

## The Study of Anti-Bacterial Effect of Eucalyptus, Thymus Vulgaris and Zataria Multi Flora Essence on E. coli and S. aureus

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### ABSTRACT

Eucalyptus is a tall tree indigenous to Australia and most of its species are cultivated in north and south of Iran. Its leaves have medical effects, for instance its leaves are anti motility, anti febrile, antisepticising, anti rheum, moreover in treating bronchitis even bronchitis chronic and asthma its leaves are effective. Thyme is an herb which has various types, this is a plant indigenous to the west of Mediterranean region and the south of Italy, in traditional medicine it's infused in the water to treat cold, coughing, bronchitis, and womb pains. Moreover, in the modern medicine, its anti microbial and anti parasitic and anti fungal effects are considered. Five microbial strains and one standard strain of *S. aureus* along with four microbial strains and one standard strain of *E. coli* were provided, then different dilutions of essence and microbial suspension in 5 cc equal to .5 Mc Farland standard were also provided, then .2 cc of each of them were combined together and after 24 hours incubation, MIC was measured. Then by placing it on the plate, after 24 hours of incubation, MBC was measured as well. The aim of this research is to define the antibacterial effect of essence of eucalyptus, thymus vulgaris and zataria multi flora on *E. coli* and *S. aureus* in laboratory with methods MIC and MBC. The gained result shows that there are useful anti microbial effects in eucalyptus and thyme; however, in comparison to eucalyptus, thyme has a stronger anti microbial effect.

**KEY WORDS:** eucalyptus, thymus vulgaris, zataria multi flora, anti bacterial, *S. aureus*, *E. coli*.

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### INTRODUCTION

Nowadays, chemical antibiotics are the most consumptive medicines in the society, these medicines are usually consumed based on individuals' self remedy. Self remedy leads to the increase of side effects of chemical medicines in body and emergence of resistant types of previously restrained bacteria. The old and rich Iranian experience in the field of herbal remedy of bacterial and infectious diseases, and having access to beneficial plants in Golestan located in the north of Iran, and the existence of plenty of infectious diseases in Iran motivated us to prove scientifically and experimentally all related issues existing in the Iranian traditional medicine. Therefore, the essence of plants such as eucalyptus camunldulensis, thymus vulgaris, zotaria multi flora and two types of bacteria causing infectious diseases in Iran ; *S. aureus* and *E. coli* were chosen for the laboratorial tests.

#### Main body

*Staphylococcus aureus* (*S. aureus*)

It is widespread in the nature. It exists in the infection of sick people, on the skin of healthy people, in the weather and dust, in the cow milk, on dishes and other stuffs.

#### MORPHOLOGY

In fact, they are spherical bacteria with diameter .8 up to 1 micro meter, they just look like cluster of grapes sitting beside one another. These clusters are bigger in the solid media and smaller in the liquid media; they usually make short and dual bonds. In infections, they are placed beside one another one by one, or two by two, or four by four.

Cell structure: the cell includes nucleotides, mesosomes, a membrane, and a three layer cytoplasm which is separated from cell wall by periplasmic space. The thickness of cell wall in young structures is from 18 to 25 nanometers. In some samples this capsule is made of hydrate of carbon. Cell wall of *S. aureus* is made of three parts, peptidoglycan, Teichoic Acid, and protein A.

Peptidoglycan: the structure of peptidoglycan includes polysaccharide structure and tetrapeptide chains. Polysaccharide structure is made up of N- acetyl glucosamine and N- acetylmuramic acid which are connected together alternatively via connection of beta 1 and 4 and make parallel chains.

Teichoic acid: in *S. aureus* there is a teichoic acid cell wall background which includes ribitol phosphate. Teichoic acid is a necessary component of phage receptor of *S. aureus*, moreover, it plays an important role in making the cell physiological functions.

Protein A ( agglutinin): protein A makes a large part of protein available in *S. aureus* cell wall, and antigens are a group specified mainly for most of the samples of *S. aureus*. 90 percent of the available protein A in the wall cell structure is on the surface which is connected to peptidoglycan by covalent bonds. Approximately, one third of protein A which is produced by bacterium is released at the time of growing.

Medium: these bacteria are parallel and do not need aerobic condition, however, under aerobic condition they grow better. In all normal media (4/5 to 6/5 degrees) they grow about PH: 4/2-9/3, but their growth in 30 up to 37 degrees is increased (PH= 7-7/5). On the simple gelose, after 24 hours round and flat and convex colons with 1-4 millimeter diameter

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appears these colons have golden pigment which is not formed in the absence of air or in liquid medium, however, it is formed successfully in a 22 degree medium and when it is exposed to air. *S.aureus* makes a complete or an incomplete beta hemolysis on Blood gelose and grows easily in media including telluride, sodium chloride(7/5 percent), and lithium chloride, while other bacteria cannot grow. This feature is used to separate *S. aureus* from other bacteria. *S. aureus* is a positive catalase and achieves its energy from oxidation and fermentation, moreover, with hydrolyzing  $H_2O_2$ , it stops aggregation of this toxic substance. This bacterium is able to do mannitol fermentation, it has DNase enzyme as well.

### **Esherichiacoli**

Position: it lives in digestive tracks of animals and humans. They exist in food, waters and they infect organisms via feces. Sometimes they are too short and some other times they are like long chains. Their size varies from 1 to 6 micrometers. They are gram-negative bacteria non spore. Some of them possess capsule or micro capsule or muculent layers. They are usually motile with quivering fibers, however, some of them are not motile. They usually have cells which have the power of creating hem agglutination, with two types; one sensitive to mannose (type 1) and the other resistant against mannose (type 2). In majority of esherichiacoli samples, there is fimbriae type one, this fimbriae has a role in bacterial virulence and it interferes with bacterial connection to mucous surfaces and urinary track epithelium. These bacteria are aerobic and non- aerobic, then it grows easily in normal media whose degree is between 15 to 45.their 24 hours colons in 37 degree media on simple gelose are as big as 1 to 3 millimeters.

### **Biochemical efficiencies**

It undergoes fermentation on Glucose and other types of sugar and it produces a kind of gas which is made up of  $CO_2$  and  $H_2$ , because of the lactose fermentation, their colon gets red, however the gelatin is not melted and  $SH_2$  is not produced. Urease is negative, lysine decarboxylase (LDC)is positive, Andel MR is positive and Citrate and VP is negative.

### **Pathogenesis in humans**

E-coli: it is the normal bacteria flora in intestine, it has made a major part of intestine aerobic bacteria, the majority of coli bacillus are not pathogenic if they exist in intestine, however, if they enter into other organs such as urinary track, appendix, around wounds and so forth, they would be able to enter blood circle and create toxins and put in danger the individual' health. This situation may be caused during papillary, agedness, being affected by diseases such as security failure, and taking drugs such as immunosuppressive. Some of the coli bacillus in rivers could act like pathogens. More than 85 percent of urinary tract infections are made by this organism and in the case of pregnant women, it is the first cause of urinary tract infection. Urinary tract infections are observed as cystitis, pyelitis, pyelonephritis, epididymitis, and so forth.

### **Laboratorial diagnosis**

To separate coli bacillus from blood, feces, infection, urine, wound and so forth, sampling is done. We should notice that just separating Esherichiacoli does not specify the diagnosis. In fact, there should be no other known pathogens. The below methods are used to achieve diagnosis.

Direct test: after providing, developing and adding color to the sample, the sample is placed under microscope to study characteristics of coli bacillus.

Medium: for this section, different media such as blood gelose and differential media such as mechanical Endo, Eosin, and methylene blue are used. In differential medium, coli bacillus colon gets red as a result of lactose fermentation. However, shigella, salmonella and some others bring about colorless colons. In the Eosin, methylene blue media, coli bacillus colon has metal gloss.

The separated bacteria should be recognized based on serological and biochemical features; moreover, to recognize diarrhea bacterium, standard serums must be used.

Quick diagnosis via immunofluorescence : to do so, the sample is put on laboratorial slide by using swab and then after fixing it via alcohol, the specific immune serum would be placed beside it . This method is very sensitive and works in less than an hour, and then it is used for quick diagnosis.

Epidemiology: seven hours after birth, they make aerobic bacteria of intestine. If they stay in intestine, they will make no diseases, but as soon as they enter other body organs, they make infection. In the nurseries, the infection transmission is done through individuals' contaminated hands. The infection may be transmitted via polluted weather of hospital or via dust. The hospital towels or even personal stuff may also cause infection.

Thyme has different names such as Avisham, Avsham, Adsham, Abshan, Afsham, Anuk, Avaj sisabz, Hasha ,Arz beh, and Sagrolhamir, it also has different types, one of the types of thyme is zataria multiflora. This is a thin shrub whose height is between 10 to 30 cm. in cold weather, it would be a one year old plant and the length of its leaves arrives at 4 to 10 millimeters. This type of thyme is indigenous to Mediterranean regions and its wild types are seen in Iran.

## **METHODOLOGY**

The present study is an experimental study which is done at microbiology laboratory of medical faculty of Gorgan medical university located in Iran. The concentration of eucalyptus and the concentration of thymus vulgaris and the concentration of zataria multi flora are quantitative variables.

Ecoli strain and *S. aureus* strain are qualitative variables

Turbidity is a qualitative variable

The stages of doing the test

1. Purifying bacteria strains:

The first step is to purify bacteria strains. Therefore, among the available colons in the plate, the large colons were chosen and were put in Muller Hinton Agar medium. The names of these strains were, S<sub>st</sub>, S<sub>2</sub>, S<sub>4</sub>, S<sub>5</sub>, S<sub>9</sub>, S<sub>14</sub> and for E-coli these names were E<sub>st</sub> (standard) and E<sub>1</sub>, E<sub>5</sub>, E<sub>21</sub>, E<sub>27</sub>.

we put them in incubator to grow, 24 hours later, absolutely pure strains with similar colons were achieved. Then, through specific biochemical tests specially catalase and clumping factor, growth in MSN medium (Agar salt monititol) of S. aureus was recognized, and then through urease test (IMVIC) and Oxide the growth of E. coli was recognized.

**Preparing microbial suspension**

In this stage, to prepare microbial suspension, in 5 cc of Nutrient Broth medium, we inseeded 4 to 5 colon of each strain, then, we put the achieved suspension in incubator for 2 to 4 hours till the media got turbid and then we accorded them with .5 Mac Farland standard concentration.

Preparing different dilutions of the essence

The preparation of eucalyptus dilutions for S. aureus is reported as following:

1. Dilution 1:  $\lambda$  1500 hexane essence
2. Dilution  $\frac{1}{2}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 essence
3. Dilution  $\frac{1}{4}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from tube no. 2
4. Dilution  $\frac{1}{8}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from tube no. 3
5. Dilution  $\frac{1}{16}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from tube no. 4
6. Dilution  $\frac{1}{32}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from tube no. 5
7. Dilution  $\frac{1}{64}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from tube no. 6

The preparation of thymus vulgaris dilutions for S. aureus:

To prepare dilution  $\frac{1}{16}$  of thyme we do 8 stages regarding the formulas shown in following:

- Dilution  $\frac{1}{4}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 essence  
 Dilution  $\frac{1}{8}$ :  $\lambda$  1500 hexane +  $\lambda$  1500 from dilution  $\frac{1}{4}$

Preparation of dilutions of zataria multiflora for S. aureus is reported in 5 stages regarding the formulas shown in following:

- Dilution  $\frac{1}{5}$ :  $\lambda$  200 zataria multiflora +  $\lambda$  800 hexane  
 Dilution  $\frac{1}{50}$ : 4/5 cc hexane + .5 cc of dilution  $\frac{1}{5}$   
 1. Dilution  $\frac{1}{500}$ : 4/5 cc hexane + .5 cc of dilution  $\frac{1}{50}$   
 2. Dilution  $\frac{1}{1000}$ : 2/5 cc hexane + 2/5 cc of dilution  $\frac{1}{500}$   
 3. Dilution  $\frac{1}{2000}$ : 2/5 cc hexane + 2/5 cc of dilution  $\frac{1}{1000}$   
 4. Dilution  $\frac{1}{4000}$ : 2/5 cc hexane + 2/5 cc of dilution  $\frac{1}{2000}$   
 5. Dilution  $\frac{1}{8000}$ : 2/5 cc hexane + 2/5 cc of dilution  $\frac{1}{4000}$

The using dilutions in E- coli strains were based on the below stages:

The below dilutions were prepared for thymus vulgaris and zataria multiflora which After 9 stages regarding the formula shown in following, the preparation of dilution would get complete.

Dilution  $\frac{1}{3}$ :  $\lambda$  200 essence +  $\lambda$  400 hexane

Also, Preparing needed dilutions of eucalyptus for E. coli regarding the formulas shown in following would get complete reporting that 8 stages are necessary in this relation.

- Dilution  $\frac{1}{2}$ :  $\lambda$  1000 hexane +  $\lambda$  1000 essence  
 Dilution  $\frac{1}{4}$ :  $\lambda$  1000 hexane +  $\lambda$  1000 from tube with dilution  $\frac{1}{2}$

E.coli

The result of created dilutions of Ecoli for eucalyptus is:

8 dilutions  $\times$  5 strains = 40 laboratorial tubes

The result of created dilutions of Ecoli for thymus vulgaris is:

9 dilutions  $\times$  5 strains = 45 laboratorial tubes

For each strain, one control was also provided, (control:  $\lambda$  200 microbial suspension +  $\lambda$  200 hexane) = 135 laboratorial tubes.

And then, we placed the provided dilution in each tube of  $\lambda$  200 microbial suspension +  $\lambda$  200 hexane. The total number of used tubes was 261. After adding suspensions to dilutions, the tubes were put in the incubator.

The combination of suspensions and dilutions media

After 24 hours, tubes were studied from perspective of turbidity; we also put them on plates having Muller Hinton agar medium which was previously prepared.

### Discussion and results

We achieved valuable findings based on results of the last dilution of essence which has had anti bacterial effect. After studying the issue of growth on plates, the results illustrated in tables below were gained. Tables no.1 and no.2 and table no. 3 show the results of growth of dilutions of *S. aureus* and their MBC for eucalyptus, thymus vulgaris, and zataria multi flora essence, moreover, tables 4 to 6 show the results of growth of dilutions of *E coli* and MBC for eucalyptus, thymus vulgaris and zataria multi flora essence. The secondary aims of this research are mentioned here:

1. Specifying minimum bacterial concentration (MBC) and minimum inhibitory concentration (MIC) of eucalyptus essence on *E. coli* and *S. aureus*.
2. Specifying minimum bacterial concentration (MBC) and minimum inhibitory concentration (MIC) of thymus vulgaris essence on *E. coli* and *S. aureus*.
3. Specifying minimum bacterial concentration (MBC) and minimum inhibitory concentration (MIC) of zataria multi flora essence on *E. coli* and *S. aureus*.

Table no. 1: The result of growth of dilutions of *S. aureus* and their MBC in the presence of different concentrations of eucalyptus essence.

15/62mg/ ml $\frac{1}{64}$ dilution	31/25 mg/ ml $\frac{1}{32}$ dilution	62/5 mg/ ml $\frac{1}{16}$ dilution	125 mg/ ml $\frac{1}{8}$ dilution	250 mg/ ml $\frac{1}{4}$ dilution	500 mg/ ml $\frac{1}{2}$ dilution	pure	Dilutions
+	+	+	+	MBC -	-	-	S <sub>1</sub>
+	+	+	MBC -	-	-	-	S <sub>2</sub>
+	+	+	MBC -	-	-	-	S <sub>4</sub>
+	+	+	+	MBC	-	-	S <sub>5</sub>
+	+	+	MBC -	-	-	-	S <sub>9</sub>
+	+	+	+	MBC -	-	-	S <sub>14</sub>

Table no. 2: The result of growth of dilutions of *S. aureus* and their MBC in the presence of different concentrations of thymus vulgaris essence.

.48mg/ ml $\frac{1}{2048}$ dilution	.97mg/ ml $\frac{1}{1024}$ dilution	1/95mg/ ml $\frac{1}{512}$ dilution	3/90mg/ ml $\frac{1}{256}$ dilution	7/81mg/ ml $\frac{1}{128}$ dilution	15/62mg/ ml $\frac{1}{64}$ dilution	31/25mg/ ml $\frac{1}{32}$ dilution	62/5mg/ ml $\frac{1}{16}$ dilution	dilutions
+	MBC -	-	-	-	-	-	-	S <sub>1</sub>
+	MBC -	-	-	-	-	-	-	S <sub>2</sub>
+	+	MBC -	-	-	-	-	-	S <sub>4</sub>
+	-	-	-	-	-	-	-	S <sub>5</sub>
+	+	+	+	MBC -	-	-	-	S <sub>9</sub>
+	MBC -	-	-	-	-	-	-	S <sub>14</sub>

Table no. 3.: The result of growth of dilutions of S. aureus and their MBC in the presence of different concentrations of essence of zataria multi flora.

125mg/ ml $\frac{1}{8000}$ dilution	250mg/ ml $\frac{1}{4000}$ dilution	500mg/ ml $\frac{1}{2000}$ dilution	1mg/ ml $\frac{1}{1000}$ dilution	2mg/ ml $\frac{1}{500}$ dilution	Dilutions
+	+	MBC -	-	-	S <sub>st</sub>
+	+	MBC -	-	-	S <sub>2</sub>
+	+	+	MBC -	-	S <sub>4</sub>
+	+	MBC -	-	-	S <sub>5</sub>
+	+	+	MBC -	-	S <sub>9</sub>
+	+	+	MBC -	-	S <sub>14</sub>

Table no. 4: The result of growth of dilutions of Ecoli and their MBC in the presence of different concentrations of essence of eucalyptus.

.97mg/ ml $\frac{1}{1024}$ dilution	1/95mg/ ml $\frac{1}{512}$ dilution	3/90mg/ ml $\frac{1}{256}$ dilution	7/81mg/ ml $\frac{1}{128}$ dilution	15/62mg/ ml $\frac{1}{64}$ dilution	31/25mg/ ml $\frac{1}{32}$ dilution	62/5mg/ ml $\frac{1}{16}$ dilution	125mg/ ml $\frac{1}{8}$ dilution	Dilutions
	+	+	+	+	MBC -	-	-	E <sub>ST</sub>
	+	MBC -	-	-	-	-	-	E <sub>1</sub>
	+	+	+	+	MBC -	-	-	E <sub>5</sub>
	+	MBC -			-	-	-	E <sub>21</sub>
			+		MBC -	-		

Table no. 5: The result of growth of dilutions of Ecoli and their MBC in the presence of different concentrations of essence of zataria multi flora.

.26mg/ ml $\frac{1}{3840}$ dilution	.52mg/ ml $\frac{1}{1920}$ dilution	1/04mg/ ml $\frac{1}{960}$ dilution	2/08mg/ ml $\frac{1}{480}$ dilution	4/16mg/ ml $\frac{1}{240}$ dilution	8/33mg/ ml $\frac{1}{120}$ dilution	66/66mg/ ml $\frac{1}{60}$ dilution	33/33mg/ ml $\frac{1}{30}$ dilution	66/66mg/ ml $\frac{1}{15}$ dilution	Dilutions
	+	+	+	+	MBC -	-	-	-	E <sub>ST</sub>
	+	+	+	MBC -	-	-	-	-	E <sub>1</sub>
	+	+	+	+	MBC -	-	-	-	E <sub>5</sub>
	+	+	MBC -	-	-	-	-	-	E <sub>21</sub>
			+		MBC -	-			

Table no. 6: The result of growth of dilutions of Ecoli and their MBC in the presence of different concentrations of essence of thymus vulgaris.

$\frac{.26\text{mg/ ml}}{1}$ $\frac{1}{3840}$ dilution	$\frac{.52\text{mg/ ml}}{1}$ $\frac{1}{1920}$ dilution	$\frac{1/04\text{mg/ ml}}{1}$ $\frac{1}{960}$ dilution	$\frac{2/08\text{mg/ ml}}{1}$ $\frac{1}{480}$ dilution	$\frac{4/16\text{mg/ ml}}{1}$ $\frac{1}{240}$ dilution	$\frac{8/33\text{mg/ ml}}{1}$ $\frac{1}{120}$ dilution	$\frac{16/66\text{mg/ ml}}{1}$ $\frac{1}{60}$ dilution	$\frac{33/33\text{mg/ ml}}{1}$ $\frac{1}{30}$ dilution	$\frac{66/66\text{mg/ ml}}{1}$ $\frac{1}{15}$ dilution	Dilutions
	+	+	+	+	+	MBC -	-	-	E <sub>ST</sub>
	+	+	+	+	MBC -	-	-	-	E <sub>1</sub>
	+	+	+	+	MBC -	-	-	-	E <sub>5</sub>
	+	+	+	+	MBC -	-	-	-	E <sub>21</sub>
			+		MBC -	-		-	

Tohid poor et al in a study observed the anti bacterial effect of eucalyptus, thymus vulgaris on Staphylococcus aureus which in the end they come into the conclusion that both these herbs have antibacterial effect, whereas Thymus vulgaris had more effect. In another study done by Khan Katrina et al in 2012, antibacterial effect of Thymus vulgaris along and in comparison with other herbs was found. Thymus vulgaris had antibacterial effect against Staphylococcus aureus which this effect increases in combining Thymus vulgaris with Cinnamomum Zeloncum. Also, Bashir Rahu et al (2008) Eucalyptus camaldulensis and Eucalyptus globules put antibacterial effect on Staphylococcus aureus and E.coli. Salari et al (2006) in their study found antibacterial effect of Eucalyptus globules on counterfoils as Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenza which MIC 50 with the counterfoils as 32-32-64-128 milligram/ liter and MBC as 64-64-128-512 milligram/deciliter were found.

## Conclusion

In this study, it was determined that the weakest MBC was related to the essence of eucalyptus and the strongest MBC was related to zataria multi flora. In the case of eucalyptus essence, S. aureus dilution is more sensitive than Ecoli to higher concentrations. It is vice versa about thymus vulgaris, the unit of concentration of thymus vulgaris essence changes into microgram to milliliter in relation to S. aureus. However, in the case of zataria multi flora essence, comparing with Ecoli, S. aureus dilution is sensitive to lower concentrations. Therefore, zataria multi flora essence with lower concentration shows the highest MBC. Here, in doing this research, various sources were used to do a perfect study.

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