

## The Inoculation Effect of *Arum conophalloides* on *Salmonella typhimurium* Bacteria Using an Antibacterial Approach at Different Temperatures, Time Intervals, and Extract Concentrations

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Received: 21 August 2016

Accepted: 21 January 2017

**ABSTRACT:** This study examines the antibacterial effects of *Arum conophalloides* on *S. typhimurium* bacteria at different temperatures ( $-18^{\circ}$ ,  $8^{\circ}$ ,  $16^{\circ}$ ,  $24^{\circ}$ , and  $32^{\circ}\text{C}$ ) and time intervals (1, 2, 3, 6, 12, 24, and 48 days). This local plant was studied to determine the greatest effect caused by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) as well as the antimicrobial effects of the extract. The *Arum conophalloides* essence was extracted via distillation or vapor using Clevenger hydro-distillation, using alcohol, chloroform, and water to extract the essence from Arum plant leaves (leading to three different extracts for comparison). In order to determine whether the extract used had minimum inhibitory and bactericidal effects, a microbial test method (isolate; 4, disk diffusion) was used in which two given dilutions (17,000 ppm and 35,000 ppm) were obtained and one of the two groups (dilutions) was selected as the control. Three groups of MIC and MBC and a control group were evaluated on plate-count agar using pour-plate technique at the designated temperatures and time intervals. Total numbers of bacterial colonies were counted. The resulting data were statistically analyzed with SPSS, with  $p < 0.05$  indicating the statistical significance. The results of the Kruskal-Wallis were only statistically meaningful for 35,000 ppm dilution; as with the 17,000 ppm dilution in the assumption of sphericity test, the difference in the total number of colonies was only meaningful at  $18^{\circ}\text{C}$  and on days 12 and 48, which had the lowest number of colonies ( $p = 0.005$ ). However, at 35,000 ppm, the assumption of sphericity test indicated strongly the significant differences in the total number of colonies at  $-18^{\circ}\text{C}$  at days 12, 24, and 48 ( $p = 0.000$ ) and at  $16^{\circ}\text{C}$  on day 48 ( $p = 0.013$ ) due to the absence of colonies at these temperatures and time points. The alcohol and chloroform extracts of different parts of the plant did not yield significant results. It might be concluded that the extracts obtained in this study have a relatively low effect concerned with MBC on *S. typhimurium* bacteria and based on our result, a connection can be made between *Arum conophalloides* extraction method and MIC, MBC.

**Keywords:** Antibacterial Properties, Aqueous Extract, *Arum conophalloides*, *Salmonella typhimurium*.

### Introduction

*Salmonella* bacteria are members of the Enterobacteriaceae family, which consists of bacteria that are Gram-negative and have peritrichous flagella. These bacteria are

transmitted to people by animals and animal products and causes typhoid fever, septicemia, and enterocolitis (Bulanov *et al.*, 1990; Kay & Palmer, 1985).

Presently, the food industry uses chemicals and synthetic materials to prevent the growth of microorganisms in food

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products. Chemical preservatives have been shown to have numerous harmful effects on human health, in that they contain carcinogens and have teratogenic and other toxic properties. Since, demands for food products with improved shelf life have been increased, the manufacturers have decided to replace the chemical preservatives with the natural additives (Candido *et al.*, 2014; Das *et al.*, 2005).

Food preservatives are additives that prevent food from spoilage (Bonora *et al.*, 2000; Bulanov *et al.*, 1990). One plant that currently is being examined for extracts that have preservative properties is the *Arum conophalloides* plant. This plant is common in the Kermanshah province, west part of Iran. The plant's lamina is 15 to 20 in. wide and long and triangular in shape, with sharp edges, corners, and sides. The ladle inflorescences of *Arum conophalloides* are 18-32 cm in diameter, with a green color and red margins, and the plant's oval-shaped petals are wide and long with spear-headed lamina. The style and sigma (spadix) form a gray purple wand shape that is a little shorter than the spathe. The receptacle of the female plant is two times longer than the male plant, with thin and narrow stamens (Koga *et al.*, 1976).

Yari (2012) investigated the antioxidant, antibacterial, and toxic properties of wild *Arum maculatum* on model human cells, with positive results. The evaluation of the antioxidant properties of *Arum maculatum* in roots and leaves revealed the total amounts (measured in mg/g of solid material) of phenol equaling 24.74 mg to 60.25 mg of gallic acid, and of flavonoid equaling 27.85 mg to 40.28 mg of quercetin, respectively. Spectrophotometry was used to measure the activity of the superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POX) enzymes. The activity of the CAT enzyme was  $45.19 \pm 82.0$ , and of POX was  $12 \pm 6.0$ .

Mohammadzadeh *et al.* (2014) investigated the antibacterial effect of *Cardoria draba* on pathogenic bacteria transmitted from food (e.g., on *S. typhimurium*) and on other types of bacteria. In this study, the results from one-way analysis of variance (ANOVA) and Duncan's multiple range test showed that the MIC of the *Cardoria draba* extract was 2.5 mg/ml and the MBC was 40 mg/ml for *Staphylococcus aureus* after the use of prepared concentrations from the extract of the plant. No inhibitory or bactericidal effect was observed on *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, or *Bacillus cereus*. Further research should be performed using different extracts from this plant.

Shakeri *et al.* (2013) modeled the growth of *Salmonella typhimurium* bacteria at different concentrations of *Carum copticum* and at different incubation temperatures, pH values, and inoculation dosages. For this study, 48 samples with different states of bacterial growth were designed, including four concentrations of *Carum copticum* extract (0.06%, 0.03%, 0.015% and 0%) at two incubation temperatures (25° and 35°C), three medium pH values (5, 6 and 7), and two inoculation dosages (10<sup>5</sup>-10<sup>3</sup> ml/cfu) based on bacterial growth during the 30-day period. During the research, the extract was analyzed using a gas chromatography/mass spectrometry (GC-Mass) device using parametric survival model log-normal distribution, then placed in a brain-heart infusion (BHI) culture medium to investigate the individual effects of the independent variables listed on the time to start of bacterial growth (determined by visible turbidity of the medium). The results demonstrated a meaningful effect ( $p < 0.05$ ) for each of these variables on growth of *S. typhimurium*. Generally, the results show that the log-normal model is suitable for estimating bacterial growth in different

medium conditions.

Mardanighahfarakhi *et al.* (2013) assessed the antioxidant and antimicrobial activity of phenol extract of *Oenothera biennis* L. For this assessment, testing the consisted of DPPH free radical scavenging activity and Fe<sup>3+</sup> reduction potential, and the results were compared to those obtained using butylated hydroxytoluene (BHT), the synthetic antioxidant. The phenol extracts showed significant antimicrobial activity for all the investigated bacteria with higher effects of the extracts on Gram-positive than Gram-negative bacteria. *S. typhimurium* showed the highest resistance among all the bacteria to acetone and ethanol extracts. The concentrations of MBC in acetone and ethanol extracts for *S. typhimurium* were equal to 10 and 20 µg/ml, respectively.

Jouda *et al.* (2013) assessed the antibacterial effects of aqueous, ethanol, and methanol extracts of the herbal plants *Eucalyptus camaldulensis* and *Ficus sycomorus* and their synergistic effects with antibiotic and non-antibiotic medicines against *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*. The results showed that the antibacterial and synergistic effects in most medicines against the three bacteria are higher in ethanol extracts as compared with the methanol and aqueous extracts. The synergistic effects of aqueous extracts with paracetamol and loperamide HCl against *E. coli* and *S. aureus* are higher than for ethanol and methanol extracts; however, the synergistic effects of ethanol extract with these two medicines against *P. aeruginosa* are higher than with methanol and aqueous extracts. This study demonstrated a decreasing MIC for methanol extracts in *Eucalyptus camaldulensis* against *E. coli* (3.125 mg/ml), methanol and aqueous extracts in *Ficus sycomorus* against *S. aureus* (6.25 to 3.125 mg/ml), and ethanol extracts in *E. camaldulensis* against *P. aeruginosa* (6.25 mg/ml). These extracts

could eventually be used to treat bacterial infections.

Husein *et al.* (2014) investigated the antimicrobial effects of the ethanol extracts of six herbal plants traditionally local to Palestine. These extracts included *Arum Palaestinum* Bioss, *Urtica pilulifera* L., *Thymbra capitata* (L.), *Origanum syriacum* L., *Teucrium creticum* L., and *Teucrium polium* L., which were tested against six bacteria: *S. aureus*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhimurium*, *E. coli*, *K. pneumoniae*. Additionally, they were tested against 5 types of *Candida*, and two dermatophytes, *microsporum canis* and *microsporum rubrum*. The results indicated that *T. capitata* and *O. syriacum* extracts had the highest activity against bacteria, while the other herbal extracts didn't demonstrate high activity.

Uzun *et al.* (2004) studied the antimicrobial activity of traditional medicines in the Sakarya Province (in the northwest of Turkey) for up to 2 months, with information gathered from 46 plant species from 30 families and 5 animal species. The plant families studied included Asteraceae, Cucurbitaceae, Lamiaceae, Rosaceae; the plants generally used included *Artemisia absinthium*, *Equisetum telmateia*, *Lavandula stoechas*, *Melissa officinalis*, *Tussilago farfara*, and *Urtica dioica*. The extracts tested were assessed for antimicrobial activity against *S. aureus* (ATCC 65538), *Staphylococcus epidermidis* (ATCC 12228), *E. coli* (ATCC 8739), *K. pneumoniae* (ATCC 4352), *P. aeruginosa* (ATCC 1549), *S. typhimurium*, *Proteus mirabilis*, and *Candida albicans* (ATCC 10231). The results indicated that the extracts of *Arum maculatum*, *Datura stramonium*, *Geranium asphodeloides*, and *Equisetum telmateia* petroleum had antimicrobial activity levels against *S. epidermidis* with MIC equal to 39.1, 78.1, 78.1, and 39.1 µg/ml, respectively, as did the

*Datura stramonium* petroleum extract, with 39.1 µg/ml MIC against *E. coli* and ethanol extract of *Trachystemon orientalis*, with 39.1 µg/ml MIC against *E. coli* (Uzun et al., 2004).

### Materials and Methods

In this study, *S. typhimurium* was purchased from the Iranian Industrial Microorganisms Collection Center (Persian Type Culture Collection) with the code of 1609. The required plant was collected, cleaned, and dried in the shade and then (after being powdered in the grinder) put in opaque glasses and kept in a dry place until required. For extraction, 50 grams of *Arum conophalloides* powder were mixed with sterile distilled water at a proportion of ¼ at room temperature and in a relatively dark place, then kept in the laboratory at room temperature for 42 hours. The mixture was stirred every 1 hour with a glass rod for the effective materials to be properly released in water, followed by placement in an ultrasonic device for 10 minutes for the process to be completed. Eventually, using 2 layers of sterile gauze and a funnel or Whatman No. 1 filter paper, the extract was separated from the residuum and put in a freeze dryer to separate water from pure extract.

A disk diffusion operation was performed to achieve the highest effect to determine MIC, MBC and the antimicrobial effects of alcohol, chloroform, and aqueous extracts from the plant leaves. Significant outcome was not observed in other cases. Disk diffusion was performed in several steps. First, an isolated bacterial colony was selected from a platinum loop and dissolved in sterile saline. The next step was to prepare and transfer the colony to an MHA culture medium so the streak could be completely cultured using a swab with transfer of the antibiogram disks taken out of the refrigerator half an hour before the test. For the culture medium, the disks must be placed

in circles, followed by closing of the plate lid. Incubation occurred at 20°C for 42 hr, and the diameter of the halo zones was obtained under the light.

In this method, 0.5 McFarland Standard bacteria is treated using a SPEC method with a 600-nm wave length. *S. typhimurium* is cultured in the medium for one day, then incubated at 37°C for 24 hours. After the response from the device becomes zero at a 600-nm wavelength, the colony and the sterilized physiology serum are placed in the device, with the absorption level becoming 1-0.8 wavelength equal to 0.5 McFarland. Eventually, three groups are formed using the designed models. The first group, MIC with 17,000 ppm dilution including 0.5 ml extract of *Arum conophalloides* plus 4.5 ml NB culture medium are mixed then, 5 ml of 0.5 McFarland Standard bacteria is added to it. The second group, MBC with 35,000 ppm dilution and 1-ml extract plus 4-ml NB culture medium, are then added to 5 ml of 0.5 McFarland Standard bacteria during the second stage of the test. The control group is set during the final step by including 4-ml NB culture medium with 5 ml of 0.5 McFarland Standard bacteria without the addition of extract. Note that one container including 50 ml of this solution must be prepared for each temperature therefore each removal is performed solely from one container and at the same conditions with respect to temperature and place. After these calculations, a pour-plate culture method is used for the test. After preparation of the 17,000 ppm and 35,000 ppm mixtures, 1 ml from the 35,000 ppm mixture is prepared in a container and poured into the first sterile test tube, after which 9 ml of physiologic serum is added. The test tube is shaken well. After preparation of this dilution, other tubes are prepared using the pour-plate serial dilution method (a total of 6 to 7 tubes). The sample is diluted using a sampler and a 1-ml sampler head by taking 1 ml of sterile solution and adding it to the first tube, which

contains diluents according to what is needed for a 1% concentration. 1-ml of sterile solution is then added to the empty plate using the same sampler from the three previous test tubes. The tube with the 1%-diluted mixture is mixed by filling and emptying. Subsequently, using another 1-ml sampler, another 1-ml is added to the tube containing diluent liquids of 0.001 concentration (that is, one-tenth of 1%, using the terminology from earlier) and the tubes continue to be diluted. The method is repeated with 17,000 ppm and control test tubes, with two plates being repeated for each dilution. Finally, the tubes are cultured on another medium. Three sterile plates are determined for each dilution and 1-ml from the three last tubes is taken and added to the related culture medium plates. 15-ml PCA culture medium is added to each plate; each medium must reach a temperature of 45°C to 50°C. All of these stages are executed for each group (MIC, MBC, control) at temperatures of -18°, 8°, 16°, 24°, and 32°C on days 1, 2, 3, 6, 12, 24, and 48.

### Results and Discussion

This research has been influenced by the importance of herbal extract and its antibacterial effect on *S. typhimurium*. After obtaining the concentrations of different culture mediums, bacteria were added and the total numbers of microorganism colonies (including *S. typhimurium*) were counted for all the temperatures on days 1, 2, 3, 6, 12, 24, 48. The logarithms of the number of colonies were calculated and applied in statistical analysis and chart generation. According to the statistical analysis of the recorded results, the numbers of colonies

were investigated on different days in the control and treatment groups of different temperatures. The results of the Kruskal-Wallis test were only significant for the 35,000 ppm dilution at all the five temperatures. The total decline in the number of colonies at the assumption of sphericity test performed in the 17,000 ppm dilution was only significant at -18°C on days 12 and 48, on which the lowest number of colonies was present ( $p=0.005$ ), while for the 35,000 ppm dilution, this test revealed a meaningful decline in colonies on more days (days 12, 24, and 48) and at more temperatures (-18°C for all three days,  $p<0.001$ , indicating a high level of statistical significance; 16°C on day 48,  $p=0.013$ ). The extracts developed using alcohol and chloroform for different parts of the *Arum conophalloides* plant did not yield any significant impact on bacterial colony growth. The results indicated that the extracts have a weak, low bactericidal effect on *S. typhimurium* bacteria. Interestingly, the related generated charts show an exceptional rise in bacterial colony count in the 17,000 ppm dilution at 24°C for half of the 6 days (on days 1, 2, and 24), while a decline is shown during the other three days and in the 35,000 ppm dilution chart. This proves that the extracts have weak antibacterial properties, and suggests that the lack of a very high bactericidal effect from a plant extract in a broader sense might be rooted in the conditions and method of extraction. The tested extract, however, likely has the advantage of being non-toxic to humans (see Table 1-5). Figure 1 to 6 show changes in the total numbers of colonies at different temperatures and concentrations.

**Table 1.** Average numbers of bacteria during storage at -18 °C

-18 Celsius Degrees Temperature							
Day	1	2	3	6	12	24	48
Control	76.6666	124.5000	24.0000	46.3333	22.3333	10.6666	1.6666
17000 ppm Concentration	118.3333	65.0000	19.6667	23.3333	0.6667	10.6667	0.6667
35000 ppm Concentration	129.3333	26.0000	2.0000	0.3333	0.0000	0.0000	0.0000

**Table 2.** Average numbers of bacteria during storage at +8 °C

+8 Celsius Degrees Temperature							
Day	1	2	3	6	12	24	48
Control	131.0000	90.0000	51.0000	103.2000	127.0000	178.6666	104.0000
17000 ppm Concentration	179.0000	70.0000	83.0000	88.0000	57.0000	196.0000	117.0000
35000 ppm Concentration	193.5000	65.0000	51.5000	71.5000	79.5000	185.0000	7.5000

**Table 3.** Average numbers of bacteria during storage at +16 °C

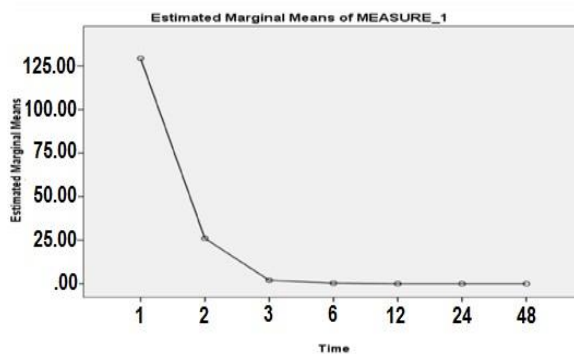
+16 Celsius Degrees Temperature							
Day	1	2	3	6	12	24	48
Control	148.0000	67.5000	73.0000	174.0000	83.5000	113.0000	104.5000
17000 ppm Concentration	219.5000	63.5000	53.0000	142.5000	105.0000	196.5000	124.0000
35000 ppm Concentration	217.5000	71.0000	45.5000	98.0000	25.0000	14.0000	0.0000

**Table 4.** Average numbers of bacteria during storage at +24 °C

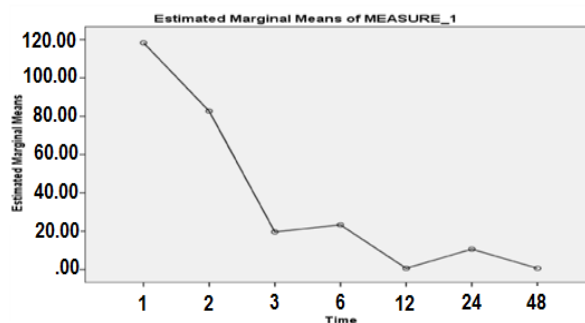
+24 Celsius Degrees Temperature							
Day	1	2	3	6	12	24	48
Control	82.0000	73.3333	43.0000	82.0000	87.3333	51.6666	45.6666
17000 ppm Concentration	38.6667	56.0000	41.6667	82.3333	83.3333	128.0000	120.0000
35000 ppm Concentration	45.6667	80.3333	40.0000	55.3333	54.3333	30.6667	23.6667

**Table 5.** Average numbers of bacteria during storage at +32 °C

+32 Celsius Degrees Temperature							
Day	1	2	3	6	12	24	48
Control	141.0000	41.5000	31.0000	58.5000	48.3333	112.6666	86.6666
17000 ppm Concentration	176.5000	46.5000	35.0000	63.0000	71.5000	57.5000	42.5000
35000 ppm Concentration	201.0000	36.5000	29.5000	19.5000	22.0000	185.5000	54.0000



**Fig. 1.** Changes in the total number of colonies with 17,000 ppm concentration (-18°C)



**Fig. 2.** Changes in the total number of colonies with 35,000 ppm concentration (-18°C)

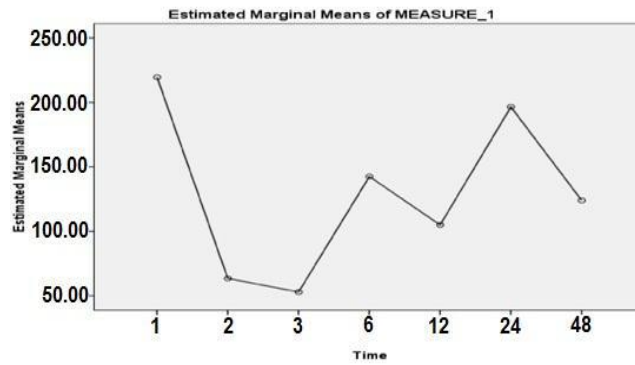


Fig. 3. Changes in the total number of colonies, 17,000 ppm concentration (+16°C)

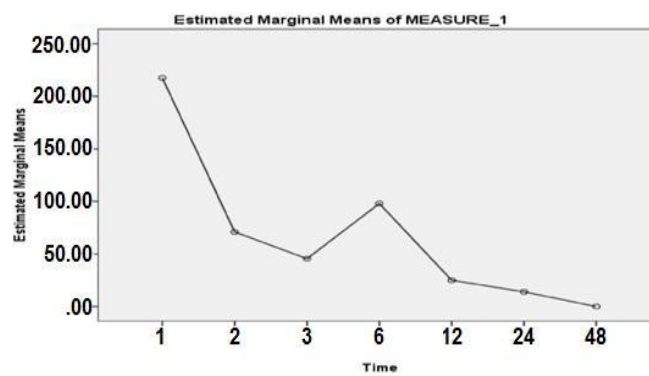


Fig. 4. Changes in the total number of colonies, with 35,000 ppm concentration (+16°C)

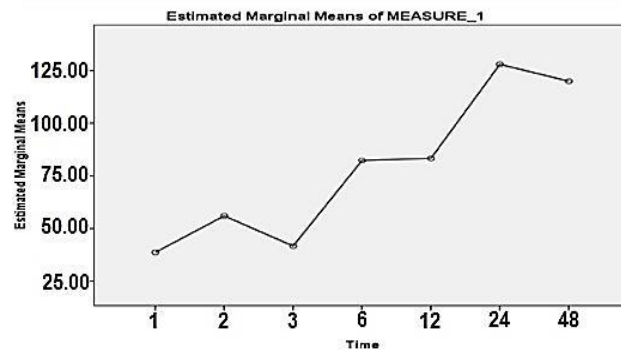


Fig. 5. Changes in the total number of colonies, 17,000 ppm concentration (+24°C)

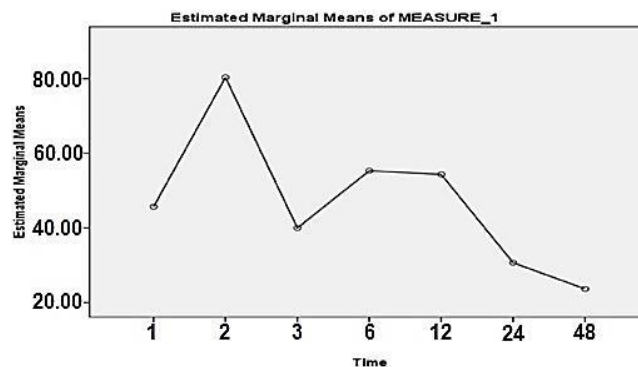


Fig. 6. Changes in the total number of colonies, 35,000 ppm concentration (+24°C)

The results of this study are related to the findings of other works related to the antimicrobial and antibacterial properties of the extracts from different types of plants. The study on the antioxidant, antibacterial, and toxic properties of *Arum maculatum* supported its positive effects on human model cells. Additionally, the enzymatic activities for SOD, CAT, and POX were measured using spectrophotometry, that supports these positive effects, in which it displays relatively weak antibacterial properties at two different extract dilutions (17,000 ppm and 35,000 ppm) (Yari et al., 2012).

Mohammadzadeh et al. (2014); Bendall, (1958) and Koga et al. (1976) investigated the antibacterial effect of *Cardoria draba* on food-borne pathogenic bacteria (*S. typhimurium* and some other species, including *S. aureus*). The statistical tests performed in this study showed that *Cardoria draba* extract had an MIC of 2.5 nmg/ml for *S. aureus* and an MBC of 40 mg/ml and bactericidal effect was not observed on *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *B. cereus*. With respect to *Cardoria draba*, more research is needed on the extract of the plant and its effects, particularly detecting the effective compounds of the plant. As noted, overall *Arum conophalloides* (with an MIC=17,000 ppm and MBC=35,000 ppm) demonstrated a weak bactericidal effect on *S. typhimurium* and counting the number of colonies to show a trend towards a decrease in their number was only worthwhile at certain temperatures and time intervals.

Other plants have been studied that specifically addressed the effect of their extracts on *S. typhimurium*. Investigated modeling of *S. typhimurium* bacteria growth under different concentrations and conditions concerned with *Carum copticum* extract, temperature, pH, and incubation. The resulting outcomes indicated the statistically significant effect ( $p < 0.05$ ) of

each of these variables on *S. typhimurium* growth. Generally, the results demonstrated the suitability of the log-normal model to estimate the growth (Mohammadzadeh et al., 2014; Shakeri et al., 2013; Das et al., 2005; Eldesouky et al., 2007; Ishida & Palmer, 1988).

Mardanighahfarakhi et al. (2013); Bulanov et al. (1990) and Osawa, (1963) evaluated the antioxidant and antimicrobial activities of the phenolic extracts of *Oenothera biennis L.*, that indicated and showed significant antimicrobial activity. *S. typhimurium* was one of the bacteria of particular interest, and showed the highest resistance of among the all bacteria studied regarding the extracts (acetone and ethanol). For their study, the MBC values of acetone and ethanol were respectively equal to 10 and 20  $\mu\text{g/ml}$ . The current study shares similarities with this study in that the aqueous extract of *Arum conophalloides* at MIC of 17000 ppm and MBC of 35000 ppm did not yield statistically significant differences in the bacterial colony number ( $p > 0.05$ ), indicating the extract's weak bactericidal effect except for the temperatures of  $-18^{\circ}\text{C}$  and  $16^{\circ}\text{C}$ .

Extracts have also been studied for their synergistic effects with other medications, but, as shown, not all the extracts will have synergistic effect when used with medications. These researchers studied the use of various herbal-plant extracts with paracetamol and loperamide HCl; the effects of these extracts when given with these medications were higher with *S. aureus* and *E. coli* with aqueous extracts rather than methanolic and ethanolic extracts (Jouda, 2013).

Husein et al. (2014) investigated the antimicrobial effects of ethanol extracts of 6 herbal medicines traditionally local to Palestine. The extracts included *Arum Palaestinum* Bioss, *Urtica pilulifera L.*, *Thymbra capaitata (L.)*, *Origanum syriacum L.*, *Teucrium creticum L.*, *Teucrium polium*



L., which were tested against six bacteria namely, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonellatyphi*, *Escherichia coli*, *Klebsiella pneumonia*, and five types of *Candida*, and two dermatophyte named *microsporum canis* and *microsporum rubrum*. The results indicated that *T.capitata* and *O.syriacumextracts* had the highest activity against bacteria while the other herbal extracts didn't demonstrate high activities except for some of the bacteria and *Candida*. Additionally, *O.syriacum* and *T. capitata* had the highest activity against *Candida* (22.5 to 29.5 mm), while *T. capitata* extract had the highest activity against Dermatophytes, and considering the fact that extract of *Arum Conophalloides* in the mentioned study with 17000 and 35000 ppm dilutions were only meaningful in three states of -18°C and +16°C Temperatures with a P-value respectively equal to 0.005, 0.000, and 0.13 and demonstrated relatively weak effect on *Salmonella typhimurium* bacteria.

Uzun (2004) performed a research on the effects of antimicrobial activities of traditional medicine in Sakarya Province in North West of Turkey on some of the species for two months. The information they obtained came from 46 plant species from 30 families and 5 animal species. Most plant families included *Asteraceae*, *Cucurbitaceae*, *Lamiaceae*, *Rosaceae*, and most plants used included: *Artemisia absinthium*, *Equisetum telmateia*, *Lavandula stoechas*, *Melissa officinalis*, *Tussilago farfara*, and *Urtica dioica*. The extracts tested in laboratory were used for antimicrobial activities against *Staphylococcus aureus* (ATCC 65538), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739), *Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 1549), *Salmonella typhi*, *Proteus mirabilis*, *Candida albicans* (ATCC 10231). The results indicated that the extracts of *Arum maculatum*, *Datura*

*stramonium*, *Geranium asphodeloides*, *Equisetum telmateia petroleum* had antimicrobial activities against *Staphylococcus epidermidis* with MIC concentrations of 39.1, 78.1, 78.1, 39.1 µg/ml, respectively, as well as *Datura stramonium petroleum* extract with 39.1 µg/ml MIC against *E.coli* and ethanol extract of *Trachystemon orientalis* with 39.1 µg/ml MIC against *E.coli*, while in the results of the mentioned research the extract was only meaningful in -18 and +16 Celsius degrees having MIC and MBC values equal to 17000 and 35000 ppm, respectively ( $p < 0.05$ ) (Uzun *et al.*, 2004).

### Conclusion

According to surveys conducted, alcohol and chloroform extracts of different parts of the plant did not yield significant results. It might be concluded that the extracts obtained in this study have a relatively low effect concerned with MBC on *S. typhimurium* bacteria.

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