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**ORIGINAL ARTICLE**

# Evaluation of the Bactericidal Amount of Alcoholic Extract of Marjoram on *Staphylococcus Aureus, E.coli, Salmonella enterica* in Chicken Batter Used in Meat Products

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**ABSTRACT**
*Herbs enjoy a unique value and importance in sustaining healthy communities in terms of disease prevention. In this regard, Marjoram is a plant of the mint family which has antibacterial properties on microorganisms. The current study aims to investigate the anti-microbial activity of the alcohol extracts (i.e., methanol or ethanol) of Marjoram plants on the bacteria of Staphylococcus (atcc: 25923), aureus E.coli (atcc: 25922), and Salmonella enterica (atcc: 13076) through utilizing disk diffusion method. Also, the minimum inhibitory concentration and the minimum bactericidal concentration were measured through tube. The measurement of minimum inhibitory concentration and minimum bactericidal concentration of ethanol and methanol extracts on E.coli were equal with 100 and 120 milligrams per milliliter, subsequently. Moreover, the measurement of the minimum inhibitory concentration and of the minimum inhibitory concentration of marjoram ethanol extraction on Staphylococcus aurous was reported to be 90 milligrams and 100 milligrams per milliliter, subsequently. In addition, the amount of ethanol and methanol extracts on Salmonella enterica was equal with 80 and 90 milligrams per milliliter, subsequently. The results showed that Marjoram alcoholic extract enjoy antibacterial properties. Also, among the alcoholic extracts, the ethanol extract has demonstrated to be the most effective extract on Salmonella enterica and E.coli.*
*Keywords: Marjoram, diffusion disk, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)*

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## INTRODUCTION

The most basic task of food is maintaining bodily health and well-being. In recent years, a plethora of studies have been carried out on the relationship between various diseases and food [1, 2]. In today's world, industrial societies increasingly are interested in fast foods such as sausages and hamburgers. These kinds of foods are prepared in many ways; however, in Iran and some of the developing countries, these foods are prepared through utilizing chicken batter [2, 3]. After the slaughtering chickens, slaughterhouses send the chickens to packing units where the chickens' skeletons remain after packing. In the past times, these bones were thrown away and were regarded as useless; nonetheless, the meat sticking to these skeletons are removed through pressure by a machine called Batter, nowadays. This machine emits both bone batter and chicken batter. However, there is an easier way to do such a task through which one can slice the chicken skeleton into the smallest pieces possible and obtain chicken batter. Since these skeletons are collected from different areas such as restaurants, canteens, and illegal slaughterhouses which are unsanitary and are transferred in trucks with no covering or in dirty sacks to factories, this product is prone to be polluted with different bacteria such as *Staphylococcus aureus E. coli*, and Salmonella enteric. These bacteria are naturally found in intestine; however, when they are in contact with outer tissues of bile and urogenital systems, they cause different diseases (1, 4, 5). The most common type of food poisoning is caused by Staphylococcal Enterotoxins. Staphylococci quickly develop resistance to many antibiotics and cause many health problems (1, 4). When Salmonella enters one's body through mouth, the way is paved for different types of diseases to enter both humans' and animals' bodies also, it causes enteritis, fever, intestinal and systemic infections [1, 4, 6, and 7]. Therefore, this product is very

difficult to judge since the earth's population is increasingly growing and people need more protein. Health authorities have always paid their utmost attention to these issues.

Based on the statistics released from world health ministry, more that 80 percent of the world population in developing countries treat themselves by utilizing herbs. The side effects of using chemical medicines lead to dangerous outcomes for patients during their treatment. Hence, herbs have become so prominent that that have a wide range of applicability. Besides, the existence of active biologic materials in herbs has caused researchers to use chemicals for their extraction [8]. Marjoram with the scientific name of Origanum Majorana from the Lamiaceae family was originally grown in the Mediterranean areas. This plant needs 25 degrees centigrade, lots of sunlight, and sandy soils contain with lime combinations of 4.5 pH to 78.7 pH [9].

The present study aims to investigate the amount of chicken batter pollution for preparing sausages and hamburgers.

**Picture 1: Sweet Marjoram**

## MATERIALS AND METHODS

Marjoram plant was collected from the Middle Alborz Mountains in the region of Baladeh Noor by an herbalist who tried to pick up healthy and fresh Marjoram without any damage. Then, the plant was dried during a period of five days and was chopped. Next, for producing methanol extract, an amount of 100 grams of the dried plant was soaked with 700 ml of 80 percent methanol. This soaked plant was circulated with 130 rpm it on a shaker for 48 hours and the whole extract was out. Afterwards, the solvent plant was passed through filter paper for the extract to be clear. Next, the extract was concentrated using the rotary at 40 ° to 50 ° centigrade for 2 days and was freeze in order to obtain pure extract without water. Also, this preparation process was carried out for obtaining 80 percent ethanol extract.

For identifying the constituents of the extract, the researchers transferred the extract to Liquid Chromatography Apparatus. Then, they made the extract concentrations of 10, 30, 40, 50, 70, 80, 90, 100, 120, and 150 milligrams per milliliter. Afterwards, the micro-organisms which were provided and lipo-freezed in the Pasteur Institute of Iran were revived and mixed with half a CC of BHI solvent to detect the kind and level of growth of the micro-organism. Then, the researchers removed the surface of the colonies and mixed it with the normal solvent of Saline Sterile to make it clear. Next, it was remained in the incubator for 24 hours with 37° centigrade to provide bacterial suspension containing CFU/mLat. Also, the suspension level of 530 nm wavelength with the absorption McFarland solvent amount of .5 will be compared.

**Picture 2: Rotary Apparatus**

**Picture 3: Marjoram Extract**

**Table 1: The Constituents in Ethanol Extract of Marjoram by Liquid Chromatography Method**

| No | Phenolic Compound | Alcoholic extract | No | Phenolic Compound | Alcoholic extract |
|---|---|---|---|---|---|
| 1 | Salicylic | 66.5 | 10 | Catechol | 23.86 |
| 2 | P-Oh Benzoic | - | 11 | P-coumaric | 3.82 |
| 3 | Caffeine | 2.49 | 12 | Gallic | 0.060 |
| 4 | Cholchecien | 11.37 | 13 | Pyrogallol | 43.24 |
| 5 | Vanillic | 16.94 | 14 | Chlorogenic | 3.18 |
| 6 | Coumarin | 19.83 | | | |
| 7 | Ferulic | 21.79 | | | |
| 8 | Ellagic | 157.98 | | | |
| 9 | Protocatechouic | 9.25 | | | |

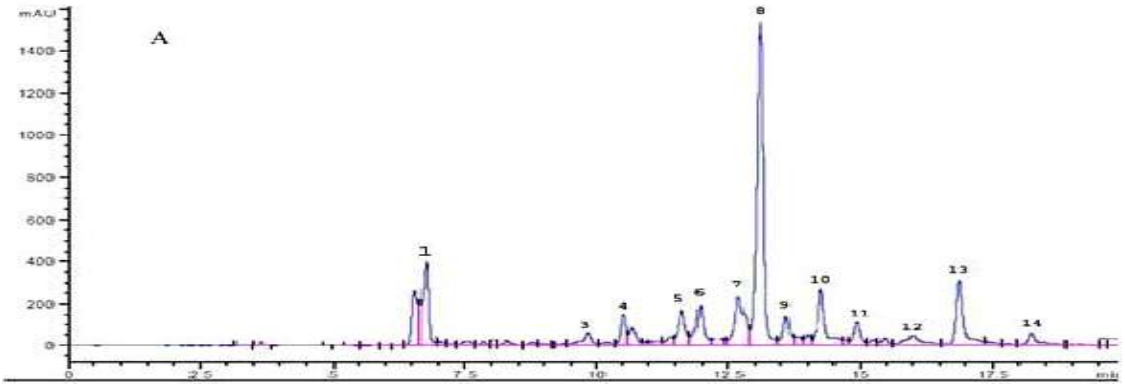



**Figure 4: The graph of Liquid Chromatography of Marjoram**

To study the anti-microbial activity, two methods of disk diffusion and tube dilution were used: 1). The disk diffusion method: 10 micro-liter microorganisms were removed and were poured on Mueller Hinton Agar; then, using a sterile swab, grass farming was applied. Afterwards, 10 micro-liters of ethanol and methanol extracts in different and separated concentrations were poured onto the blank disks and were put in the incubator with 37° centigrade for 18-24 hours. Then, the non-growth materials will be measured using Caulis. a caliper of the channel. Also, a 10-microgramant Biotic Gentamicin disk was utilized for positive control. Also, a disk having a 10-microgram 100 percent DMSO solvent was used for negative control in all the stages of experiments.

**Picture 5: Disk Diffusion**

**Tube Dilution Method**

First, one milliliter of Molar Hinton Bras was poured in the tubes; then, they were autoclaved and .1 microgram of alcoholic extracts with different concentrations were added to them. Afterwards, they were put in the incubator for 24 hours in 37 degrees centigrade to compare the tubes with reference to being murky. For determining the least amount of inhibition and bactericidal concentration, the lowest murky tubes with the least concentration was determined for the least inhibition concentration.

For identifying the least bactericidal concentration, the tubes with no murky environment was used for Agar Nutrient Environment to be farmed. Also, the Pilates were put in 37 °C for 24 hours. The

concentration with the number of colonies of 9.99 was determined for the minimum bactericidal concentration.

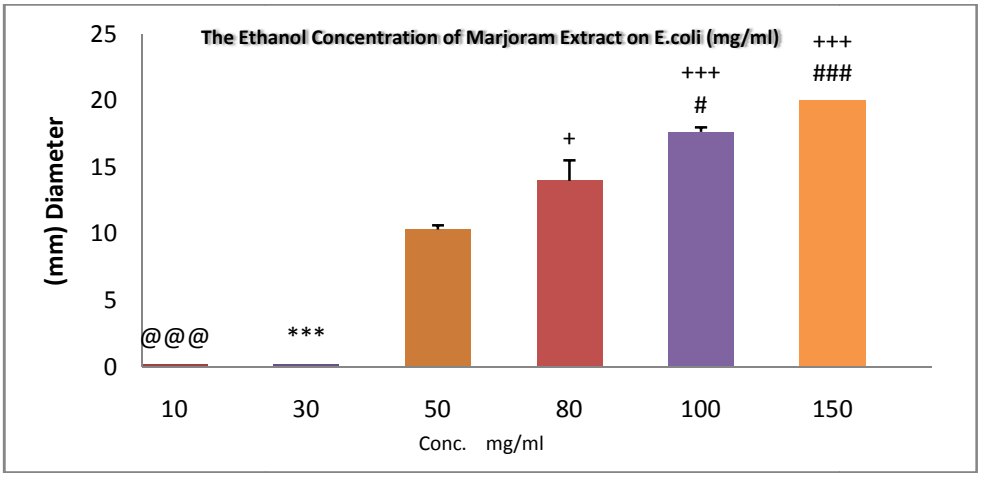
**Picture 6: Tube Dilution Method**

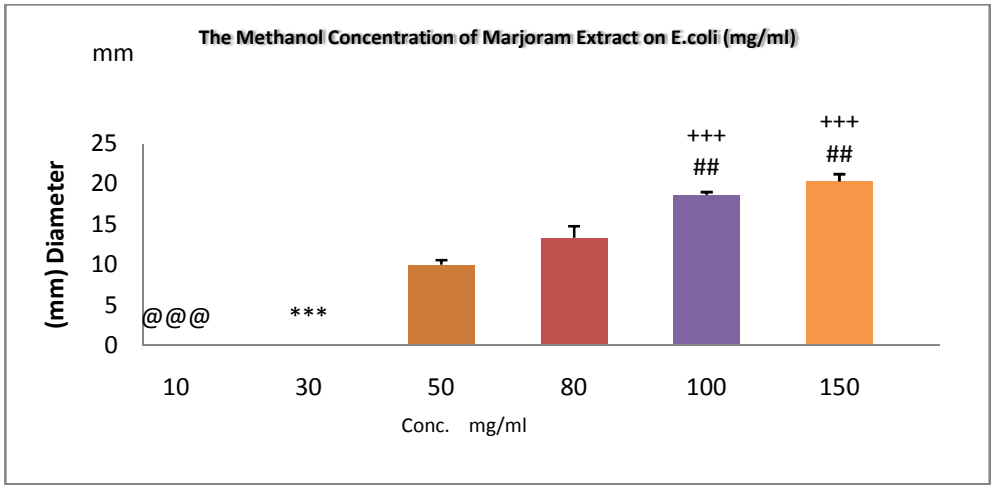
**RESULTS**

Among the alcoholic extracts, ethanol extract was the most effective. Also, it had the most significant impact on the microorganisms of *Salmonella enterica* and E.coli so that they showed the highest resistance. Besides, in terms of measurement, they were equal regarding the least amount of inhibition and bactericidal concentration of ethanol and methanol extracts and were reported to be 100 and 120 mg/l, respectively. Also, for the measurement of the minimum inhibitory concentration, the Marjoram's minimum inhibitory concentration based on the ethanol extract of this plant were counted to be 90 mg/ml and the minimum amount of bactericidal concentration was reported to be 100 mg/ml. In addition, for the methanol extract, the minimum inhibitory concentration and the minimum amount of bactericidal concentration were 100 and 120 mg/l and this amount for ethanol and methanol extracts on Salmonella enterica were equal and were reported to be 80 and 90 mg/ml, respectively. Moreover, the alcoholic extract of Marjoram has a significant effect on Salmonella; nevertheless, it had a non-significant impact on Staphylococcus.

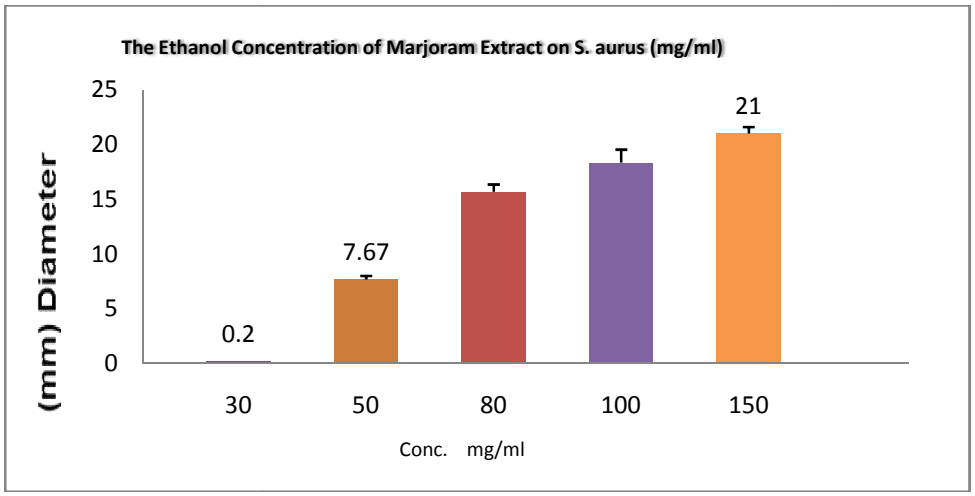
The graph of ethanol and methanol extracts of Marjoram indicates that they have significant effect on E. coli @@@p≤0.001 or other groups except dose 30, *** p≤0.001 or other groups except dose 10, + p≤0.05, +++ p≤0.001, or dose of 50, # p≤0.05, ## p≤0.005, ### p≤0.001, or dose of 80. Also, the graph of ethanol and methanol extracts of Marjoram indicates that effect of ethanol and methanol extracts are significantly related to S. aureus @p≤0.001, or other groups except dose 30, *** p≤0.001, or other groups except dose 10, ## p≤0.005, dose of 80, ### p≤0.001, groups which share similar methanol % p≤0.05, or groups with dose 80 (i.e., p≤0.05 with methanol 80). Also, the graph of ethanol and methanol extracts of Salmonella illustrates that @p ≤0.001, or other groups except dose 30, *** p≤0.001 or other groups except dose 10, &&& p≤0.001, or other groups, # p≤0.05, ### p≤0.001 dose of 80, %%% p≤0.001 with the same methanol groups, p≤0.001 with groups of 80, 100, and 150.

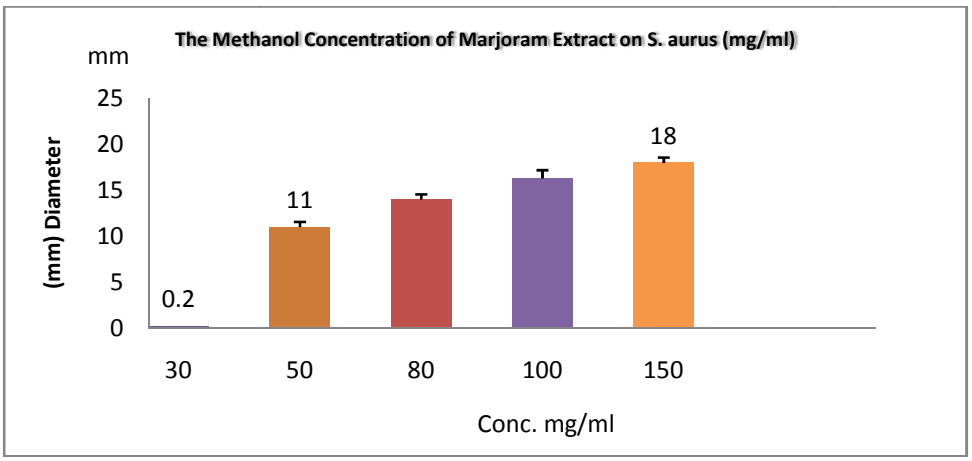
**Graph 7 -SPSS statistical analysis for Ethanol and Methanol Extracts of Marjoram on E.coli**



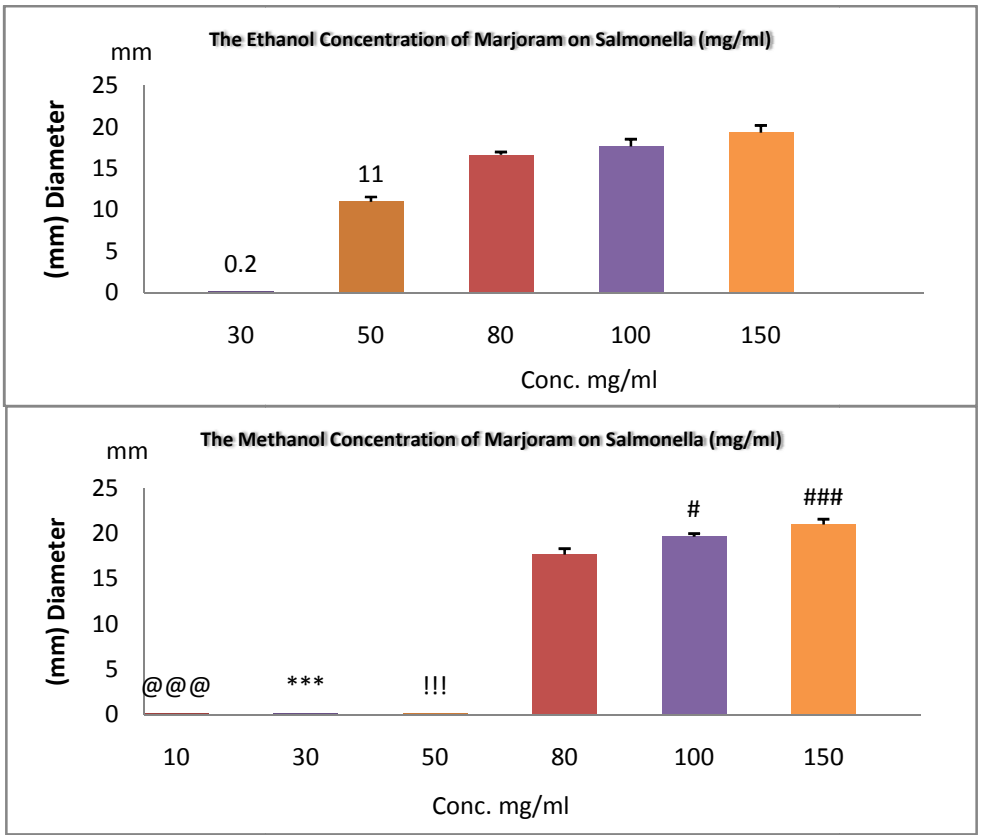



**Graph 8: SPSS statistical analysis for Ethanol and Methanol Extracts of Marjoram on S. aureus**



**Graph 9: SPSS statistical analysis for Ethanol and Methanol Extracts of Marjoram on Salmonella entrica**




**DISCUSSION**

The results of the present study showed that the alcoholic extract of Marjoram enjoys a high potential to be regarded as a substitution for industrial materials. In this regard, the quality of our production and health will be improved. Sherafat *et al.* [10] carried out their study on the antioxidant properties of ethanol extracts of *Thymus vulgaris* and investigated its antimicrobial effect on *Staphylococcus aureus*. They concluded that *Thymus vulgaris* extract significantly resulted in hindering the growth of S. aureusregarding hamburger in refrigerated conditions, which is in line with the results of the current study. Also, Haddad Khodaparast *et al.* [11] investigated the antimicrobial effect of Nowrook leaf extract powder against Staphylococcus aureus in hamburger and concluded that at the high concentration above 20,000 milligrams per liter, this powder reduces the microbial load.

Furthermore, Mahboubi *et al.* [12] investigated the antimicrobial effect of thyme, marjoram, Savory, and Eucalyptus on *Escherichia coli, Salmonella typhimurium, Aspergillus fungus*, and *Aspergillus flavus*. They found that the effect of ethanol extracts of thyme and Marjoram enjoyed a greater impact on the bacteria than the fungi which is in line with the results of current research. In another study, Alvandi *et al.* [13] carried out their research on the chemical composition and antimicrobial effect of peppermint on Escherichia coli and Staphylococcus aureus and Salmonella typhimurium Mornium and concluded that the minimum level of essence deterrence on *Staphylococcus aureus*, E. coli, and Salmonella typhi Mornium reported to be 150, 300, and 250 ppm, respectively, which is consistent with the results of this research. In another study by Jodi *et al* [14] on anti-microbial properties and chemical composition of Marjoram, oregano, and peppermint extracts on the bacteria of Staphylococcus aureus, E.coli, and the yeast of *Candida albicans*, she found that the Ethanol extract of Marjoram had the most significant effect on Staphylococcus and enjoyed the highest level of resistance to E. coli. Khosro Mohammadi *et al.* [15] investigated the effect of Thyme essence on *E. coli* bacterium in white cheese in salty water during the production and maintenance. They concluded that this plant which is from the Mint family has a higher concentration than 200ppm with antibacterial properties. The reason for this change may be due to the variability in the type of plant; nevertheless, they are related to the same family. Hence, in the same concentration, they show antibacterial effect. Moreover, Olaee *et al.* [16] carried out their research on the antimicrobial effect of ethanol extract of *Thymus vulgaris* on standard *Staphylococcus aureus*; also, they

investigated the changes in the number of bacteria within 8 hours of exposure to concentrations of 25 and 30 percent of the extract and 4 Mg/ml of Oxacillin. The results of their experiments demonstrated that the minimal inhibitory concentration (MIC) and minimum bactericidal concentration of the extract equaled to the concentration of 25 percent. In addition, the minimum inhibitory concentration and minimum bactericidal concentration for the extract of Oxacillin were also 0.5 mg/ml and 2 mg/ml, respectively [17]. Nonetheless, regarding the current study, the reason behind the change in the minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the extract is due to the duration and type of plant.

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