64	NT
Proteus vulgaris ATCC 33420	256	-	256	64	NT
Listeria monocytogenes ATCC 19112	64	256	256	32	NT
Pseudomonas aeruginosa ATCC 35032	128	-	256	64	NT
Micrococcus luteus ATCC 9341	128	64	-	32	NT
Bacillus cereus ATCC 11778	32	256	128	64	NT
Bacillus subtilis ATCC 6633	256	256	64	64	NT
Candida albicans ATCC 10231	-	-	256	NT	64
Candida utilis ATCC 9950	64	256	64	NT	64
*Candida tropicalis**	256	256	256	NT	64
*Candida glabrata**	256	-	256	NT	64

2: n-Propanol, 3: Ethanol and 4: Acetone
Methanol, hexane, ethyl acetate extracts did not show antibacterial activity.
Str = Streptomycin, NS100 = Nystatin.
(–) = No effect.
* From Faculty of Medicine, Adnan Menderes University, Aydin, Turkey

In addition, n-propanol and acetone extracts of *A. graveolens* demostrated against *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Proteus vulgaris* ATCC 33420, *Pseudomonas aeruginosa* ATCC 35032 and *Candida glabrata*. However, methanol, hexane and ethyl acetate extracts of *A. graveolens* did not show any antimicrobial effects against used microorganisms. Only acetone extract of *A. graveolens* showed effect on *Candida albicans* ATCC 10231. In addition to this, only n-propanol extract of *A. glaveolens* Mill. was effective on *Streptococcus pneumoniae* ATCC 27336. While acetone extract of *A. graveolens* demonstrated stronger activity against *C. xerosis* ATCC 373, *Serratia marcescens* ATCC 13880, *Bacillus subtilis* ATCC 6633 and *Candida utilis* ATCC 9950 the n-propanol extract of *A. graveolens* indicated stronger activity against *Stapylococcus epidermidis* ATCC 12228, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Listeria monocytogenes* ATCC 19112, *Bacillus cereus* ATCC 11778 and *Candida utilis* ATCC 9950. In addition to this, the ethanol extract of *A. graveolens* showed high effect only against *Serratia marcescens* ATCC 13880 (Figures 2a,b,c,d). However, methanol, hexane and ethyl acetate extracts of *A. graveolens* did not show any antimicrobial effects against used microorganisms.

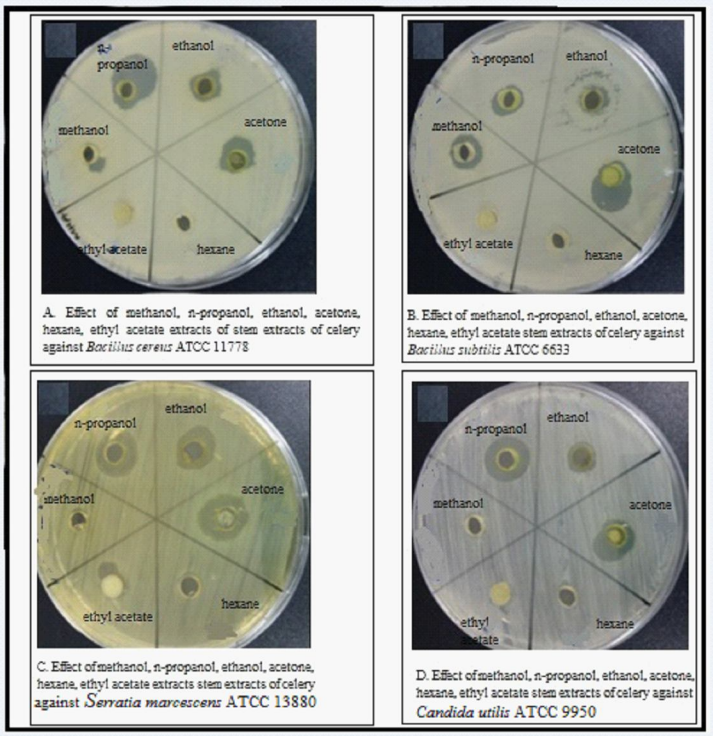

Figure 2: (a): Effect of methanol, n-propanol, ethanol, acetone, hexane, ethyl acetate extracts of stem extracts of celery against *Bacillus cereus* ATCC 11778, (b): Effect of stem extracts of celery against *Bacillus subtilis* ATCC 6633, (c): Effect of stem extracts of celery against *Serratia marcescens* ATCC 13880 and (d): Effect of stem extracts of celery against *Candida utilis* ATCC 9950 1: Methanol, 2: n-Propanol, 3: Ethanol, 4: Acetone, 5: Hexane, 6: Ethyl Acetate.

The MIC values in Table 2 showed that n-propanol, ethanol and acetone extracts demostrated remarkable effect (32-64 μgml^{-1}) on *S. epidermidis* ATCC 12228, *C. xerosis* ATCC 373, *S. pneumoniae* ATCC 27336, *S. marcescens* ATCC 13880, *L. monocytogenes* ATCC 19112, *M. luteus* ATCC 9341, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6633 and *C. utilis* ATCC 9950.

However, n-propanol, ethanol and acetone extracts showed low effect (128-256 μgml^{-1}) against *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *E. aerogenes* ATCC 13048, *S. typhimirium* ATCC 14028, *K. pneumoniae* ATCC 13882, *M. smegmatis* ATCC 607, *E. faecalis* ATCC 29212, *P. vulgaris* ATCC 33420, *P. aeruginosa* ATCC 35032, *C. tropicalis* and *C. glabrata*.

According to statistical values, n-propanol extract is seen the most effective among the used solvents (Figure 1).

Nilugal *et al*. (2015) evaluated the aqueous and ethanol extracts of *A. graveolens* at concentration range between 130000 µg/ml to 13 µg/ml against *Neisseria gonorrhoeae* and *Candida albicans* for antimicrobial potentiality. They found that both extracts had different sensitivity levels for the tested strains of microorganism and the inhibition zones ranged between 20.00 ± 2.00 to 6.67 ± 0.58.

Shanmugapriya and Ushadevi (2014) examined methanol, diethyl ether and aqueous extracts of *A. graveolens* seeds against *Escherichia coli* and *Pseudomonas aeruginosa* isolated from urinary tract infected patients.

Edziri *et al*. (2012) investigated antimicrobial activity of aqueous and methanol extracts obtained from *A. graveolens* Among tested extracts, the methanol extract of *A graveolens* Mill. showed the best antifungal activity against *Candida albicans*, *Candida kreussei* and *Candida parapsilosis*.

Shad *et al*. (2011) researched antimicrobial effect of methanol, hexane, chloroform and water extracts of *A. graveolens* and found that these extracts showed various degrees (6-14 mm) of inhibition of antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*.

Zhou *et al*. (2009) characterized an antimicrobial component from celery (*A. graveolens*) seeds. Therefore, a crude alcoholic extract of celery seeds was fractionated by organic solvent extractions, column chromatography and HPLC. Fractions were tested for antimicrobial activity against *Helicobacter pylori*, *Campylobacter jejuni* and *Escherichia coli*. According to results, the extract showed effects on *H. pylori* and was not active against *C. jejuni* and *Escherichia coli*.

In this study, n-propanol extract of of *A. graveolens* showed high antimicrobial activity against *Stapylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Listeria monocytogenes* ATCC 19112, *Bacillus cereus* ATCC 11778, *Candida utilis* ATCC 9950 and the inhibition zones ranged between 12-16 mm. Moreover, acetone extract of *A. graveolens* indicated high antimicrobial activity against *Corynebacterium xerosis* ATCC 373, *Serratia marcescens* ATCC 13880, *Bacillus subtilis* ATCC 6633, *Candida utilis* ATCC 9950 and the inhibition zones ranged between 12–15 mm. However, ethanol

extract of *A. graveolens* demostrated slightly effect while the hexane and ethyl acetate extracts did not show any antimicrobial effects against used microorganisms. Therefore, the extracts of the n-propanol and acetone of *A. graveolens* were found most effective among the solvents.

4. Conclusion

The extracts of the n-propanol and acetone of *A. graveolens* stem showed high effect comparing to other solvents. The ethanol extracts demonstrated moderate effect, while the extract of methanol, n-hexane and ethyl acetate showed no effect against tested bacteria and yeasts. The n-propanol and acetone reveal effective secondary metabolites like alkaloids, tannins, and flavonoids against microorganisms from *Apium graveolens* Mill. stem. This could be the reasons of different uptake mechanisms of these chemicals. Solvents that has easy uptake in microorganisms can be more effective. More research was needed to prove the reasons behind this and also the need to find new compounds from plants with antimicrobial effect.

Acknowledgments

This work was carried out in the Department of Microbiology, Adnan Menderes University, Aydin, Turkey.

Conflict of interest

We declare that we have no conflict of interest.

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