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EVALUATION OF ANTIBACTERIAL ACTIVITY AND SYNERGISTIC EFFECT BETWEEN TEA TREE OIL (*Melaleuca alternifolia*), BURDOCK (*Aractium lappal*) AND LICORICE (*Glycyrrhiza glabra*) ROOT EXTRACT AGAINST *Propioni bacterium acnes, Staphylococcus aureus* AND *Staphylococcus epidermidis*

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ABSTRACT

The use of antibiotics for the treatment of infection promotes the development of antibiotic-resistant bacteria, and it leads to serious problems in the field of medical science in the whole world. The aim of this study is to evaluate the antibacterial activity and synergistic effect between burdock" root extract (Aractium lappal), "tea tree" oil (Melaleuca alternifolia) and "licorice root" extract (Glycrrhiza glabra), which are useful as alternative drugs to control acne lesions and pimples caused by bacteria. The antibacterial activity is studied against *Propioni bacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis* by using

disc diffusion assay. The dilution methods are used to determine the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentration (MBC), and gas chromatography is also used to identify the compounds of these alcoholic extracts. The results showed that a 4% concentration of "tea tree" oil has a strong antibacterial activity more than "burdock" and "licorice" root extract. Furthermore, a 1.5 % concentration of different combination of three extracts (blend)is more effective against infections. Therefore, combination of their extracts can provide synergistic inhibitory effect making them more effective as antibacterial agent without having side effect and using chemicals.

Keywords: *Propioni bacterium acnes*; *staphylococcus aureus*; *staphylococcus epidermidis*; anti-bacterial property; herbal extracts

Abbreviations: minimum inhibitory concentrations (MIC), the minimum bactericidal concentration (MBC)

INTRODUCTION

Antibiotic resistance occurs over time against different types of bacteria like *Propioni bacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis* due to the rapid emergence of antibiotic resistant strains [1, 2]. It has become so difficult for doctors to deal with because the amount of antibiotic has reduced for treating infections. Therefore, ongoing efforts are made to find new antimicrobial drugs [3]. One way to solve this problem is to use herbal extracts. Some plant extracts have anti-bacterial properties because of having unique compounds [4]. Plants contain various secondary compounds that are important in biological activities, and are used in pharmaceutical and cosmetics industry [5]. In recent years, many researchers have studied the effects of anti-bacterial properties in different extracts. So far microbial resistance has not been reported against using medicinal plants to treat skin lesions [6, 7, 8, 9, 10, 11]. So, the use of herbs has been studied instead of using chemical drugs and antibiotics. Since Iran is rich in medicinal plants, and the identification of herbal plants has been taken into consideration by domestic and foreign researchers. Consequently, "tea tree" oil (*Melaleuca alternifolia*), "burdock" (*Aractium lappal*) and "licorice" (*Glycrrhiza glabra*) have been studied in terms of unique properties to treat bacterial infections. These plants were used in traditional medicine for treating inflammatory and non-inflammatory acne lesions. Various studies have been conducted for the sake of anti-bacterial activities of these plants [12, 13, 14, 15, 16, 17]. In this study, the overlapping activities

of three herbal ethanolic extracts ("tea tree" oil, "licorice" and "burdock") have been investigated against *Propioni bacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis* by disc diffusion method.

MATERIALS AND METHODS

Plant material and extract preparation

The leaves and terminal branches of *Melaleuca alternifolia ("*tea tree*", Mirtaceae)* and the roots of *Arctium lappal L. ("*burdock*", Asteraceae)* and *Glycyrrhiza glabra L. ("*licorice*", Fabaceae)* have been bought from a grocery in Tehran at the end of harvesting season, and identified by a botanist. Next, these were converted into powder by mechanical milling, and prepared for extraction. Then, 100 g of powder from the roots of *Arctiuml appal L.* and *Glycyrrhiza glabra L* were extracted with ethanol 70 % (400 ml) in Soxhlet apparatus for 1.5 hours. Next, extracts were condensed with a rotary machine for 1 hour at 60°C.Besides,"tea tree" oil extract obtained from100 g of power from leaves and terminal branches of *Melaleuca alternifolia* with 500 ml ethanol 70 %. Next, capped sample was put at microwave for 30 seconds, and kept it for 2 days in order to extract oil completely.

Also, 100 g of each plant was weighed and then blended and extracted by above mentioned method for the preparation of blend extract. Finally, herbal extracts were dissolved in 10% dimethyl sulfoxide (DMSO), and obtained final concentration of 100 mg/ml, and stored at 4°C.

Gas chromatography was used to identify the types and percentage of compounds in these extracts. Besides, the *Propioni bacterium acnes* (ATCC 11827) was prepared from Razi Vaccine and Serum Research Institute, and cultivated in Brain Heart Infusion Agar for 7 days at 37 °C. Also, *Staphylococcus epidermidis* (ATCC 49461) and *Staphylococcus aureus* (ATCC 29213) were cultivated into Blood Agar for 24 hours at 37 °C.

Anti-bacterial activity assay

The evaluation of anti-bacterial activity of these extracts was conducted by disc diffusion. Then, all strains were prepared on half of McFarland$1/5 \times 10^8$ $\frac{cfu}{ml}$. Next, 100 µL of bacterial suspension from each strain was cultivated in Mueller Hinton agar. Sterile filter disks (Padtan Teb Co, Tehran, Iran) were impregnated with different concentrations of ethanolic extracts. Each disc was placed at a certain distance on the agar surface. Plates were incubated for 24 hours at 37 °C. The diameter of the zones of inhibition was measured, and results were analyzed. Inhibition zone with diameter lower of 10 mm; resistant, 11 to 15 mm; intermediate and 16 mm or more; sensitive have been

considered. Also, disc which contains H_2O was used as negative control, and disc which contains 30µg vancomycin was used as positive control. Based on CLSI protocol, the diameter of the zones of inhibition for vancomycin for *Staphylococcus aureus* is 17 to 21mm; sensitive, 15 to 16mm; intermediate and lower of 14 mm; resistant. Also, the diameter of the zones of inhibition for vancomycin for *Propioni bacterium* acnes and *Staphylococcus epidermidis* are more than 17mm; sensitive, 15 to 16mm; intermediate and lower than 14 mm, resistant.

This test was repeated for 3 times, the average diameter was recorded as final diameter.

Determination of MIC and MBC

The minimum herbal extract concentration was checked out for inhibiting the bacterial growth for the determination of MIC. A dilution of concentration 1:1000 of these bacteria was prepared which contains 10^6-10^5 bacteria per ml.10 tubes of dilution were prepared for each extract. 1 mm of Brain Heart Infusion Agar was added into the tube from 2 to 10. The tube 1 was void of medium, and used as negative control. 1 ml of extract solution (200mcg/ml) was added intothe tube 1 and 2. Then, 1 ml of the solution 2 was added into tube 3. This work was continued until the tube 9. Tube 10 was void of extract which are used as positive control. Then, 1 ml of concentration 1:1000 of each bacteria solution was added. Tube contains *Staphylococcus epidermis* was incubated for 12 hours at 37 °C, and *Propioni bacterium acnes* was kept for 7 days at anaerobic environment. All plates were placed for 24 hours at appropriate temperature in order to grow each bacterium. The lowest concentration of bacteria was considered asminimum bactericidal concentration (MBC).

Synergy studies

In this study, synergy according to FIC indices was observed for blend extract, and calculated using the following equation:

$\Sigma \text{ FIC} = \frac{1}{3}(FIC1 + FIC2 + FIC3)$

FIC1 (FIC of tea tree oil)= $\frac{MIC\ (blend)}{MIC\ (tea\ tree\ oil)}$

FIC2 (FIC of burdock root extract)= $\frac{MIC\ (blend)}{MIC\ (burdock\ root\ extract)}$

FIC3 (FIC of licorice root extract)= $\frac{MIC\ (blend)}{MIC\ (licorice\ root\ extract)}$

Value less than 0.5 as synergistic, value between 0.5-1 as additive, value between 1-4 as indifferent and value greater than 4 as antagonistic.

RESULTS

The result of gas chromotography have been shown that "tea tree" oil contains a variety of compounds that terpineol-4-olis the most component of "tea tree" oil. It was

about 49.3 % [2]. Also, caryophyllene oxide as main component of "burdock" and glycyrrhizic acid and glabridin are the main components of " licorice" root extract.These compounds were about 52.5 % at "burdock" and 22.3% and 2.2 % in "licorice" root extract, respectively [3, 4].

The diameter of zone of inhibitions, minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) have been assayed(table 1).According to obtained results, a 4% concentration of "tea tree" oil can significantly inhibit the growth of Bactria more than "burdock" and "licorice" root extract. The zones of inhibition obtained with blend extract were larger than other plants. Synergistic effect of a combination of tea tree oil, licorice and burdock were obtaind 0.072, 0.05 and 0.041 for *Propionibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis* respectively.

Table 1: The diameter of zone of inhibitions, MIC and MBC

Plants	Bacterial species	Inhibition zone (mm)	MIC (%)	MBC (%)	Dose (%)
"Licorice"root extract	*Propionibacterium acnes*	14.5±0.5	1.3	2.4	7
	staphylococcus epidermidis	16±0.5	3.5	7.1	
	staphylococcus aureus	14±0.25	3.56	6.8	
"Burdock" root extract	*Propionibacterium acnes*	15±0.5	1.39	2.6	7
	staphylococcus epidermidis	14.5	3.75	7.2	
	staphylococcus aureus	14±0.5	3.32	5.76	
"Tea tree" oil	*Propionibacterium acnes*	19	0.35	0.6	4
	staphylococcus epidermidis	18±0.5	0.86	1.9	
	staphylococcus aureus	16	0.89	1.6	
Three herbal extracts (blend extract)	*Propionibacterium acnes*	22	0.05	0.11	1.5
	staphylococcus epidermidis	19.8	0.073	0.15	
	staphylococcus aureus	18.5	0.09	0.17	

Table 2: Compounds of "tea tree" oil analyzed by GC

Identified compound	Area (%)
a-terpinene	7.1
p-cymene	4.8
Eucalyptol	5.6
Limonene	2.3
γ –terpinene	16.5
Terpinolene	2.7
terpineol-4-ol	49.3
a- terpineol	4.1

Table 3: Compounds of "burdock" root extract analyzed by GC

Identified compound	Area (%)
α-Pinene	0.06
Aromadendrene	17.3
Carvomenthone	0.05
Trans-β-farnesense	0.12
ϒ-cadinene	2.84
Caryophyllene oxide	52.5
β-copaen-4α-ol	1.8
Isoaromadendrene	6.6
β-costol	2
Eicosane	0.5
Tetracosane	0.11
Pentacosane	0.23
Hexacosane	1.44
Heptacosane	0.2

Table 4: Compounds of"licorice" root extract analyzed by GC

Identified compound	Area (%)
Pyrogallol	3.3
Gallic acid	3.2
Caffeic acid	4.7
Vanillic acid	2.13
Caffeine	2.1
Rutin	4.1
Quercitrin	2.7
Coumaric acid	2.3
Naringin	2.16
Benzoic acid	4.99
Ellagic acid	5.12
Liquiritin	7.9
Naringenin	3.9
Myricetin	4.98
Quercetin	5.3
Glycyrrhizic acid	22.3
glabridin	2.2
Liquiritigenin	4.1
Cinnamic acid	4.66
Apigenin	3.9
Kaempferol	7.9

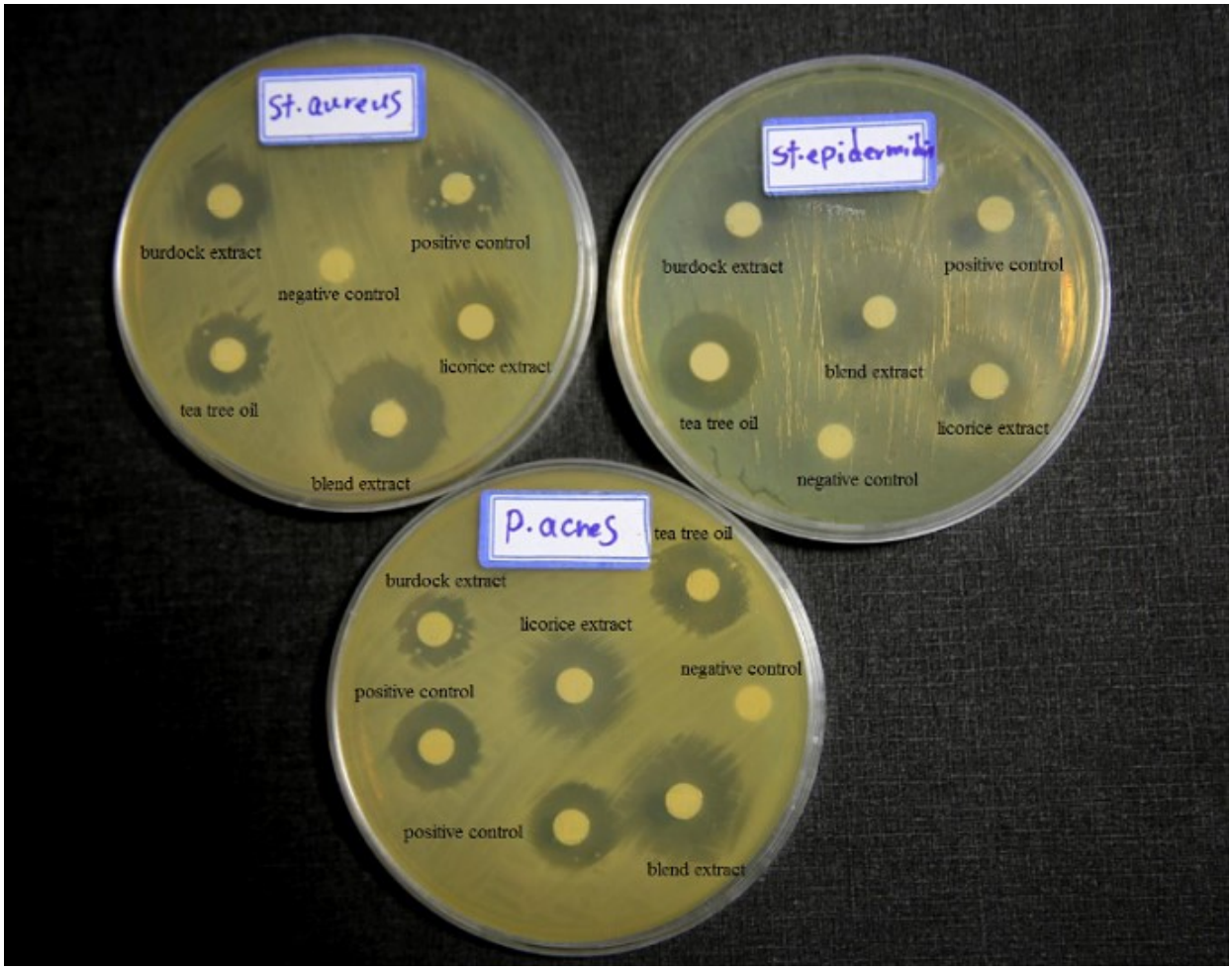

Figure1. In vivo antibacterial activity of the herbal extracts against*Propionibacterium acnes*, *staphylococcus aureus* and *staphylococcus epidermidis.*

DISCUSSION

Nowadays, the use of medicinal plants has increased for different reasons. Including, these plants are safe, have no known harmful side effects and do not show any antibiotic resistance.

But, a main problem associated with medicinal plants is related to less effective in comparison with antibiotic. Therefore, the aim of this study is to evaluate the overlapping activity of several medicinal plants in order to obtain an activity like antibiotic for treating acne lesions and pimples caused by bacteria. First, the efficacy of each plant was evaluated separately, and measured inhibition zone, MBC and MIC. Based on observations of this study on antimicrobial activities of "tea tree" oil, "licorice" and "burdock", it can be concluded that these plants can be used as effective antibacterial agent against *Propioni bacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis.* Next, the mixture of these plants (blend) was treated with above mentioned bacteria in order to measure the synergistic effect

between them. Result showed that the efficacy of blend increased more than using just one plant against above mentioned bacteria, and a significant difference has shown in the level of inhibition zone, MBC and MIC of blend and each plant. Therefore, this result confirmed the synergistic inhibitory effect of blend, and making them more effective as antibacterial agent. According to previous studies, assumptions about the mechanism of action of herbal extracts were made based on its hydrocarbon structure, and this hydrocarbon partition penetrates into the biological membranes of bacteria and disrupts the structural and functional integrity of bacterial membrane, and prevents the amplification of RNA and DNA of microorganism [31]. Even though, previous research showed that hydrocarbon structure not found in tea tree oil, and terpenes found in tea tree oil, and these compounds caused to lysis and the loss of membrane integrity and function of microorganism by using the leakage of ions and the inhibition of respiration. The treatment of *S. aureus* with tea tree oilleads to the leakage of potassium ions and inhibits respiration [32]. Therefore, "tea tree" oil could disrupt the structural and functional integrity of bacterial membranes [32]. Also, the effects of terpinen-4-ol, α-terpineol, and 1,8-cineole on *S. aureus* were tested [33]. All compounds like "tea tree" oil inhibit the respiration of microorganism after treatment whit a mechanism of action involving the loss of membrane integrity and function [33]. With regard to the fact that antibiotics could promote the spread of antibiotic resistance like antibiotic-resistant of *Propioni bacterium acnes* against different types of antibiotics like azithromycin, erythromycin, clindamycin, and it is accompanied with the formation of biofilm, and increases the resistance against antimicrobial agents [18, 19, 20, 21], and may cause the failure of antibiotic treatment. Several researches illustrated the antimicrobial activity of "tea tree" oil, "burdock" and "licorice" separately [12, 13, 14, 15, 16, 17]. But, a few studies have been done to find a strong mixture of herbs for treating bacterial infections [22, 23, 24, 25]. Also, the possibility that "tea tree" oil may have synergistic or antagonistic interactions with other essential oils were evaluated in previous study with lavender and other plants [26, 27, 28, 29, 30, 34], but no study has been done to evaluate on the overlapping activities of "tea tree" oil with "licorice" and "burdock". The result of this study showed that this mixture can be used in different types of products as anti-bacterial agent, and is more effective and safer rather than synthetic products without

any severe side effects. Consequently, this blend could be used as alternative treatment to antibiotic without having any side effect and antibiotic resistance.

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Conflict of interest statement

The authors have declared no conflict of interest.

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