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Effect of Hydro-alcohol Extract of Six Medical Plants on the Antibiotic resistant pseudomonas Aeroginosa and Staphylococcus Aureus Bacteria

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ABSTRACT

Objective: As the resistance of pathogen bacteria is increasing against antibiotic, the researchers attempt to find drug and the new compounds in medical plant as an alternative for the ineffective antibiotic.
The objective of the present study was to determine the properties of antibacterial hydro-alcohol extract of medical plants on clinical and standard strains of pseudomonas aeruginosa and staphylococcus aureus.
Method and Materials: First, the hydro-alcohol extract of the intended plants were prepared by soaking in alcohol 70% (Maceration). In order to determine the sensitiveness of bacteria to the herbal extracts, agar diffusion Method and determine minimal inhibitory concentration, dilution and growth in blood agar invironment were applied. The results were compared with gentamycin, cotrimoxasol, amikasin, vancomycin, ceftazidime, ciprofloxacin, imipenam, chloramphenicol and streptomycin antibiotics.
Results: when the results were evaluated, it was found that the hydro-alcohol extracts in compared to the aqueous extracts were more effective on the both species of staphylococcus aureus and pseudomonas aeruginosa
The maximum diameter for non-growth zone and minimal inhibitory concentration (MIC) were related to the salvia multicaulis on staphylococcus aureus which the mean of the zone diameter for non-growth and MIC measure were 30 mm and 6 mg/ml respectively. The mean of zone diameter for non-growth bacteria staphylococcus aureus to hydro-alcohol extract of allium gestianum were 27mm, asafetida stingingassa were 27mm, malvasilvestries were 22mm and teucraumpolium were 25mm. The aqueous and hydro-alcohol extracts of alyssum homolocarp had no effect on the mentioned bacterials.
Conclusion: with regard to this fact that the resistance of bacterial against the antibiotics is increasing rapidly، it is recommended to apply the natural alternatives such as the extracts of medical plants.
Keywords: Hydro-alcohol, Antibiotic resistant, pseudomonas Aeroginosa, Staphylococcus Aureus

INTRODUCTION

Natural plant products have been used throughout human history for the treatment of patient's sufferings. In recent years, herbal drugs gain skyrocket popularity due to low cost, high compatibility and lack of known side effects of chemical drugs. It has been estimated that one third of all medicinal products have plant origin or changed after extraction of plant (Buhner, 1999).

Infectious diseases pose a great deal of problem to human's health, and many efforts has been done to control and treating them. Form the past, antibiotics were the main treatment for infectious diseases, but due to drug's side effects and also increasing resistance of bacteria to antibiotics; other remedies and complementary sources particularly phytomedicinal drugs got special importance (Abbasi et al. 2007).

The therapeutic application of plant's components and also research on their antimicrobial properties has had long history. For example, research on the medical and medicinal properties of extracts (essences) of labiates have begun from the late 1980s and results shown that labiate species have some bactericide components such as saponins and cyclic alcohols (Larrondo et al., 1995).

The current study was conducted with purpose of identification of bactericidal effects of hydroalcoholic extracts of medicinal plants Salvia multicaulis (Lamiaceae family), Teucraumpolium (Lamiaceae family), Assefetidastingingassa (Apiaceae family), Allium gestianum (Amaryllidaceae family), Malvasilvestris (Malvaceae family), and Alyssum homolocarp (Brassicaceae family) on pathogenic bacteria staphylococcus aureus and pseudomonas aeruginosa.

There are cineol in Salvia multicaulis; asaresinotannol, mucilage, and ferulic acid in Assefetidastingingassa; sulfide components in Allium gestianum;anthocyanins in Malvasilvestris; terpenoid and glycosidic component in Teucraumpolium; Mucilage, lipids, sulfide, terpenoid, anthocyanins, glycosidic components, and volatile essences in Alyssum homolocarp (Azar-Nivand, 1384).

For preparation of plant's extract, chloroform, methanol, ethanol, acetone, and water was used as solvent; results shown that ethanolic extract of all plants was more effective and lead to widest bacterial growth inhibition zone.

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Pseudomonas aeruginosa is a gram-negative, compulsory aerobic, motile bacillus with size of 0.6-2 micron which observed as single, dual or sometimes short chain of rod cocci.

Pseudomonasa eruginosa is ubiquitous bacteria that observed abundantly in the nature and is an opportunistic pathogen which can cause urinary tract infection, respiratory system infections, skin inflammation, soft tissue and bacteremia, joint and gastrointestinal infections (Hauser and Rello, 2003; Doring and Pier Gerald, 2008).

Staphylococcus aureusis a gram-positive, aerobic and facultativeanaerobic cocci. It is a shared bacteria between the human and animal– member of the skin normal –and colonized around the belly button of infants and front part of the nose in adults and sometimes colonized in the adult intestine. Staphylococcus aureus lead to a wide range of infections from simple skin infections such as acne, boil, carbuncle, sty and abscess to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and septicemia. Staphylococcus aureus is one of the five most common cause of nosocomial infection, particularly the cause of surgical wound infections.

The issue of incidence and prevalence of antimicrobial resistance, particularly resistance in Gram-negative bacteria is considered as one of the main obstacles on the way to cure infectious diseases.

Research and studying herbal plants as novel antimicrobial agents has gotten widespread due to increasing resistance of bacteria to chemical antibiotics, which is the result of the indiscriminate use of antimicrobial drugs to treat infectious diseases.

MATERIAL AND METHODS

Table 3-1 Plant extracts used in present study

Plant	Scientific name	Medicinal part	Extract Alcohol content (%)	Extraction method
Salvias	Salvia multicaulis	Flower	70	Maceration
cheeses	Malvasilvestries	Flower	70	Maceration
Freula	Ferulaassafoetida	Seed	70	Maceration
Hedge mustard	Sisembrium officinal	Seed	70	Maceration
felty germander	Teucraumpolium	Bloom	70	Maceration
Alium	Aliumjestianum	Leaves	70	Maceration

Boiling method was used to prepare the aqueous extract of this plants. 10 g of each plant would be mixed with 100 ml of distilled water in a suitable container. Then we boiled it with a gentle heat for 30-20 minutes until its residual volume reaches one-third of the initial volume. Then the mixture was centrifuged with the 3000rpm for 10 min and the supernatant was separated.

Maceration was used to prepare alcoholic extract of plants. To perform this method, the plants were cut into small pieces and powder, then 10 g of the resulting powder of each plant was added to 100 ml of ethanol 70%, methanol 70%, acetone70 %, and chloroform 70% as solvent. Then the resulting mixtures were kept for 24 hours at laboratory temperature, and the mixtures was stirred every five hours using a glass rod. Then the mixtures were kept in an oven at 50-40°C for another 48 to 72 hours. After this period, the resulting solution was centrifuged with 3000 rpm for 10 min (Hettich, Germany), and the supernatant aliquot was separated

3.8 Evaluation of the antimicrobial effect of the extracts on bacterial isolates.

After preparation of the aqueous, alcoholic, acetone, and chloroform extracts, the antibacterial effect of the extracts were investigated on isolates using well diffusion method.

First the cell suspension of each bacterial have been prepared with 0.5 Mc Farland concentration (1.5×10^8 cell), and then streaked by sterile swab on Muller Hinton 10 cm plates with 4 mm thickness. Then diffusion wells were created by sterile Burrell on the plate (5 wells per plate). 100 µl of desired extract was poured into each well (by 100 micro liters sampler), and then let the juices are absorbed into the agar. The plates inoculated by Staphylococcus aureus and Pseudomonas aeruginosa were incubated at 37°C(Bassam et al., 2004)

Finally after 24-48 hours, resulting growth inhibition zone of extracts around the well on Pseudomonas aeruginosa and Staphylococcus aureus was measured by a caliper.

3.9.1 Preparing various effective dilution of plant extracts on bacteria

At this point dilutions of ½, ¼, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024 were prepared respectively from each one of extract effective on the bacteria. Plate was composed of 8 rows of 12 cells. The required volume for each cell is 200 ml. Culture medium that used in this study was Muller Hinton broth.

- Cell one: it was used as control for viability of bacteria, thus containing 100 ml medium without any extract
- Cell 2 to 11:contains 100 µL of extracts with dilutions of ½, ¼, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, respectively.
- Cell 12: it was used as control for non-contaminated extract, thus it was contain 100 µL of medium and 100 µL of extracts.

3.9.2 Bacterial suspension and inoculation

- 10 ml of the bacterial suspension at a concentration equivalent to 0.01 of half- McFarland were prepared and 100 µL of it was added to cells 1 to 11 of microplate and then incubated at 37 degree of Celsius.

3.9.3 Determination of minimum inhibitory concentration (MIC)and minimum bactericidal concentration (MBC) of each extract.

After streak and culturing the bacteria for testing, some wells were made on the surface of medium with tip of sterile Burrell (5 wells per plate). Then 100 µL of each extracts was added to each well (using 100 µL sterile sampler) and let the extract was absorbed into the agar.The plates were put in the incubator at 37 degree of centigrade under suitable conditions. After 24-48 hours, the diameter of zone of inhibition around the wells was measured by caliper, so that, MIC and MBC were determined (Bakri and Douglas, 2005).

3.10 medicinal plant extracts purification using column chromatography

In the current study, medicinal plant extracts have been purified by column chromatography using silica gel as the stationary phase and ethanol solvent as the extraction solvent.

For this operation, 100 ml of ethanol were cast to the top of column of chromatography, then samples leaked out of the bottom of the column werecollectedin micro- tubes A, B, C and D,respectively; as volumes of 25 mL, which after extraction of solvents, the extracted material was used in the study.

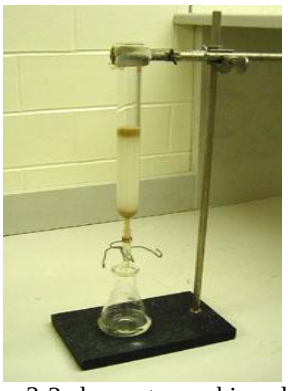


Figure 3-2 chromatographic column

3.11 Effect of purified extracts of medicinal plants on bacterial isolates

After purification of herb extracts using column chromatography, the effect of antibacterial compounds of extracts was investigated.

First, bacterial cell suspension was prepared with a concentration of 0.5 McFarland (1.5×10^8 cell), and then the agar were streaked by a sterile swab on Muller Hinton plates 10 cm in diameter and 4 mm thickness. Then the wells was created in the medium using a sterile Burrell tip (5 wells per plate). 100 µL of desired extracts were poured into each well (using µL sampler) and left until let the extract was absorbed into the agar. The plates of Pseudomonas and Staphylococcus aureus were incubated at 37°C (Bassam and Ghaleb, 2004).

Finally, after 24-48 hours, the zone of growth inhibition of Staphylococcus and Pseudomonas for each extract that formed around each of the wells was measured by a caliper.

3.5 Phenotypic identification of the isolates using API kit

API20E kit is used for the detection of bacteria in the Enterobacteriaceae family and other gram-negative bacilli that are not hard growing. This kit includes 20 biochemical tests, which form as a strip of microtubes.

RESULTS

The effect of antibiotics on bacterial isolates

Antibacterial activity of antibiotics on Staphylococcus aureus and Pseudomonas aeruginosa was examined. The results could be seen in Table 4-1. According to the results obtained in this phase of the research, Pseudomonas aeruginosastrains are resistant to streptomycin, chloramphenicol, cotrimoxazole, vancomycin and cefazolin.

Table 4-1 Effects of antibiotics on bacterial isolates

The percent of bacterial resistant to antibiotics									No	Bacteria
Ciprofoxacin	Cefazolin	Gentamicin	Vancomycin	Cotrimoxazole	Chloramphenicol	Imipenem	Streptomycin	Amikacin		
85	100	70	100	100	100	40	100	45	30	**Pseudomonas aeruginosa clinical**
S	R	S	S	R	R	S	R	S	1	**Pseudomonas aeruginosa - ATCC**
40	0	100	20	50	25	0	60	50	40	**Staphylococcus aureus - clinical**
S	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**1**	**Staphylococcus aureus - ATCC**

Table 4-1 shows that gram-negative bacteria such as Pseudomonas aeruginosa compared with gram-positive bacteria such as Staphylococcus aureus, are more resistant to certain antibiotics.

Table 4.2 Minimum, maximum and mean diameter of zone of growth inhibition (mm) by antibiotic - sensitive strains.

Staphylococcus aureus			Pseudomonas aeruginosa			Bacteria Antibiotics
mean	Max	Min	Mean	Max	Min	
23	26	16	20	29	10	**Amikacin**
11	14	10	-	-	-	**Streptomycin**
36	42	27	27	42	10	**Imipenem**
26	35	20	-	-	-	**Chloramphenicol**
24	26	18	-	-	-	**Cotrimoxazole**
13	21	10	-	-	-	**Vancomycin**
21	26	18	-	-	-	**Cefazolin**
29	31	20	21	34	10	**Ciprofoxacin**

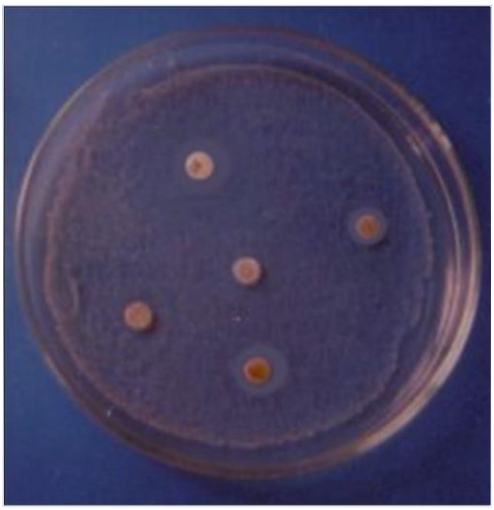


Figure 4.2 The effect of antibiotics on Pseudomonas aeruginosa

4.4 Determination of the inhibitory effect of plant extracts on bacteria

4.4.1 The inhibitory effect of hydro alcoholic extracts of herbs on bacteria

Results obtained from analysis of zone of growth inhibition of Pseudomonas aeruginosa and Staphylococcus aureusin comparison with the alcoholic extract is shown in Table 4-5.

Table 4-5 Average diameter of zone of growth inhibition (mm) of bacterial strainscaused by the influence of alcoholic extract of herbs.

Alyssum homolocarp	Teucraumpolium	Malvasilvestries	Assefetidastingingassa	Aliumjestianum	Salvia multicaulis	Bacteria
_	12	8	_	10	15	Pseudomonas aeruginosa-clinical
_	14	-	8	12	17	Pseudomonas aeruginosa-ATCC
_	25	22	27	27	30	Staphylococcus aureus-clinical
_	27	20	18	20	32	Staphylococcus aureus-ATCC

4.4.2 Determination of the inhibitory effect of aqueous extracts of plants on bacteria

Results obtained from analysis of zone of growth inhibition of pseudomonas aeruginosa and Staphylococcus aureus due to aqueous extracts are shown in Table 4-6.

Table 4-6 Average diameter of zone of growth inhibition (mm) of bacterial strains, caused by the effects of extracts of medicinal herbs.

Alyssum homolocarp	Teucraumpolium	Malvasilvestries	Assefetidastingingassa	Aliumjestianum	Salvia multicaulis	Plant extract Bacteria
-	-	-	-	-	8	Pseudomonas aeruginosa-clinical
-	-	-	-	8	10	Pseudomonas aeruginosa-ATCC
-	15	16	14	10	18	Staphylococcus aureus-clinical
-	17	15	12	21	16	Staphylococcus aureus-ATCC

As can be seen in Table 4-5 and 4-6, Pseudomonas aeruginosa and Staphylococcus aureusare more sensitive to aqueous extract. These bacteria are also most sensitive to the Salvias(Figures 4-3 and 4-4).

According to Table 4-5 and 4-6, diameter of zone of growth inhibition of Staphylococcus aureusagainst alcoholic and aqueous extracts indicate that these bacteria are sensitive to hydro-alcoholic extract of salvias, cheeses, alium, felty germander. Also, the bacteria scored the highest sensitivity towards hydro-alcoholic extracts of Salvias and felty germander (Figure 4-9).

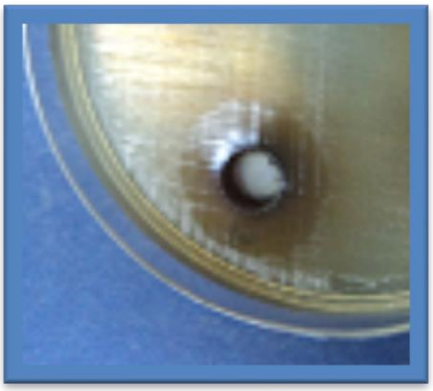


Figure 4-3 Zone of growth inhibition of Pseudomonas aeruginosa in the presence of hydro-alcoholic extracts of salvias

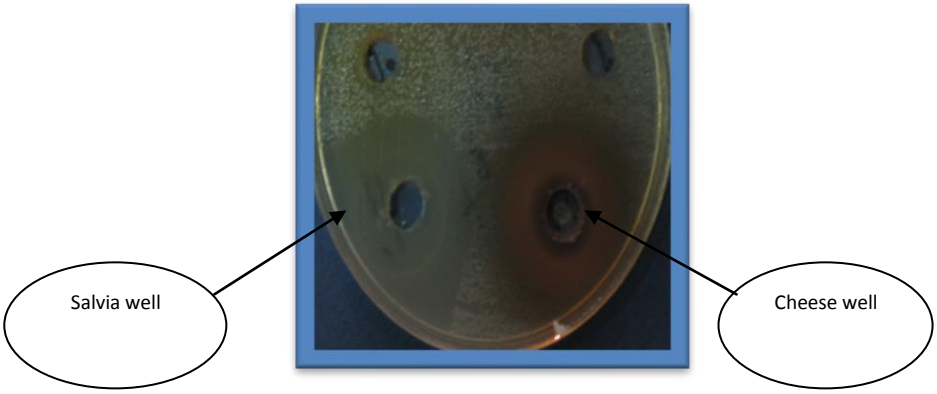


Figure 4-4 Zone of growth inhibition of Staphylococcus aureus in the presence of hydro-alcoholic extracts of salvia and cheeses

4-5 Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hydro-alcoholic extracts of plants

The results of the determination of MIC and MBC of hydro-alcoholic extracts on pseudomonas aeruginosa and Staphylococcus aureus are both shown in Tables 4-7 and 4-8.

Table 4-7. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hydro-alcoholic extracts of plants on study's bacteria

Teucraumpolium		Alyssum homolocarp		Aliumjestianum		Malvasilvestries		Assefetidastingingassa		Salvia multicaulis		Plant extract
MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	Bacteria
16	8	-	-	150	75	75	75	90	30	[12]	6	**Staphylococcus aureus-clinical**
24	8	-	-	50	75	150	75	180	60	24	12	**Staphylococcus aureus-ATCC**
54	27	-	-	230	164	460	1150	-	-	115	57	**Pseudomonas aeruginosa-clinical**
27	13	-	-	230	180	230	230	-	-	115	151	**Pseudomonas aeruginosa-ATCC**

* values that listed in Table are based onmg/ml

According to Table 4-6, salvias with the lowest concentration had highest inhibitory effect on Staphylococcus aureus Pseudomonas aeruginosa; and then, felty germander with the lowest concentration of have the highest inhibitory effect on pseudomonas aeruginosa and Staphylococcus aureus.

4.6 Effect of segregated compounds of plant extracts.

Table 4-9 shows the effect of compounds that separated partially in the chromatographic on bacterial isolates.

Table 4-9.Zone of growth inhibition (mm) caused by pure compounds of plant extracts on staphylococcus aureus

Teucraumpolium				Malvasilvestries				Aliumjestianum				Assefetidastingingassa				Salvia multicaulis				
4	3	2	1	4	3	2	1	4	3	2	1	4	3	2	1	4	3	2	1	**Bacteria**
14	22	22	20	11	25	27	15	10	25	13	10	8	25	12	14	-	15	26	24	**Staphylococcus aureus**
-	8	10	-	-	-	-	-	-	-	8	10	-	-	-	-		8	15	-	**Pseudomonas aeruginosa**

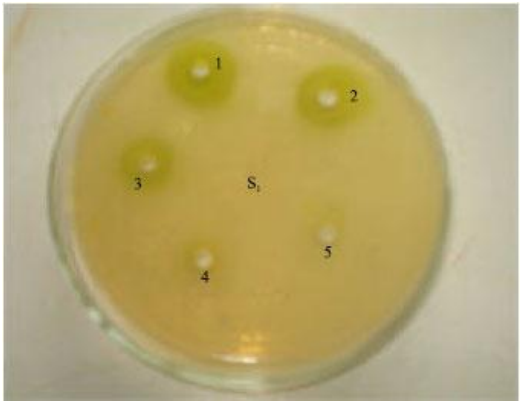
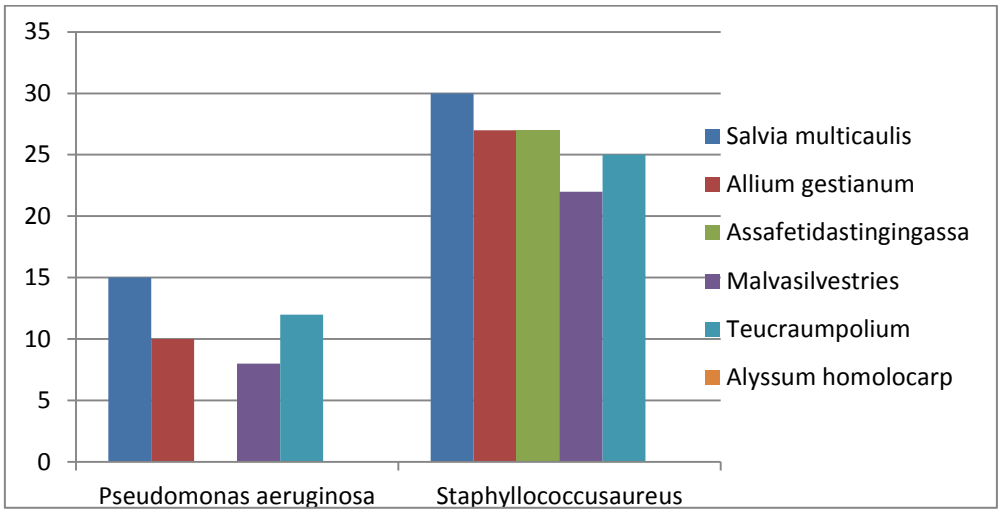


Figure 4-5 the effects of pure component of salvias flowers on S. aureus

4.7 Statistical Results

Following Charts shows the growth inhibition effects (diameter of zone of growth inhibition) of hydro-alcoholic extract on Staphylococcus aureus and Pseudomonas aeruginosa



Average inhibition zone diameter in millimeters

The following table shows the descriptive statistics including sample number, mean and standard deviation and 95% confidence interval for the mean of diameter and the diameter of the minimum and maximum diameter of each plant extract for clinical Pseudomonas aeruginosa bacteria. For example, salvias 30 samples and average diameter of 15 and a standard deviation of 2.7 and the minimum and maximum sample diameter is 10 and 21 respectively. The 95% confidence interval for the mean diameter is in the range of (16. 0096 and 13. 9904). Means with 95% confidence interval, the mean diameter of the population fall in the range of (16. 0096 and 13. 9904).

Descriptive statistics of zonediameter per extract for the clinical pseudomonas aeruginosa

	No	Mean	SD	95% significance interval		Min	Max
				Upper limit	Lower limit		
salvias	30	15. 0000	2. 70376	13. 9904	16. 0096	10. 00	21. 00
Alium	30	10. 0000	2. 36352	9. 1174	10. 8826	5. 00	15. 00
cheeses	30	8. 0000	2. 79161	6. 9576	9. 0424	4. 00	15. 00
felty germander	30	12. 0000	2. 82843	10. 9438	13. 0562	8. 00	18. 00
Total	120	11. 2500	3. 70612	10. 5801	11. 9199	4. 00	21. 00

The following table shows the analysis of variance table comparing the four extracts of salvias, Alium, cheeses, felty germander in terms of their effects on the diameter of growth inhibition zone of clinical pseudomonas aeruginosa. Due to the probability value in the last columns which is less than 0.05, it is assumed that extracts are not the same based on their effects on clinical pseudomonas aeruginosa and the hypothesis is rejected. In the other word, with 95% confidence we accept that there is a difference between the extracts.

Table Analysis of Variance (ANOVA) of zone of inhibition diameter for each extract on clinical pseudomonas aeruginosa

	The sum of squares (SS)	degrees of freedom (df)	mean squares (MS)	test statistic (F)	Probability value (P)
extracts	802. 500	3	267. 500	37. 296	. 000
error	832. 000	116	7. 172		
sum	1634. 500	119			

The following table compares the combined two of four extracts of salvias, alium, cheeses and felty germander in terms of their effects on the clinical pseudomonas aeruginosa and with the LSD method. Given the probability values of all which are less than 0.05, the pairs of different extracts are significantly different base on the diameter of zone of inhibition on the clinical pseudomonas aeruginosa with 95% confidence.

Table of comparing pairs of four extracts of salvias, alium, cheeses and felty germander based on their effects on clinical pseudomonas aeruginosa with LSD method

(I)	(J)	mean difference (I-J)	Probability value (P)	95% confidence interval of mean differences	
				lower limit	Upper limit
salvias	alium	5. 00000(*)	. 000	3. 6304	6. 3696
	cheeses	7. 00000(*)	. 000	5. 6304	8. 3696
	felty germander	3. 00000(*)	. 000	1. 6304	4. 3696
alium	Salvias	-5. 00000(*)	. 000	-6. 3696	-3. 6304
	cheeses	2. 00000(*)	. 005	. 6304	3. 3696
	felty germander	-2. 00000(*)	. 005	-3. 3696	-. 6304
cheeses	Salvias	-7. 00000(*)	. 000	-8. 3696	-5. 6304
	alium	-2. 00000(*)	. 005	-3. 3696	-. 6304
	felty germander	-4. 00000(*)	. 000	-5. 3696	-2. 6304
felty germander	Salvias	-3. 00000(*)	. 000	-4. 3696	-1. 6304
	alium	2. 00000(*)	. 005	. 6304	3. 3696
	cheeses	4. 00000(*)	. 000	2. 6304	5. 3696

* The mean difference is significant at 5 % or 95% of significance level

The following table shows the descriptive statistics of the sample mean and standard deviation, 95% confidence interval for the mean diameter of zone and the diameter of the population and the minimum and maximum diameter of the zone extract on clinical Staphylococcus aureus. For example, the number of samples for salvias is 40 and the average zone diameter is 30 and a standard deviation of samples is 33.3, and mined max diameter of the zone are 23 and 37,respectively. Although, with 95% confidence interval for the mean zone diameter of the population for salvias is in range of (31. 0669 and 28. 9331). Means with 95% confidence interval, the mean of zone diameter of the population is fall in the range of (31. 0669 and 28. 9331).

Descriptive statistics of the zone diameter for each extract onclinical Staphylococcus aureus

	No	Mean	SD	95% significance interval		Min	Max
				Upper limit	Lower limit		
salvias	40	30. 0000	3. 33590	28. 9331	31. 0669	23. 00	37. 00
Alium	40	27. 0000	3. 77577	25. 7925	28. 2075	20. 00	36. 00
Assefetida	40	27. 0000	3. 56622	25. 8595	28. 1405	21. 00	36. 00
cheeses	40	22. 0000	2. 53134	20. 7404	22. 3596	16. 00	27. 00
felty germander	40	25. 0000	2. 50128	24. 2001	25. 7999	20. 00	30. 00
Total	200	26. 1100	4. 21148	25. 5228	26. 6972	16. 00	37. 00

ANOVA table below show the results of comparing extracts of five plants of salvias, Alium, Assefetida, cheeses, felty germander based on their effects on the inhibition zone of clinical Staphylococcus aureus. Due to the probability values in the last column which is less than 0.05, we can assumed that the hypothesis of equal effect of extracts on clinical Staphylococcus aureus zone diameter is rejected. In other word, with 95% confidence interval, there is difference between the extracts.

Table Analysis of Variance (ANOVA) of zone of inhibition diameter for each extract on clinical Staphylococcus aureus

	The sum of squares (SS)	degrees of freedom (df)	mean squares (MS)	test statistic (F)	Probability value (P)
extracts	1549. 680	4	387. 420	38. 157	. 000
error	1979. 900	195	10. 153		
sum	3529. 580	199			

The following table compares the combined two of four extracts of salvias, alium, cheeses and felty germander in terms of their effects on the clinical Staphylococcus aureus and with the LSD (least significant differences) method. Given the probability values of all which are less than 0.05, the pairs of different extracts are significantly different base on the diameter of zone of inhibition on the clinical Staphylococcus aureus with 95% confidence.

Table of comparing pairs of four extracts of salvias, alium, cheeses and felty germander based on their effects on clinical Staphylococcus aureus with LSD method

(I)	(J)	mean difference (I-J)	Probability value (P)	95% confidence interval of mean differences	
				lower limit	Upper limit
salvias	alium	3. 00000(*)	. 000	1. 5948	4. 4052
	cheeses	3. 00000(*)	. 000	1. 5948	4. 4052
	felty germander	8. 45000(*)	. 000	7. 0448	9. 8552
alium	Salvias	5. 00000(*)	. 000	3. 5948	6. 4052
	cheeses	-3. 00000(*)	. 000	-4. 4052	-1. 5948
	felty germander	. 00000	1. 000	-1. 4052	1. 4052
cheeses	Salvias	5. 45000(*)	. 000	4. 0448	6. 8552
	alium	2. 00000(*)	. 006	. 5948	3. 4052
	felty germander	-3. 00000(*)	. 000	-4. 4052	-1. 5948
felty germander	Salvias	. 00000	1. 000	-1. 4052	1. 4052
	alium	5. 45000(*)	. 000	4. 0448	6. 8552
	cheeses	2. 00000(*)	. 006	. 5948	3. 4052

* The mean difference is significant at 5 % or 95% of significance level

5.1 DISCUSSION AND CONCLUSIONS

This study was conducted to answer this question, which medicinal plants have antimicrobial compounds, and are they inhibitory and bactericidal effects on antibiotic-resistant bacteria. To answer this question, the effects of six plant extracts native to the northern Fars were assessed on clinical and standards sample of pseudomonas aeruginosa and Staphylococcus aureus. For the preparation of medicinal plant extracts, ethanol, methanol, acetone, chloroform and water were used as solvents. The antibacterial effect of the extracts was determined by the agar diffusion method. Zone of growth inhibition showed that the antibacterial effect of the solvent 70% methanol is more than 70% ethanol, 70% acetone, 70%chloroform, and water.

In the study of antibacterial activity of the leaf extract of Trifoliumal exandrinum against seventeen pathogenic gram- positive and negative bacteria, it was found that polar extracts shown certain antimicrobial activity against pathogens; and methanolic extracts have highest antibacterial activity with maximal inhibition zone compared to the other solvents (Abdul et al., 2002).

First, comparing antibacterial activity of herbs with conventional antibiotics listed in table 4-3 and table 4-5 shown that antibiotic-resistant bacteria are sensitive to plant extracts. Pseudomonas aeruginosa and its standard sample, ATCC isolates are fully resistant to standard antibiotics streptomycin, cothrimoxazole, chloramphenicol, cefazolin, while sensitive to hydro-alcoholic extract of salvias and alium. In previous research, the antibacterial effects of essential oils or extract of many medicinal plants have been shown on bacteria resistant to antibiotics. For example, in the study of antibacterial and phyto-chemical activity of 45 medicinal plants in India against MDR (multi drug resistant) bacteria which are human pathogens showed that more than 92 percent of the plants had antibacterial compounds. In this research after investigation and analysis of phyto-chemical properties of plants, the main compounds of tested plant were include alkaloids, phenols, flavonoids, glycosides, saponins and tannins (Iqbal and Arina., 2001).

Another research have been done on the relationship between the synergistic effect of various antibiotics and plant extracts. The antibacterial activity of some plants alone and along with cephalexin, penicillin G, oxy-tetracycline, ciprofloxacin and sulfa-dimethoxin were assessed. They found that pseudomonas aeruginosa was resistant to tetracycline and ciprofloxacin, but it was sensitive to combination of different extracts with tetracycline or with ciprofloxacin. In the present study, reduced minimum inhibitory concentration in the combination of ethanol extract and antibiotics in pseudomonas aeruginosa was results of efflux pump inhibitor activity of Pseudomonas aeruginosa (Bassman, 2002).In study of efflux pump, it was found that the role of efflux pomp in the pseudomonas aeruginosa was excreting anti-Pseudomonas component from the bacteria; such

as a multigene efflux system called MEX-A, OPR-M led off antibiotics, quinolones, aminoglycosides, tetracycline, chloramphenicol, Novobiocine and often beta - lactam antibiotics, but no effect on imipenem (Xavier et al., 2010), which is consistent with our antibiogram test results.

One way of Staphylococcus aureus resistant to aminoglycosides is the presence of bacterial membrane efflux pump. In the present study, S. aureus isolates were 100 percent resistant to gentamicin.

Plants are rich of secondary metabolites contents such as alkaloids, flavonoids and tannins. The antimicrobial of these substances are proved in the in vitro studies (Lewis, 2006).

In a study, the essence of achilaellifora was extracted through evaporation with distilled water and using Clevenger apparatus, then the resulted extract were analyzed by gas chromatography (GC) linked to mass spectrometry (GC / MS) and components were determined based on deterrence and inhibitory indices. With above mentioned method, 36 compounds identifiedin the essential oils which mainly include camphor, cineol, camphene, pinene and borneol (Oroojalian and kasra, 1389).

In table 4-7, the MIC of salvias flower against gram-positive staphylococcus aureus was 6 mg/ml and against gram-negative bacteria pseudomonas aeruginosa was 57 mg/L, which indicates that the compounds of plant extracts are more effective on gram-positive bacteria than gram-negative bacteria. Based on researches, gram-positive bacteria are more sensitive of plant extracts against gram-negative bacteria (Burt s, 2004).The results obtained in this study (higher MIC of hydro-alcoholic extracts of plants against pseudomonas vs Staphylococcus aureus) showed more sensitivity of gram-positive bacteria than gram negative, which is due to the presence of the outer membrane that surrounds the cell wall in gram-negative bacteria (Oroojalian, 1389).

Comparing the results of current study with the results of other researchers showed that Salvias, alium, Assylum, cheeses, felty germander have antibacterial activity.

The diameter of zone of growth inhibition of Staphylococcus aureus in the presence of hydro-alcoholic extract of Lawsoniainermis and Emblicaofficinalis was 30 (Iqbal, 2002); and also the zone of inhibition of Pseudomonas aeruginosa growth in the presence of Lawsoniainermis was 17 (Dhanalakshmi, et al. 2013). The diameter of zone of inhibition of growth of Staphylococcus aureus in the presence of Hyperiumperforatum extract was 27 mm and zone of pseudomonas aeruginosa was 14 mm (Gonel et al. 2008); or the MIC of Staphylococcus aureus in the presence of Kampanula Lyrata extract was 14/5 mg/ml (Mehica et al., 2007).

The phyto-chemical properties of the essential oils of Achillea Shirazi on clinical samples and standards sample of S. aureus (Oroojalian, 1389) in which the extract was prepared through the vacuum distillation method were studied and the minimum concentration of growth was 15 mg/ml. One of the reasons for low levels compared with other findings is the methods used to extract plant substances. In addition to the extracted material, the concentration are effective too. The results of studying Yarrow essential oil components by GC-MS (gas chromatograph connected to a mass spectrometry) proved the presence of 36 compound in the Yarrow.

The lowest MIC in investigated medicinal plants was seen in salvias and alium. The hydro-alcoholic extract of Salvia multicaulis (Lamiaceae family) with inhibition zone of 30 mm against Staphylococcus aureus and 15 mm against pseudomonas aeruginosa, and also Teucraumpolium (Lamiaceae family) with inhibition zone of 25 mm against Staphylococcus aureus and 12 mm against pseudomonas aeruginosa shown that they have compounds with anti- bacterial effects,

The results of comparing the MIC and MBC of salvias and felty germander (tables 4-7 and 4-8) with previous studies shown that they had enough and desirable amount of antibacterial compounds. In future research, it is better to explore the compounds and properties of local plants first. In the second step, it's better to assess the method for extracting herbal compounds. Because the method of extraction is different for various parts of plant and it must be appropriate for certain part, final step is analysis of plants compounds, understanding the mechanisms of plant compounds and their effect are topics for future research. Therapeutic applications of plant constituents as well as study of antimicrobial compounds of them have long history. Example, research on the medicinal properties of plant extracts and dark mint began in the late 1980s, Research shows that Dark Mint plants possess antibacterial such as saponins and cyclic alcohols (Larrondo et al., 1995).

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