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Original Article

Investigating the effect of aqueous extracts (*Oregano* and *Marjoram*) on the wild strain of *Escherichia coli* in chicken farms with colibacillosis in Yasuj, Iran

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ABSTRACT

Aqueous extracts were prepared from two plants (*Oregano* and *Marjoram*) using the percolation method. The antibacterial activity of *Escherichia coli* (colibacillosis) in specific culture media was assessed by dipping sterile filter paper, sized like antibiogram tablets, into aqueous extracts of varying concentrations. These were then subjected to diffusion and incubation to evaluate microbial sensitivity. The microbial sensitivity test of the extracts showed that the diameter of the non-growth halo of *Oregano* aqueous extract was 18.3 mm at 100% concentration, 15.1 mm at 75%, and 11.2 mm at 50%, with an insignificant halo at 25% concentration. For the mixed extract, the growth inhibition halo was 19.4 mm at 100%, 16.3 mm at 75%, and 11.4 mm at 50%, with a very small halo at 25%. The largest halo for the wild *Marjoram* aqueous extract was 11.5 mm at 100% concentration, decreasing to 10.1 mm at 75%, and was very insignificant at 50% and 25% concentrations. The minimum inhibitory concentration (MIC) of bacteria by *Oregano* extract was 20 mg/ml, mixed extract was 15 mg/ml, and *Marjoram* extract was 130 mg/ml, showing a direct relationship with the increase in dilution of the extracts. The minimum bactericidal concentration (MBC) for *Oregano* extract was 60 mg/ml, for the mixed aqueous extract was 55 mg/ml, and for the wild *Marjoram* aqueous extract was 220 mg/ml. The results showed that the aqueous extract of *Oregano* and the mixed extract (*Oregano* and wild *Marjoram*) had greater antibacterial properties than the aqueous extract of *Marjoram*. Thus, aqueous extracts of plants can be used as a cheap and available source for therapeutic use and even prevention against bacterial infections and are a good alternative to antibiotics.

بررسی اثر عصاره‌های آبی (پونه کوهی و مرزنگوش وحشی) بر روی سویه وحشی باکتری *اشریشیاکولای* در مرغداری‌های مبتلا به کلی باسیلوز در یاسوج، ایران

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چکیده

از دو گیاه پونه کوهی و مرزنگوش وحشی به روش پرکولاسیون عصاره ابی تهیه شد. در محیط کشتهای اختصاصی فعالیت ضد باکتری اشریشیاکولای (کلی باسیلوز) به روش آغشته کردن کاغذ صافی استریل به اندازه قرص آنتی بیوگرام (در غلظت‌های مختلف) به عصاره‌های آبی و انتشار و انکوباسیون جهت انجام حساسیت میکروبی انجام شد. آزمایش حساسیت میکروبی عصاره‌ها نشان داد که قطر هاله عدم رشد عصاره آبی پونه به ترتیب مربوط به غلظت ۱۰۰درصد (۱۸/۳ میلیمتر) و ۷۵ درصد (۱۵/۱ میلیمتر) و ۵۰ درصد (۱۱/۲ میلیمتر) بود و در غلظت ۲۵ درصد ناچیز بود. قطر هاله عدم رشد عصاره (مخلوط) در غلظت ۱۰۰ درصد (۱۹/۴ میلیمتر) و ۷۵ درصد (۱۶/۳ میلیمتر) و ۵۰ درصد (۱۱/۴ میلیمتر) و درغلظت ۲۵ در صد، قطرهاله بسیار ناچیز بود. بیشترین هاله عصاره آبی مرزنگوش وحشی به ترتیب مربوط به غلظت ۱۰۰ درصد (۱۱/۵ میلیمتر) و بعد ۷۵ درصد (۱۰/۱ میلیمتر) و غلظت ۵۰ درصد و در غلظت ۲۵ در صد، هاله بسیار ناچیز بود. میزان حداقل غلظت بازدارنده (MIC) باکتری توسط عصاره پونه کوهی ۲۰ mg/ml ، عصاره مخلوط ۱۵ mg/ml و عصاره مرزنگوش ۱۳۰ mg/ml بود و با افزایش رقت عصاره ها رابطه مستقیم داشت. حداقل غلظت باکتری‌کشی (MBC) عصاره پونه کوهی ۶۰ mg/ml، عصاره آبی مخلوط ۵۵ mg/ml و عصاره آبی مرزنگوش وحشی ۲۲۰ mg/ml بود. نتایج نشان داد که عصاره آبی پونه کوهی و عصاره مخلوط (پونه کوهی و مرزنگوش وحشی) خواص ضد باکتریایی بیشتری نسبت به عصاره آبی مرزنگوش داشتند. بنابراین، عصاره‌های آبی گیاهان می‌توانند به عنوان منبعی ارزان و در دسترس برای مصارف درمانی و حتی پیشگیری در برابر عفونت‌های باکتریایی مورد استفاده قرار گیرند و جایگزین مناسبی برای آنتی‌بیوتیک‌ها باشند.

واژه های کلیدی: پونه کوهی، مرزنگوش وحشی، عصاره مخلوط، ماکیان، کلی باسیلوز

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INTRODUCTION

Colibacillosis is one of the most significant diseases caused by *Escherichia coli*, leading to substantial economic losses in the poultry industry. Due to the growing trend of antibiotic resistance, it is essential to prevent the disease from entering farms by adhering to proper nutritional and hygiene principles. Timely vaccination can also significantly prevent poultry from contracting infectious diseases, thereby reducing the use of various antibiotics and moving towards the production of antibiotic-free products [1]. *Oregano*, a popular edible plant from the mint family, grows wild near springs, with the best harvesting season from early April to mid-May. Drinking a glass of oregano brew can also relieve fatigue [2]. However, those allergic to plants in the mint family, such as basil, hyacinth, peppermint, and sage, should exercise caution as they may also have allergic reactions to *Oregano* [3]. Plants are the most common source of antimicrobial substances, with about 80% of the world's population in Asia, Latin America, and Africa using them in traditional medicine due to their fewer side effects. The genus *Mentha*, from the *Lamiaceae* family, has 20 species, with *Mentha pulegium L.* being one of them [4]. *Oregano* is commonly known as pennyroyal and is important for treating human diseases due to its chemical compounds with therapeutic effects. It is very similar to mint and belongs to the mint family [6, 7]. Many organic components and therapeutic properties of these two plants are similar. *Oregano* leaves are used more than other parts of the plant, and their aroma and taste are more pronounced after drying, which is unusual for medicinal plants [8]. Due to the increase in antibiotic use against bacterial infections and the emergence of resistant strains, researchers are focusing on finding auxiliary and alternative treatments, including medicinal plants [9].

The wild *Marjoram* genus belongs to the mint family and includes many species found wild in the Mediterranean regions, exhibiting high morphological and chemical diversity globally. The species *Origanum vulgare L.* has six subspecies worldwide, but only three subspecies including *viride*, *vulgare*, and *gracile* have been identified in Iran. The active ingredients of *Marjoram* include carvacrol and thymol, along with other compounds such as gamma-terpinene, p-cymene, linalool, and sabinene. The *Marjoram* plant is used as a spice, antioxidant, pain reliever, disinfectant, diuretic, and in the treatment of stomach and intestinal diseases [10]. Its disinfecting property is notable, as it is used orally for the digestive system and externally on wounds. The extract of this plant serves as a suitable antibacterial agent to prevent and treat infections caused by this group of bacteria [11]. This research investigates the effect of two extracts of *Oregano* and wild *Marjoram* on the wild strain of *Escherichia coli* bacteria in chicken farms with colibacillosis in Yasuj.

MATERIALS AND METHODS

The study involved visiting chicken farms infected with general bacillus disease in Yasuj. A total of 150 infected chickens with typical symptoms were collected, and 115 wild strains of *E. coli* were isolated. The isolates were identified using culture media and biochemical tests. *Oregano* and wild *Marjoram* were prepared and collected from the Dana Mountains of the Si-Sherd region, and their authenticity was confirmed by Sinre Pharmaceutical Company. The determination of the minimum inhibitory concentration (MIC) of bacterial growth was done using the broth microdilution method and 96-well microplates. To determine the minimum bactericidal concentration (MBC) of *Oregano* and wild

Marjoram extracts and the mixed extract (*Oregano* and *Marjoram*), 10 microliters were used. The contents of each well of the 96-well microplate (MIC well and three pre-MIC wells) were cultured separately on Mueller Hinton agar medium. After 24 hours of incubation at 37°C, the lowest concentration of the extract in which bacteria did not grow (99% non-growth) was reported as MBC. The Kirby-Bauer disc diffusion method was also used to measure the antimicrobial effect of the extracts. In the disc diffusion method, 24-hour colonies of cultured bacteria tested in the culture medium were removed by a loop and mixed completely in a sterile test tube containing 5 ml of sterile physiological serum. A uniform suspension of the test bacteria was prepared similar to the turbidity of the McFarland half standard tube. Then, they were cultured using a swab on Muller Hinton Agar media. In the next step, 50 microliters of extracts dissolved in DMSO were added on sterile blank discs. After 2 hours, when the extracts were absorbed by the paper discs, the discs were placed on the plate at appropriate intervals, and the samples were incubated at 37°C for 18 hours. The inhibition zones were then evaluated and calculated based on millimeters. Experiments were repeated twice [1, 12].

Statistical Analysis

The collected data were analyzed with SPSS statistical software using one-way analysis of variance (ANOVA) along with Tukey's test.

RESULTS

The data collected from chicken farms infected with general bacillus disease involved 150 infected chickens, among which wild strains of E. coli were isolated. The data obtained from this method were collected, coded, and entered into the computer and analyzed using SPSS software in two sections: descriptive and inferential. Frequency distribution tables, graphs, and statistical indices such as mean, mode, median of variance, percentage of frequency, and standard deviation were used to describe the obtained results. Inferential statistics of the data collected with SPSS statistical software and one-way analysis of variance (ANOVA) along with the Tukey test were used for analysis. Accordingly, comparative results of this research are presented in Tables 1-8 and Figures 1-3.

Table 1: Distribution of the sample population based on the wild strain of *E. coli*

Type of wild strain	Frequency	percent
wild strain *E. coli*	115	76.7
Other strains	35	23.3
Total	150	100

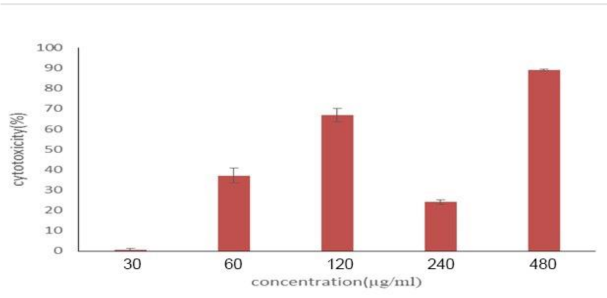

Figure 1: Sample population distribution based on wild strains of *E. coli*. The diagram specifies the dangerous level of wild strains of *Escherichia coli* at different concentrations.

Table 2: Descriptive statistics of the collected data

Variables	Mean	Mode	Median	Variance	Standard deviation
E. coli wild strain	1.23	1	1	0.18	0.42

Table 3: The amount of halo of non-growth of the samples in (mm)

Aqueous extract (%)	*Oregano*	*Oregano* and *Marjoram* (mixed)	Wild *Marjoram*
25	0	0	0
50	11.2	11.4	0
75	15.1	16.3	10.1
100	18.3	19.4	11.5

Table 4: Minimum inhibitory concentration (MIC) and Minimum Bacteriocidal concentration (MBC) mg/ml of samples

Aqueous extract concentration	*Oregano*	*Oregano* and *Marjoram* (mixed)	Wild *Marjoram*
MIC (mg/ml)	20	15	130
MBC (mg/ml)	60	55	220

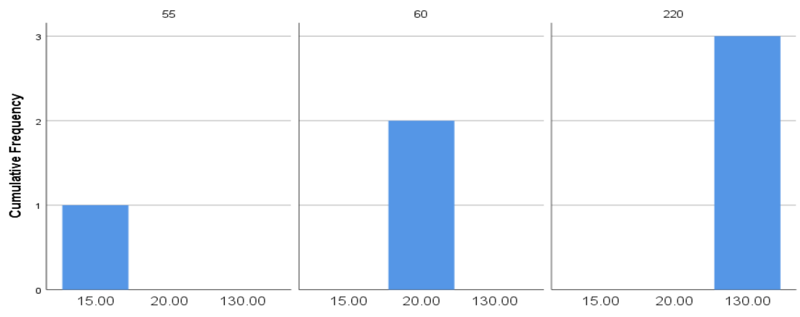

Figure 2: Minimum inhibitory concentration of samples**.** The highest value is for mixed extracts, *Oregano*, and *Marjoram*, respectively.

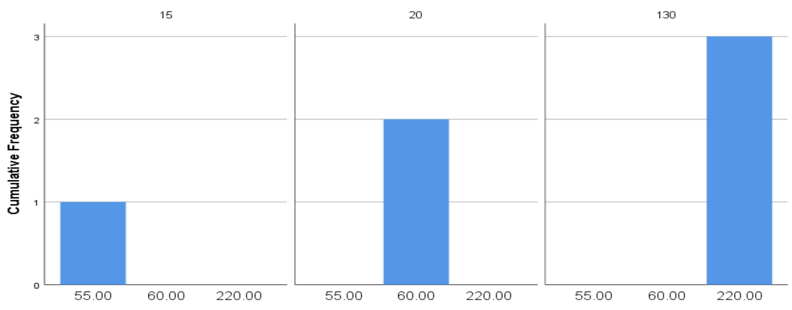

Figure 3: Minimum bactericidal concentration (MBC) of samples (mg/ml). The highest MBC is for mixed extracts, *Oregano*, and *Marjoram*, respectively.

Table 5: Variables of *Marjoram* extract and *E. coli* wild strain at a concentration

Concentration (%)	P value	F	Mean square	Sum of square	Variables
25	0.065	67.654	5.830	5.830	between groups
			0.042	6.176	within groups
			-	12.006	total
50	0.055	61.432	5.290	5.290	between groups
			0.031	5.899	within groups
			-	11.179	total
75	0.052	59.691	5.160	5.160	between groups
			0.029	5.280	within groups
			-	10.440	total
100	0.059	58.891	6.430	6.430	between groups
			0.041	6.120	within groups
			-	12.550	total

Table 6: Variables of *Oregano* extract and wild strain of *E. coli* at a concentration

Concentration (%)	P value	F	Mean square	Sum of square	Variables
25	0.061	60.432	7.231	7.231	between groups
			0.045	6.693	within groups
			-	13.924	total
50	0.053	66.895	7.003	7.003	between groups
			0.041	6.198	within groups
			-	13.201	total
75	0.050	62.472	6.361	6.361	between groups
			0.038	5.921	within groups
			-	12.282	total
100	0.049	62.821	6.630	6.630	between groups
			0.043	6.340	within groups
			-	12840	total

Table 7: Variables of the mixture of extracts (*Marjoram* and *Oregano*) of the wild strain of *E. coli* at a concentration

Concentration (%)	P value	F	Mean square	Sum of square	Variables
25	0.055	58.543	5.010	5.010	between groups
			0.034	5.120	within groups
			-	10.130	total
50	0.050	55.487	4.950	4.950	between groups
			0.033	5.010	within groups
			-	9.990	total
75	0.049	58.654	5.870	5.870	between groups
			0.043	6.350	within groups
			-	12.220	total
100	0.045	59.342	6.980	6.980	between groups
			0.043	6.750	within groups
			-	13.730	total

Table 8: Pearson's correlation coefficient for investigating the relationship between *Oregano*, *Marjoram* and Mixed (*Marjoram* and *Oregano*) plants and wild strain of *E. coli*

Variables / Statistical indicators	Number of items	Average	Standard deviation	Regression	P value
E. coli	115	1.230	0.424	-0.721	0.000
Oregano	10	0.43	0.305		
E. coli	115	1.230	0.424	-0.835	0.000
Marjoram	10	0.210	0.266		
E. coli	115	1.231	0.423	-0.712	0.000
Mixed (marjoram and oregano)	10	0.280	0.224		

$p<0.05$ is significant.

DISCUSSION

Based on the results, 150 infected and suspected chickens with general symptoms of colibacillosis were necropsied. Among them, 115 samples (76.7%) were identified as wild strains of Escherichia coli during isolation using culture medium (EMB) and test (IMViC). Thirty-five samples (23.3%) of chickens had other strains, such as *Salmonella* and others. In Table 3, the microbial discovery test with filter paper soaked in *Oregano* extract, mixed extract (*Oregano* and wild *Marjoram*), and water extract of wild *Marjoram* showed:

- The non-growth halo of *Oregano* aqueous extract was significant at 100% (18.3 mm), 75% (15.1 mm), and 50% (11.2 mm) concentrations and negligible at 25%.

- The minimum growth halo of the mixed aqueous extract (*Oregano* and wild *Marjoram*) was 100% (19.4 mm), 75% (16.3 mm), 50% (11.4 mm), and negligible at 25%.

- The non-growth halo of wild *Marjoram* aqueous extract was 100% (11.5 mm) and 75% (10.1 mm), with very small diameters at 50% and 25% concentrations.

In Table 4, the results of tests (MIC) showed the minimum capacity for aqueous extracts: oregano (20 mg/liter), mixed aqueous extract (*Oregano* and wild *Marjoram*) (15 mg/liter), and marjoram (130 mg/liter). There was a direct relationship with dilution, and as the water extract increased in each dilution, the number of *Escherichia coli* colonies from the culture decreased. The minimum bactericidal concentration (MBC) for *Oregano* aqueous extract was 60 mg/ml, for mixed *Oregano* and *Marjoram* aqueous extract was 55 mg/ml, and for wild *Mrjoram* aqueous extract was 220 mg/ml. The strongest bactericidal effect was related to the mixed aqueous extract (*Oregano* and wild *Marjoram*), followed by the aqueous extract of *Oregano* and then the aqueous extract of wild *Marjoram*.

Table 5 shows that the F value with 1 and 148 degrees of freedom is more than 0.05 ($p \geq 0.05$), so the hypothesis is rejected, and *Marjoram* extract alone is not found to have an antibacterial effect on the strain.

Table 6 shows that the F value with 1 and 148 degrees of freedom is less than 0.05 ($p< 0.05$), so the hypothesis is researched, and *Oregano* extract has an anti-inflammatory effect on the wild strain. *Oregano* extracts (75% and 100%) has more antibacterial properties compared to the aqueous extract of wild *Marjoram*.

Table 7 shows that the degree of freedom of 1 and 148 is less than 0.05 ($p < 0.05$). Therefore, the hypothesis was researched, and the mixed extract (*Oregano* and wild *Marjoram*) has an antibacterial effect on the wild strain of *Escherichia coli*, increasing with its growth. The mixed extract has more antibacterial properties compared to wild *Marjoram* extract alone and *Oregano* extract alone.

In Table 8, the Pearson correlation coefficient shows a strong and negative relationship (-0.721, -0.835, -0.712) between *Oregano*, *Marjoram* and mixed plants (*Marjoram* and *Oregano*), and the wild strain of *Escherichia coli*. The significance level of the obtained correlations is less than 0.05 ($p \leq 0.05$), indicating a significant relationship with 95% confidence, it can be said that there is a relationship between the aforementioned water extracts and the wild strain. The more aqueous extracts used, the lower the amount of *Escherichia coli* wild strain.

Several studies have investigated the effect of volatile oils of *Oregano*, *Mint*, *Tarragon*, *Cumin*, and *Thyme* on *Escherichia coli* and *Staphylococcus aureus* in liquid culture medium [13]. The results of their study, like ours, showed that the volatile oils of *Mint*, *Oregano*, and *Cumin* plants had a moderate effect on the studied bacteria. Another study investigated the combined effect of mint essential oil and *Nisin* on the growth of *Escherichia coli* [14]. In their study, the minimum inhibitory concentration of peppermint essential oil was 40 μg/ml [15]. In our study, the minimum inhibitory concentration of *Oregano* aqueous extract was 20 mg/ml, mixed aqueous extract (*Oregano* and wild *Marjoram*) was 15 mg/ml, and *Marjoram* extract was 130 mg/ml.

Another study investigated the antimicrobial effect of *Marjoram* alcoholic extract on *Escherichia coli* and *Salmonella enterica* using the disc diffusion and tube dilution method. The minimum lethal concentration of ethanolic and methanolic extracts of this plant against *Escherichia coli* was 100 and 120 mg/ml, respectively [16]. In our study, the minimum lethal concentration (MBC) of oregano aqueous extract was 60 mg/ml, mixed aqueous extract was 55 mg/ml, and wild *Marjoram* aqueous extract was 220 mg/ml.

The antibacterial effect of ethanolic and methanolic *Oregano* extracts was also investigated in laboratory conditions. The results showed that both extracts of this plant have a significant antibacterial effect on some Gram-negative bacteria, such as *Escherichia coli*, which was also obtained in our study [17]. The antibacterial properties of *Oregano* essential oil on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* were checked. The results showed that oregano essential oil has an antibacterial effect against these pathogens. In our study, which was conducted on Oregano and *Marjoram* extract, the results were favorable [18].

CONCLUSION

The obtained results showed that the aqueous extract of *Oregano* and the mixed extract (*Oregano* and wild *Marjoram*) had better antibacterial properties than the aqueous extract of *Marjoram*. Aqueous extracts of plants can be used as a cheap, good, and available source for therapeutic purposes and even prevention of bacterial infections. They are a suitable alternative to antibiotics and have a good complementary effect. In this research, the wild strain of *Escherichia coli* bacteria was tested in four concentrations of 25, 50, 75, and 100%, and in each concentration, it was investigated with water extract of *Marjoram*, wild *Oregano* extract, and mixed extract (*Marjoram* and *Oregano*). They have a positive antibacterial effect, and their effectiveness increases as the concentration of the extracts increases.

ETHICS

Approved.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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