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The Effectiveness of Thyme and Ferula Mixture İn Multi-drug Acinetobacter Baumannii species

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Abstract: *In recent years, infections caused by Acinetobacter strains has been increasing in our country and around the world. Treatment of Acinetobacter infections are diffucult due to multi-drug resistant(MDR). Infections caused by resistant Acinetobacter strains lead to very high mortality. Therefore, alternative treatment methods are needed against MDR Acinetobacter strains and in recent years herbal treatment methods are promising. First, this study is intend to research the antibacterial effectiveness of a a mixture of Thymus longicaulis C. Presl. var. Subisophyllus(thyme) and Ferula elaeochytris(ferula) against MDR Acinetobacter baumannii(Ab). 20 MDRAb species were enrolled into the study. All of the strains are worked through both quantitative suspension test as well as the agar well diffusion method. In the quantitative suspension test method, the colony count is performed and it is observed that there is no reproduction in the bacteria species. In the agar well diffusion method, the antimicrobial activity of the test solution is evaluated by measuring inhibition zone diameter. The inhibition zone diameter are more than 15 mm in all of the bacteria species. Mixture of thyme and ferula is found to be effective against MDRAb and it could be used for infections caused by Acinetobacter strains.*

Keywords – *Acinetobacter baumannii, ferula, thyme*

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I. Introduction

Nosocomial infections caused by *Acinetobacter* species lead to significant morbidity and mortality, and lead to the increased hospitalization duration as well as increased treatment costs. The largest problem in the treatment of *Acinetobacter* species infection is the resistance problem [1]. The main risk factors leading to resistance development in '*Acinetobacter*'s are defined as the usage of antibiotics (carbapenems, third generation cephalosporins, fluoroquinolones, aminoglycosides, and metronidazoles), mechanical ventilation, long term stay in the intensive care unit, long term stay at hospital, severe disease, emergency surgery, and previous invasive procedures [2]. There are a very few number of antibiotics which can be used against this resistant bacteria, and the production of new antibiotics is also extremely limited [3]. In recent years, the emergence of antibiotic-resistant strains and the frequency of antibiotic side effects in synthetic drugs, which are rarely experienced in natural origin drugs, have driven scientists to examine the natural origin drugs [4].

Acinetobacter spp. is an aerobe, gram-negative coccobacillus and causes nosocomial infections. *Acinetobacter* species lead to surgical wound infections, skin and soft tissue infections, hospital-acquired pneumonia, and urinary infections. Mechanical ventilation, the usage of broad spectrum antibiotic, and catheterization can be considered risk factors [1].

Acinetobacter baumannii is extremely resistant to the environmental condition. The bacteria are a species of *Acinetobacter* exist that in nature, soil, and on animals as a saprophyte, and the virulance of the bacteria is low. Since *Acinetobacter spp* adapts to the external ambient conditions easily and has the ability to develop multiple resistance to antibiotics, it leads to infections. The frequency of infection is increasing gradually for hospitalized patients, especially in intensive care units. Control of multidrug-resistant *Acinetobacter baumannii* (MDRAb) is very difficult [5,6].

In a study published between 2002-2006 in our country and in which the resistance of *Acinetobacter baumannii* was researched, the sensitivity ratios obtained according to results of 15 articles are as follows: Amikacin 14-98%, ampicillin-sulbactam 15-59%, imipenem 38-72%, meropenem 40-77%, piperacillin-tazobactam 2-37%, cefepime 5-38%, ciprofloxacin 7-50% [7]. This indicates that the treatment of *Acinetobacter* infections will be more difficult as time goes on.

According to research performed in 91 countries by World Health Organization (WHO), the quantity of medical plants used for medicinal purpose is about 20,000. Production of about 500 are actually made and utilized. Furthermore, very few plants used for various purposes are registered into Codex. For example, the number of plants which are registered into the Turkish codex is about 140 [4].

The type of *Thymus* is one of the aromatic plants which belong to the *Lamiaceae* family. There are roughly 400 species in this type, many sub-species which are aromatic, evergreen, or semi-evergreen herbaceous plants. This type grows in temperate climates such as southern Europe and Mediterranean regions. The plant is used fresh and dry as a common kitchen plant. It is used in fats, food, drinks, and cenfectionery products, and is also used in various aroma agents. Moreover, it is used in odorization of soap, lotion, and perfumery. Due to its antiseptic and antimicrobial properties, it is used as well for medical purposes. *T. longicaulis C. Presl* is a kind of medical plant, which is flowerless, crawling, long, woody, as well as traditional, and its antiseptic effectiveness is reported in literature [8].

The type of *Ferula* is from the *Apiaceae* family. It includes roughly 170 species. It is known to grow in Northern Africa and Central Asia. Its anti-fungal, anti-microbial, and anti-inflammatory effectiveness is reported [9].

In literature, no publication has been encountered which researches the effectiveness of these two natural products against MDRAb species. First, this study is intended to research the antibacterial effectiveness of a mixture of *Thymus longicaulis C. Presl. var*. *Subisophyllus*(thyme) and *Ferula elaeochytris*(ferula) against MDRAb.

II. Material-Method

2.1.Definition of MDRAb:

MDRAb is defined as the resistance to three or more drug groups used in the treatment of *Acinetobacter* infections [10].

2.2.Bacteria Species

20 MDRAb species, which have been isolated from hospitalized patients staying at our hospital with infection (wound, urine, blood), were enrolled into the study. The antimicrobial sensitivity of all the strains is determined through the Disc Diffusion Method of Kirby Bauer, according to the suggestions of Clinical and Laboratory Standards Institute (CLSI). The antibiotic sensitivity profiles of *A. baumannii* strains are shown in Table 1.

Table-1: The antibiotic sensitivities of A. baumannii strains

A. baumannii strains	AK	CAZ	CİP	IMP	SXT	CN	TZP	CRO	SAM
1	R	R	R	R	R	R	R	R	R
2	R	R	R	R	R	R	R	R	R
3	R	R	R	R	R	R	R	R	R
4	R	R	R	R	R	R	R	R	R
5	R	R	R	R	R	R	R	R	R
6	R	R	R	R	R	R	R	R	R
7	R	R	R	R	R	R	R	R	R
8	R	R	R	R	R	R	R	R	R
9	R	R	R	R	R	R	R	R	R
10	R	R	R	R	R	R	R	R	R
11	R	R	R	R	R	R	R	R	R
12	R	R	R	R	R	R	R	R	R
13	R	R	R	R	R	R	R	R	R
14	R	R	R	R	R	R	R	R	R
15	R	R	R	R	R	R	R	R	R
16	R	R	R	R	R	R	R	R	R
17	R	R	R	R	R	R	R	R	R
18	R	R	R	R	R	R	R	R	R
19	R	R	R	R	R	R	R	R	R
20	R	R	R	R	R	R	R	R	R

R:resistant

The bacteria are kept in skim milk medium at -20°C until the day of study. On the day of study, the bacteria are inseminated into the agar with 5% sheep blood and are incubated for 24 hours at 37°C. Through the use of Tryptic Soy Broth (TSB), the bacteria suspension with Mc Farland 0.5 turbidity ($1.5*10^8$ cfu/mL) is prepared. All of the strains are worked through both quantitative suspension test as well as the agar well diffusion method. 3% Tween 80 (polyoxyethylene sorbitan monooleate, ICN Bromedicals Inc.) is used as a neutralization agent.

2.3.Mixture of Thyme-Ferula

10 grams of thyme (Thymus longicaulis C. Presl. var. subisophyllus) leaves, dried at room temperature were broken into pieces with 100 ml methanol by using Waring-branded Blender, and the mixture is kept (waiting) for two days. The liquid portion is filtered and separated. The extract the concentration of which is 16.3 g/L is then obtained.

The ferula extract is obtained using the same method and the concentration of which is 34.4 g/L is obtained with 29 grams of ferula (Ferula elaeochytris) body, and 200 ml methanol was used.

The mixture which contains 1.72% ferula and 0.81% thyme is prepared with the use of extracts prepared in order to use in the anti-bacterial studies.

2.4.Quantitative Suspension Test

The bactericidal activity is detected according to DIN EN 1040[2] standard. 1 ml bacteria suspension and 1 ml distilled water are mixed and incubated for 2 minutes. Next, 8 mL of test agent is added to this mixed solution. The solution formed is kept (waiting) for 1, 5, 10, 30, and 60 minutes. At the end of contact time, 1 ml of this solution, 8 mL neutralization agent, and 1 ml distilled water are incubated for 5 minutes. 1 ml solution from the last mixture is transferred to the agar with 5% sheep blood. After it is incubated for 24 hours at 37º C, the colonies are counted.

As negative control, 8 mL sterile water is used instead of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* mixture, and all of the procedures are carried out simultaneously through the primary test.

2.5.Agar Well Diffusion Method

The modified agar well diffusion method of Perez et al. is used in this study. Microorganism Mueller-Hinton inoculated into the agar. 8-ml wells are opened on the prepared agar and 50 uL from test material (*Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* mixture) is formed. The plaques are incubated for 18-24 hours at 37°C. After 18-24 hours, the inhibition zone diameter on plaques is measured in mm.

III. Results

In this study, the antibacterial activity of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* mixture against MDRAb is evaluated with the use of quantitative suspension test as well as with the agar well diffusion method.

In the quantitative suspension test method, the colony count is performed and it is observed that there is no reproduction in the bacteria species.

In the agar well diffusion method, the antimicrobial activity of the test solution is evaluated by measuring inhibition zone diameter and given in Table 2. The inhibition zone diameter are more than 15 mm in all of the bacteria species.

Table2: Inhibition zone diameter of Thymus longicaulis C. Presl. var. subisophyllus and Ferula elaeochytris mixture against MDRAb.

A. Baumannii strains	Zone diameter/mm of Thymus longicaulis C. Presl. var. subisophyllus and Ferula elaeochytris mixture
1	18
2	26
3	20
4	20
5	20
6	20
7	25
8	20
9	22
10	22
11	25
12	25
13	32
14	26
15	16
16	22
17	35
18	32
19	30
20	17

In conclusion, Mixture of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* is found to be effective against MDRAb.

IV. Discussion

In this study, it has been shown that the mixture of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* elicits good antibacterial activity against MDRAb strains. The inhibition zone diameter is found to be >15 mm on all *A. baumannii* strains in agar well diffusion method, while no reproduction occurs in all of the contact times from the quantitative suspension test. As far as we can tell, this is the first study in literature to research the effectiveness of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* mixture against multidrug-resistant *A. baumannii* strain.

Lysakowska et al. researched the antimicrobial effectiveness of thyme volatile fat (*thymus vulgaris*) against multidrug-resistant *Acinetobacter* spp strains and they conducted the study using agar diffusion method. In conclusion, they showed that *T. Vulgaris* fat has a strong effect against *Acinetobacter* strains [11].

The studies related to the subject are summarized in Table 3.

Table-3:The studies related to subject

Researcher	Source	Year	Bacteria	Agent	Result
Azaza et al	12	2003	*C albincans*	*Thymus zygioides var. lycaonicus and Thymus longicaulis subsp. chaubardii var. Chaubardii*	Successful
Azaza et al	12	2003	*Staphylococcus aureus*	*Thymus longicaulis subsp. Longicaulis var. subisophyllus*	Successful
Martino et al	8	2009	Gram(+) bacteria	*Thymus longicaulis* and *T. pulegioides*	More effective than Gentamicin and tetracycline
Sanda et al	13	2011	*Haemophilus influenzae* and *Streptococcus pneumoniae*	*Thymus longicaulis C. Presl*	Successful
Sanda et al	13	2011	*Staphylococcus aureus*	*Thymus longicaulis C. Presl*	Successful
Sienkiewicz et al	14	2011	*Staphylococcus, Enterococcus, Escherichia,* and *Pseudomonas*	*Thymus vulgaris*	Failed
Pavel et al	15	2010	*E coli, S typhimurium, S. enteritisdis, E cloacae, P aeruginosa, P mirabilis, S aureus, S. epidermidis, S faecalis, B subtilis, M luteus, M. flavus* and *L monocytogene* and *C albicans*	*Thymus pulegioides L. and T. glabrescens Willd*	Successful
Köse et al	16		*H influenza*	*Ferula Lycia*	Effective
Köse et al	16	2010	*S aureus,E faecalis,P vulgaris*	*Ferula Lycia*	Poor effective

The effectiveness of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* mixture in MDRAb species has been tested in this study for the first time. The current number of medications which can be used on *Acinetobacter baumannii* species is relatively limited. According to the results of this study, the mixture of *Thymus longicaulis C. Presl. var. subisophyllus* and *Ferula elaeochytris* is found to be extremely effective against *Acinetobacter* species. We thus consider that this mixture could be a good alternative wound antiseptic for skin and soft tissue infections caused by *Acinetobacter*. However, new and well-designed in vitro and in vivo studies are needed.

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