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Determination of Antimicrobial Effect and DNA Interaction of Two Endemic *Rhaponticoides* Species (*R. mykalea* and *R. hierroi)*

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Research Article

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Abstract

Purpose: This study was carried out to determine the antimicrobial effect and DNA interaction of two endemic *Rhaponticoides* species (*R. mykalea* and *R. hierroi*) which are distributed in Turkey. Ethanol and methanol extracts of leaf and stem parts of *R. mykalea* and *R. hierroi* were used in this study. **Material and Methods:** The antimicrobial activities of the extracts were determined by agar well method and evaluated on *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumaniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258 and *Candida tropicalis* Y-12968. For comparison, ampicillin, chloramphenicol (antibacterial) and ketoconazole (antifungal) were used as standard antibiotics. The diameter of the inhibition zones, formed after incubation, is measured in mm. The DNA interaction of plants extracts were determined by agarose gel electrophoresis method. The effect of extracts on DNA was measured for 24 and 48 hours. Furthermore, the nucleotide linkage of the substances was investigated by restriction enzyme digestion. **Results:** *R. hierroi* methanol extract formed against to *E. coli* ATCC 35218, 10.67 ± 0.47 mm; against to *S. aureus* ATCC 25923, 12 ± 0.82 mm and against to *K. pneumaniae* ATCC 13883, 13.33 ± 0.47 mm inhibition zone diameter. *R. hierroi* ethanol extract formed against to *S. aureus* ATCC 25923, 12.67 ± 0.94 mm; against to *B. subtilis* ATCC 6633, 10.33 ± 0.47 mm inhibition zone diameter. *R. mykalea* methanol extract formed against to *P. vulgaris* RSKK 96029, 13 ± 1.41 mm; against to *K. pneumaniae* ATCC 13883, 12.33 ± 0.47 mm; against to *B. cereus* NRRL B – 3711, 11.67 ± 0.47 mm and against to *P. aeruginosa* ATCC 27853, 12 ± 0 mm inhibition zone diameter. *R. mykalea* ethanol extract formed against to *E. hirae* ATCC 9790, 11.67 ± 0.47 mm; against to *K. Pneuma niae* ATCC 13883, 11.33 ± 0.47 mm and against to *P. aeruginosa* ATCC 27853, 12 ± 0 mm inhibition zone diameter. Likewise, the extracts were observed to cause DNA breaks and bound to both A/A and G/G nucleotides by restriction enzyme digestion experiments. **Conclusion:** In this study, it has been determined that the extracts obtained from *R. hierroi* and *R. mykalea* plants have antimicrobial activity on at least one or more microorganisms. The

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R. mykalea methanol extract was found to be more effective than the others. The highest inhibition zone diameter was determined against *R. hierroi* methanol extract *K. pneumoniae* ATCC 13883 strain (13,33 ± 0,47 mm). It has been determined that the extracts had a concentration and time-dependent effect on the DNA and this effect and is in the form of DNA cutting activity. The strongest effect was observed at high concentration, while at other concentrations, form III DNA was observed which formed a double chain fracture outcome.

Keywords: Antimicrobial effect, DNA interacton, Endemic, Herbal plant, *Rhaponticoides*, Turkey

1. Introduction

Rhaponticoides include lots of endemic species which belongs to the *Asteraceae* family. The previous *Centaurea* sect. *Centaurea* has been segregated from the *Centaurea* genus and named as *Rhaponticoides Vaill* [12]. Rhaponticoides are found in Portugal and Monaco in the west, and in Mongolia in the east with 32 species [12]. Most of these species are either rarely endemic or spatially distributed [9]. Only a very small number of species show wide spread. In our country Rhaponticoides genus is represented by 8 species. *R. hierroi* is endemic for the region of Antalya and *R. mykalea* is endemic for the region of Aegean. *R. mykalea* categorized as CR (Critically Endangered, Very Endangered). This species called as a "peygamber çiçeği" in Turkey [9].

This study was carried out to determine the antimicrobial effect and DNA interaction of two endemic *Rhaponticoides* species (*R. mykalea* and *R. hierroi*) which are distributed in Turkey.

Photo 1. R. mykalea capitula

2. MATERIAL AND METHODS

2.1 Material

Ethanol and methanol extracts of leaf and stem parts of *R. mykalea* and *R. hierroi* were used in this study. Plants materials were collected in May, August and September 2017 from Konya and Isparta. The voucher specimens were stored at the KNYA herbarium. The antimicrobial activities for the extracts were evaluated on *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumaniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258 and *Candida tropicalis* Y-12968. We were also used ampicillin, chloramphenicol and ketoconazole, standard antibiotics. Muller Hilton Agar, Nutrient Broth, Malt Extract Agar, % 0.9 Serum physiologique, Distilled water, DMSO, ethanol and methanol were used for the analysis.

Photo 2. R. hierroi capitula

2.2 Methods

Leaf and stem parts of two endemic *Rhaponticoides* species (*R. mykalea* and *R. hierroi*) were dried in a cool and dry environment. Leaf and stem parts of this dried plants were milled. Milled plant samples and 10 times as much solvent as the sample were mixed and left in the dark for 7 days at room temperature. The mixture filtered through Wathman number 1 paper filter. The solvent evaporated at 50° C with the low pressure.

2.3 Antimicrobial Activity

The antimicrobial activities of the extracts were determined by agar well method and evaluated on *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumaniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258 and *Candida tropicalis* Y-12968. For comparison, ampicillin, chloramphenicol (antibacterial) and ketoconazole (antifungal) were used as standard antibiotics. The diameter of the inhibition zones, formed after incubation, is measured in mm.

In the agar well method, bacterial strains were allowed to incubate at 37 ° C for 24 hours in Nutrient Agar medium and yeast strains were incubated for 48 hours at 30 ° C in Malt Extract Agar medium. The post-incubation microorganisms were adjusted to 0.5 McFarland blur. Muller – Hinton Agar (for bacterial strains) and Malt Extract Agar (for yeast strains) were spread on a petri with a 1% suspension of microorganism suspension. With the punch, 6 mm in diameter wells are opened at specific points of the medium. The opened wells were placed in a volume of 50 µL from plant extracts at a concentration of 100 mg / mL and left to incubate. The diameter of the inhibition zones formed after incubation is measured in mm.

Chloramphenicol, ampicillin and ketocanosol were used for antimicrobial activity.

2.4 DNA Interaction

The DNA interaction of plants extracts were determined by agarose gel electrophoresis method. The effect of extracts on DNA was measured for 24 and 48 hours. Furthermore, the nucleotide linkage of the substances was investigated by restriction enzyme digestion.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

R. hierroi methanol extract formed against to *E. coli* ATCC 35218, 10.67 ± 0.47 mm; against to *S. aureus* ATCC 25923, 12 ± 0.82 mm and against to *K. pneumaniae* ATCC 13883, 13.33 ± 0.47 mm inhibition zone diameter.

R. hierroi ethanol extract formed against to *S. aureus* ATCC 25923, 12.67 ± 0.94 mm; against to *B. subtilis* ATCC 6633, 10.33 ± 0.47 mm inhibition zone diameter.

R. mykalea methanol extract formed against to *P. vulgaris* RSKK 96029, 13 ± 1.41 mm; against to *K. pneu-*

Table 1. Antimicrobial effect results

Plant **Microorganism**	*R. hierroi* **Methanol**	*R. hierroi* **Ethanol**	*R. mykalea* **Methanol**	*R. mykalea* **Ethanol**	**Amp**[a]	**C**[b]	**Keto**[c]
E. coli ATCC 35218	10,67 ± 0,47	-	-	-	-	8 ±0	nw
E. coli ATCC 25922	-	-	-	-	18 ± 0	25 ± 0	nw
S. aureus ATCC 25923	12 ± 0,82	12,67 ± 0,94	-	-	44 ± 1	24 ± 1	nw
S. typhimurium ATCC 14028	-	-	-	-	19 ± 1	38 ±1	nw
P. vulgaris RSKK 96029	-	-	13 ± 1,41	-	-	32 ± 1	nw
E. hirae ATCC 9790	-	-	-	11,67 ± 0,47	9 ± 1	22 ± 1	nw
E. faecalis ATCC 29212	-	-	-	-	27 ± 0	20 ± 0	nw
K. pneumaniae ATCC 13883	13,33 ± 0,47	-	12,33 ± 0,47	11,33 ± 0,47	-	31 ± 1	nw
B. subtilis ATCC 6633	-	10,33 ± 0,47	-	-	23 ± 1	21 ± 0	nw
B. cereus NRRL B-3711	-	-	11,67 ± 0,47	-	-	-	nw
P. aeruginosa ATCC 27853	-	-	12 ± 0	12 ± 0	60 ± 0	34 ± 0	nw
C. albicans ATCC 14028	-	-	-	-	nw		11 ± 1
C. krusei ATCC 6258	-	-	-	-	nw		18 ± 1
C. tropicalis Y-12968	-	-	-	-	nw		34 ± 2

Amp: ampicillin, C: chloramphenicol, Keto: ketoconazole, nw: not worked

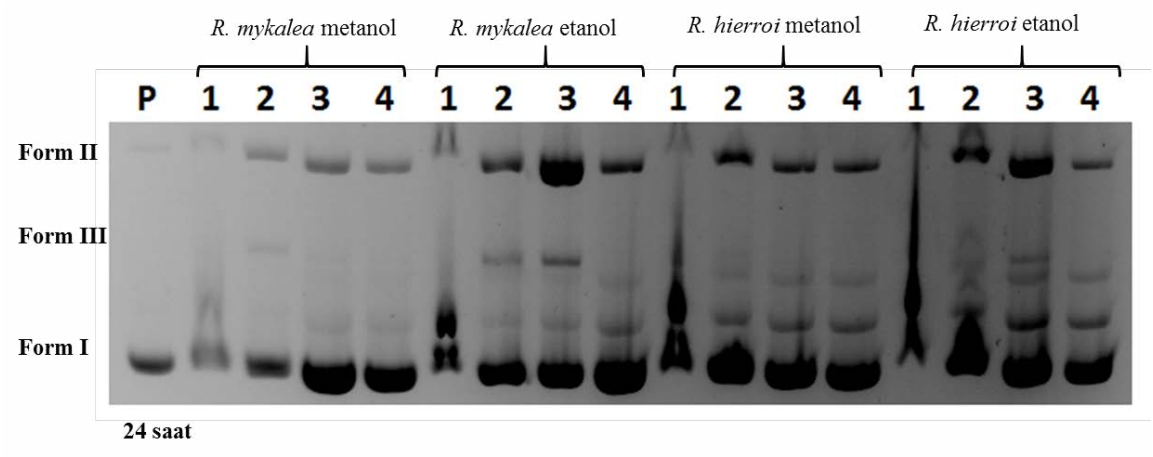

Figure 1

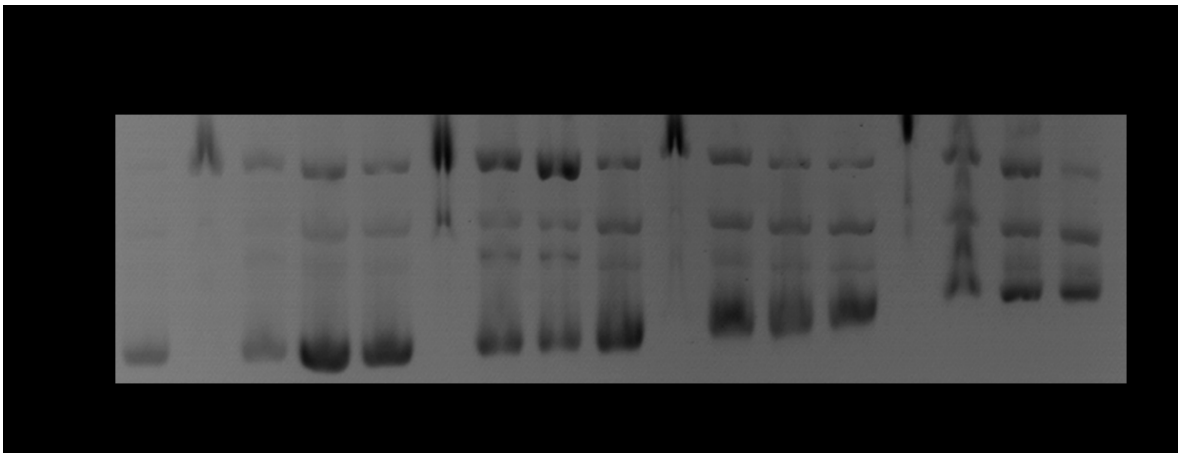

Figure 2

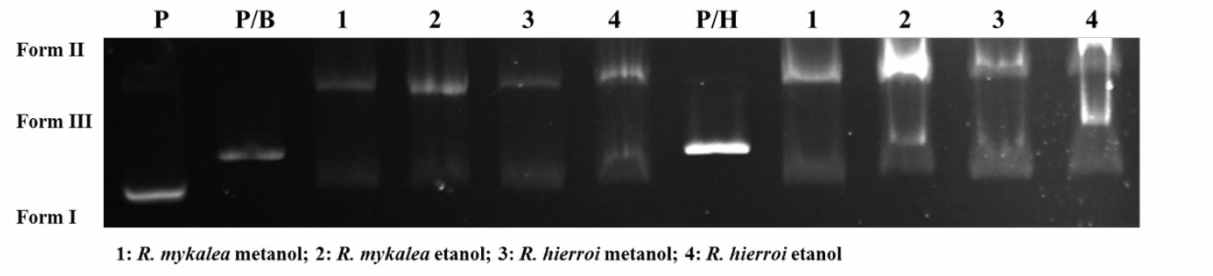

Figure 3

maniae ATCC 13883, 12.33 ± 0.47 mm; against to *B. cereus* NRRL B – 3711, 11.67 ± 0.47 mm and against to *P. aeruginosa* ATCC 27853, 12 ± 0 mm inhibition zone diameter.

R. mykalea ethanol extract formed against to *E. hirae* ATCC 9790, 11.67 ± 0.47 mm; against to *K. Pneuma niae* ATCC 13883, 11.33 ± 0.47 mm and against to *P. aeruginosa* ATCC 27853, 12 ± 0 mm inhibition zone diameter.

3.1 DNA Interaction

The effect of the extracts on DNA was measured at 24 and 48 hours (Figure 1 and 2). The extracts were observed to cause DNA breaks. In addition, the nucleotide linkage of the products was investigated by restriction enzyme digestion experiment (Fig. 3) and was found to bind both A / A and G / G nucleotides.

4. CONCLUSION

4.1 Antimicrobial Activity

It has been determined that the extracts obtained from *R. hierroi* and *R. mykalea* plants have antimicrobial activity on at least one or more microorganisms. None of the extracts showed antimicrobial activity against *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028 and *E. faecalis* ATCC 29212 bacterias. It was also determined that the extracts do not have any antifungal activity. Compared with the standard antibiotics, like ampicillin and chloramphenicol, extracts showing low level antimicrobial activity. The *R. mykalea* methanol extract was found to be more effective than the others. The highest inhibition zone diameter was determined against *R. hierroi* methanol extract *K. pneumoniae* ATCC 13883 strain (13,33 ± 0,47 mm).

4.2 DNA Interaction

The effect of extracts on DNA was measured for 24 and 48 hours. Furthermore, the nucleotide linkage of the substances was investigated by restriction enzyme digestion. The extracts were observed to cause DNA breaks and bound to both A/A and G/G nucleotides by restriction enzyme digestion experiments. It has been determined that the extracts had a concentration and time-dependent effect on the DNA and this effect and is in the form of DNA cutting activity. The strongest effect was observed at high concentration, while at other concentrations, form III DNA was observed which formed a double chain fracture outcome.

4.3 Conflict of Interest

The authors declare that there is no conflict of interest.

5. Acknowledgement

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