The Journal of Medicinal Plants and By-products
Original Article

The Effect of *Satureja bachtiarica*, *Carum carvi*, and *Thymus daenensis* Essential Oil on *Cronobacter sakazakii*, a Foodborne Opportunistic Pathogen

Samira Abbasi[1], Mohammad Goli[1], Hasan Momtaz[2], Fatemeh Malekpoor[3*], Abdollah Ghasemi Pirbalouti[4] and Tayebeh Ahmadi[5]

[1] *Department of Food Science and Technology, Khorasgan Branch, Islamic Azad University, Isfahan, Iran*
[2] *Department of Microbiology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
[3] *Department of Biology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
[4] *Medicinal Pants & Nutraceuticals Program, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*
[5] *Department of Medical Laboratory Science, College of Science, Knowledge University, Kirkuk Road, Erbil 44001, Iraq*

*Corresponding Author: Email: Fmalekpoor87@yahoo.com

Article History: Received 07 May 2025/Accepted in revised form 27 October 2025

ABSTRACT
Cronobacter sakazakii is one of the main pathogens transmitted to infants through the consumption of contaminated infant formula and baby food. The bacterium is a major cause of mortality, digestive diseases, and meningitis in newborns. The present study was performed to determine the microbiological effect of *C. Sakazakii* in the infant formula and baby food samples and to investigate the effect of 3 plant essential oils on bacterial isolates. A total of 200 samples of baby food and infant formula of different brands were collected, and the inhibitory effects of bacterial growth by essential oils of *Satureja bachtiarica*, *Carum carvi*, and *Thymus daenensis* were evaluated by the disc diffusion method. The most sensitive bacteria isolated at 1000 micrograms per milliliter were *T. daenensis*, *S. bachtiarica*, and *C. carvi*, respectively. Inhibitory effects of growth were not observed in the concentration of 31 micrograms per ml in any of the essential oils. Infection outbreaks caused by bacteria can be reduced by careful inspection of the quality of infant formula and baby food, strict supervision of the production process, adherence to hygiene rules, and the use of natural antimicrobials.
Keywords: *Cronobacter sakazakii*, Baby food, Milk powder (infant formula), *Carum carvi*, *Thymus daenensis*, *Satureja bachtiarica*.

INTRODUCTION
Infant formula and baby food are widely available and often serve as a supplement or substitute for breast milk. Although infant formula is subjected to heat treatment during production, this process, unlike that for liquid formulations, does not render the product sterile. Consequently, this production necessitates stringent sanitary measures and rigorous monitoring of environmental conditions. These measures are crucial for reducing the microbial load in the final infant formula product [1].

C. sakazakii is a Gram-negative, straight rod bacterium belonging to the family *Enterobacteriaceae*. It is a motile, non-spore-forming, facultatively anaerobic organism possessing a peripheral flagellum. This organism has been isolated from a wide range of clinical sources, including blood, sputum, urine, wounds, intestinal contents, and purulent abscesses, as well as environmental sources such as water, soil, sewage, animal droppings, and various food products. Prior to 1980, this bacterium was known as *Enterobacter cloacae* biogroup, recognized by its production of a yellow pigment. Key distinguishing features from other *Enterobacter* species include its inability to ferment D-sorbitol and the production of extracellular ribonuclease. *Enterobacter sakazakii* comprises four categories and fifty-seven strains; currently, the organism is classified as *C. sakazakii* [2–6].

Since microbial infections are a serious threat to human health and have endangered the lives of people, people are always looking for medicines to cure the disease and reduce its effects. In this regard, the use of herbs has long been considered and traditionally used among different nations. Since the useful medicinal plants grow in Iran in abundance, studying the effective combinations and medicinal effects of these plants can be a positive step in the identification and optimal use of this valuable national wealth [7-9].

In many instances, medicinal plants exhibit a favorable biological profile wherein the synergistic action of active ingredients, associated with other co-occurring compounds, often results in fewer adverse side effects during disease treatment. In recent years, synthetic drug side effects and a growing human inclination toward natural health maintenance have significantly increased attention directed toward medicinal plants. Although the use of plant-derived therapeutics has a long history, the perception that they are entirely free from side effects must be tempered; nevertheless, they remain a valuable cultural heritage for many nations. Furthermore, the secondary metabolites produced by plants are crucial for their protective role against pathogens such as fungi, bacteria, and viruses. Compounds exhibiting antibacterial, antifungal, and antiviral effects in certain plant species are termed phytoalexins, and their presence is considered a key factor in the curative efficacy of herbal medicines. Upon pathogen attack or contamination, the plant initiates the regulation (coding) of biosynthesis enzyme genes, leading to the transcription and translation mediated by messenger RNA. Consequently, these protective compounds are synthesized in specific cells and tissues, gradually increasing in concentration to ultimately prevent the invasion by pathogens, pests, or germs [7–10].

The plants studied belong to the families Lamiaceae and Apiaceae. T. daenensis is an aromatic species belonging to the family Lamiaceae. All aerial parts, especially the branches, are utilized in traditional medicine [11]. *S. bachtiarica* is a species belonging to the family Lamiaceae, which contains high levels of essential oils. *S. bachtiarica* has tannins, fats, and different sugars as well as terpene and a kind of phenol.

Carvacrol is one of the main constituents of the essential oil of S. bachtiarica [12]. *C. carvi* is a species belonging to the family Apiaceae, which contains tannins, waxes, resinous materials, fixed green oil, mucilage, sugars, nitrogen compounds, and essential oils containing dihydrocarvone, carvone, and two types of setan derivatives. The fruit of *C. carvi* is the harvestable part, collected in July of the second year by cutting the plant and allowing it to ripen [13]. Several studies have investigated the antimicrobial activity of secondary plant compounds, particularly those from medicinal plants, using various extraction technologies and methods of evaluating their effects both in vitro and in vivo. The results of most studies confirm the antimicrobial or phytoalexin properties of certain secondary compounds found in medicinal herbs and aromatic plants. This study aims to investigate the effects of the essential oils of *T. daenensis, S. bachtiarica,* and *C. carvi* on *C. sakazakii* isolated from various baby foods produced in Iran.

MATERIALS AND METHODS

This was a cross-sectional study examining infant formula and baby food samples collected from pharmacies during the winter and spring of 2020.

Samples and Separating *C. sakazakii*

In this cross-sectional study, 100 samples of 8 commercial infant formula brands and 100 samples of 4 different baby food brands marketed in drugstores were purchased. For each sample, 25 g of the contents of three packs from one production series were mixed and then poured into a sterile glass vessel under sterile conditions. All testing phases were done under a laminar hood with a HEPA filter. The identification tests for *C. sakazakii* were done according to the Iversen and Forsythe method [14].

Valuating the Medicinal Plant Effects

The medicinal plants listed in Table 1 were used to evaluate the antimicrobial effects of native Iranian medicinal plants. These plants were collected from the mountainous areas of the Zagros Mountains, in the Chaharmahal va Bakhtiari district (altitude: 2000-2500 m asl, latitude: 30°-31°, longitude: 50°-51°), during May–Sep, 2020. The plants were collected and dried after ensuring accurate identification. Dried plants were chopped into pieces of 0.5 to 1 cm using a laboratory mill (Moulinex Model, Spain), and 50 grams of each plant were weighed with an accuracy of 0.001g for distillation using a digital scale (Sartorius, Germany) and transferred into a flask. To extract the volatile oil, the dried aerial parts powder was mixed with water and placed in a flask connected to the condenser of a Clevenger apparatus (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran). The extraction continued for 150 min, and the volatile oil and water mixture was finally separated by decantation. The volatile oils were dried over Na2SO4, stored in a dark glass bottle, and kept at a low temperature (5 °C) until analysis.

Table 1 Profile of plants used in this study

scientific name	plant families	used parts	used extracts
T. daenensis	Lamiaceae	Arial parts (stem, leaves, flowers)	essential oil
S. bachtiarica	Lamiaceae	Arial parts *(stem, leaves, flowers)*	essential oil
C. carvi	Apiaceae	Arial parts *(stem, leaves, flowers)*	essential oil

Making Different Concentrations of the Essential Oils

To determine the effective concentration of essential oils with antibacterial activity, different concentrations were prepared. For this purpose, 0.01 g of each essential oil was added to a test tube, followed by the addition of 10 mL of 70% ethanol. This resulted in Solution 1, with a concentration of 1000 μg/mL. To produce a 500 μg/mL concentration, 5 mL of Solution 1 was mixed with 5 mL of ethanol. Similarly, to obtain a 250 μg/mL concentration, 5 mL of the 500 μg/mL solution was mixed with 5 mL of ethanol. Concentrations of 125 and 62 μg/mL were prepared using the same serial dilution method. Thus, five different concentrations of each extract were prepared and used in the subsequent experiments.

Determining the Antibiotic Resistance Model

To determine the antibiotic resistance profile of separated isolates in this study, a simple disk diffusion method was used. For this purpose, the separated isolates were cultured into Tryptic Soy Broth liquid medium at 37 ° C overnight and after preparing the thinness of 0.5 McFarland, they were continuously cultured into the solid medium of agar Mueller-Hinton and were cultured in the presence of antibiotics discs (ampicillin (10 μg/disk) amoxicillin (20 μg/disk), aztreonam (30 μg/disk), cefotaxime (30 μg/disk), amikacin (30 μg/disk), streptomycin (10 μg/disk), meropenem (10 μg/disk), mezlocillin (30 μg/disk), nalidixic acid (30 μg/disk), tigecycline (15 μg/disk), tetracycline (30 μg/disk), ticarcillin (30 μg/disk), chloramphenicol (30 μg/disk), ceftazidime (30 μg/disk), ciprofloxacin (5 μg/disk), cefepime (30 μg/disk), imipenem (10 μg/disk), levofloxacin (5 μg/disk), minocycline (30 μg/disk), piperacillin (30 μg/disk), piperacillin-tazobactam, carbenicillin (100 μg/disk), tobramycin (10 μg/disk), cotrimoxazole (30 μg/disk), moxifloxacin (5 μg/disk), gentamicin (10 μg/disk), colistin (10 μg/disk) at 37 °C for 24 hours. The susceptibility or resistance of the isolates to each antibiotic was determined and reported according to the CLSI guidelines [15].

Statistical Analysis

All test results were transferred to a Microsoft Excel spreadsheet for data compilation and analysis. Statistical analysis was performed using SPSS version 18.0 software, employing a completely randomized design (CRD). Significant differences between treatment groups were determined at a probability level of $P<0.05$.

RESULTS

Comparing Growth Inhibition Zone Diameter by Different Concentrations of the *T. daenensis* Essential oil on *C. sakazakii*

The effect of different concentrations of *T. daenensis* essential oil on the growth inhibition zone diameter of *C. sakazakii* isolates separated

from milk powder has been shown in Fig. 1. As can be seen, by increasing the concentrations of *T. daenensis* essential oil, the growth inhibition zone diameter of *C. sakazakii* significantly increases (at 5%). The largest diameter of the growth inhibition zone was related to the *T. daenensis* sample with a concentration of 1,000 micrograms per milliliter. However, no halo was observed at concentrations of 31 and 62.5 mg/ml, which indicated that *C. sakazakii* has not shown any sensitivity to this concentration of given essential oil, and its growth has not decreased.
Growth inhibition zone diameter of baby food of *C. sakazakii* isolates when using the different concentrations of *T. daenensis* essential oil has been shown in Fig. 2. As can be seen, the essential oil concentration causes a significant difference in the microbe growth so that by increasing the essential oil concentration, the growth inhibition zone diameter increases and the largest diameter of growth inhibition zone was related to 1000 mg/ml concentration of *T. daenensis*, while the lowest diameter of growth inhibition zone was related to *T. daenensis* samples with 31 mg/ml and 62.5 mg/ml concentration. The growth of bacteria did not decrease in these concentrations.

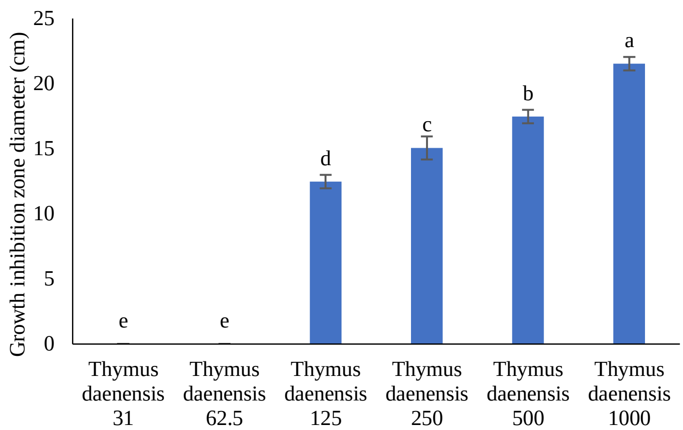

Fig. 1 Comparing the growth inhibition zone diameter by different concentrations of the *T. daenensis* essential oil on *C. sakazakii* in milk powder.

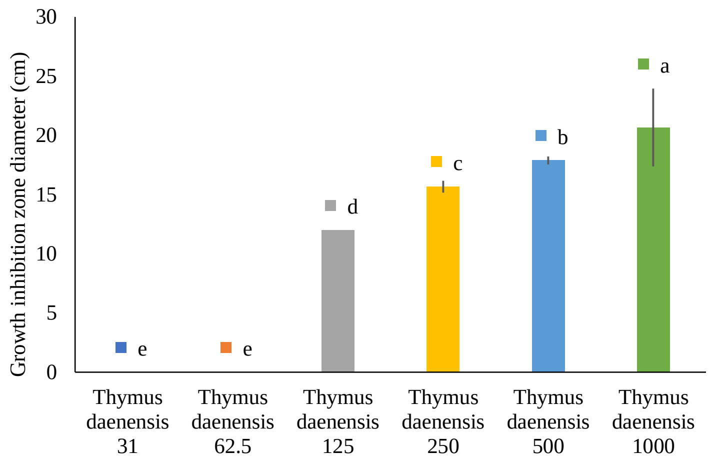

Fig. 2 Comparing the growth inhibition zone diameter by different concentrations of *T. daenensis* essential oil on *C. sakazakii* in the baby food.

Comparing the Growth Inhibition Zone Diameter by Different Concentrations of *S. bachtiarica* Essential Oil on *C. sakazakii*

Our study results showed that the *C. sakazakii* isolates separated from infant formula (Fig. 3) and baby food (Fig. 4) had the most sensitivity to high concentrations of *S. bachtiarica* essential oil. Among the *S. bachtiarica* concentrations studied, the concentration of 1000 mg/ml of *S. bachtiarica* had the most bacterial isolate sensitivity. However, the lowest diameter of the growth inhibition zone was related to a concentration of 31 mg/ml of *S. bachtiarica* essential oil.

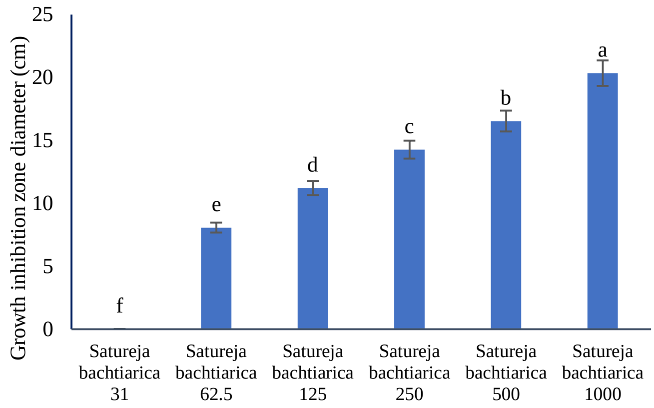

Fig. 3 Comparing the growth inhibition zone diameter by different concentrations of *S. bachtiarica* essential oil on *C. sakazakii* in the milk powder.

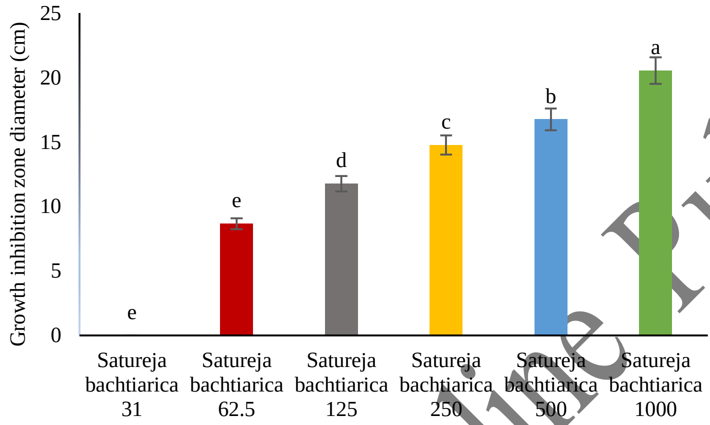

Fig. 4 Comparing the growth inhibition zone diameter by different concentrations of *S. bachtiarica* essential oil on *C. sakazakii* in the baby food.

Comparing the Growth Inhibition Zone Diameter by Different Concentrations of *C. carvi* Essential oil on *C. sakazakii*

The growth inhibition zone diameter of *C. sakazakii* isolates separated from milk powder (Fig. 5) and baby food (Fig. 6) when using different concentrations of the *C. carvi* essential oil has been shown in Figs. 5 and 6. As can be seen, the essential oil concentration causes a significant difference (at 5%) in the above-mentioned microbe growth, so that the growth inhibition zone diameter increases with increasing essential oil concentration. The largest diameter of the growth inhibition zone was related to the concentration of 1000 mg/ml of *C. carvi*, while the lowest diameter of the growth inhibition zone was related to *C. carvi* samples with concentrations of 31, 62.5, and 125 mg/ml, in which no bacterial growth inhibition zone was observed. Thus, the bacterium does not show any sensitivity to 3 concentrations of 31, 62.5, and 125 mg/ml and completely resists three concentrations.

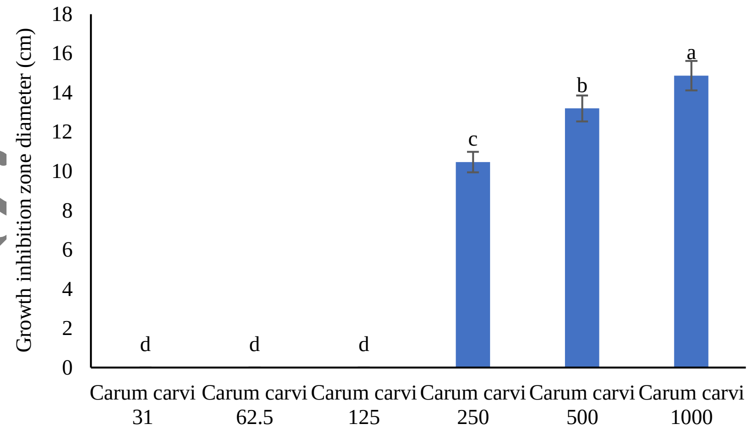

Fig. 5 Comparing the growth inhibition zone diameter by different concentrations of *C. carvi* essential oil on *C. sakazakii* in milk powder

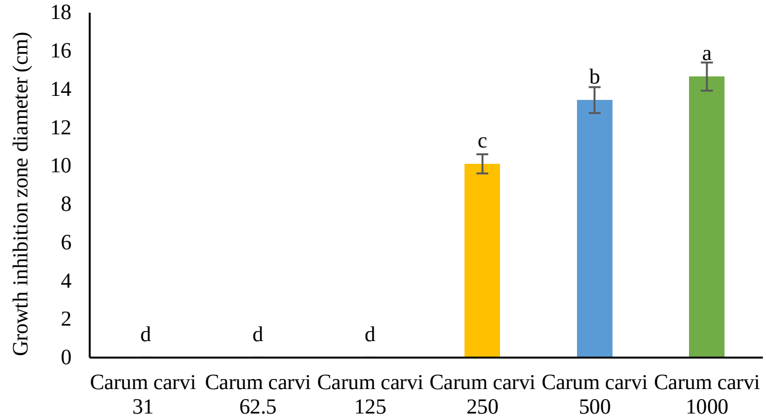

Fig. 6 Comparing the growth inhibition zone diameter by different concentrations of C. carvi essential oil on C. sakazakii in baby food

Comparing the Growth Inhibition Zone Diameter by Different Essential Oil Concentrations and Comparing it with Antibiotics on *C. sakazakii*

The effect of different essential oil concentrations and comparing them with a variety of antibiotics in the growth inhibition zone diameter of bacteria isolates of baby food and infant formula have been shown in Fig. 7. As can be seen, the highest diameter of growth inhibition zone was related to *T. daenensis* with concentrations of 1000 micrograms per milliliter, *S. bachtiarica* with concentration of 1000 mg/ml, ciprofloxacin, meropenem and imipenem, respectively. However, the lowest diameter was related to low essential oil concentrations, in which no growth inhibition zone was observed. Also, according to the essential oil comparison, *T. daenensis* showed the highest antimicrobial properties among the 3 essential oils, and then *S. bachtiarica,* with the growth inhibition zone diameter of 20.5 mm in the concentration of 1000 mg/ml had the highest diameter of growth inhibition zone. *C. sakazakii* was the most resistant to *C. carvi* essential oil, in particular in low concentrations.

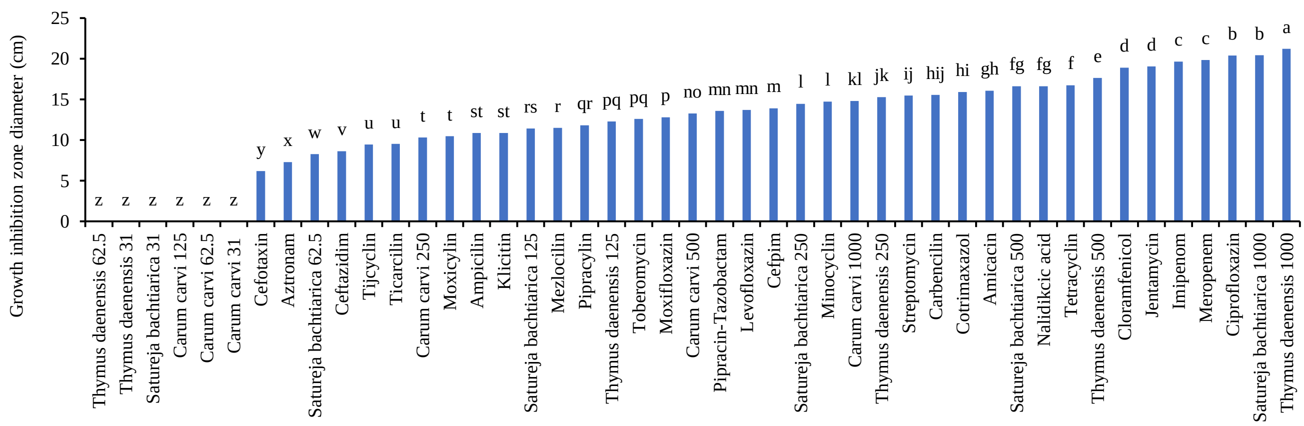

Fig. 7 Comparing the growth inhibition zone of *C. sakazakii* by plant essential oils with different concentrations of a variety of available antibiotics

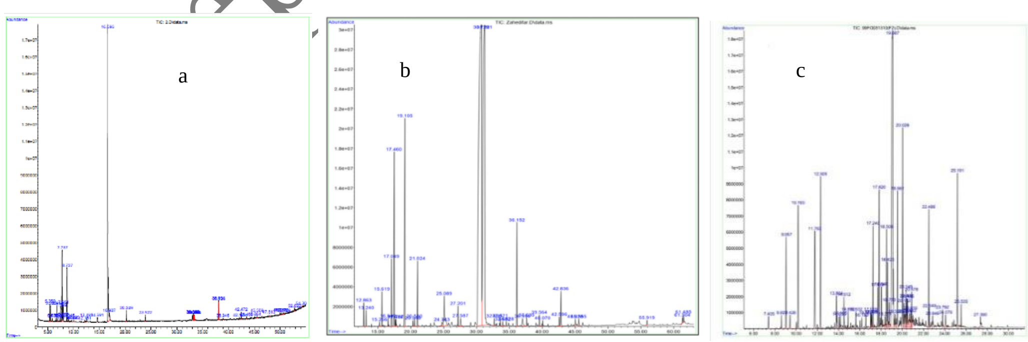

Fig. 8 GC-MS chromatogram of *Thymus daenensis* (a), *Satureja bakhtiarica* (b), and *Carum carvi* (c)

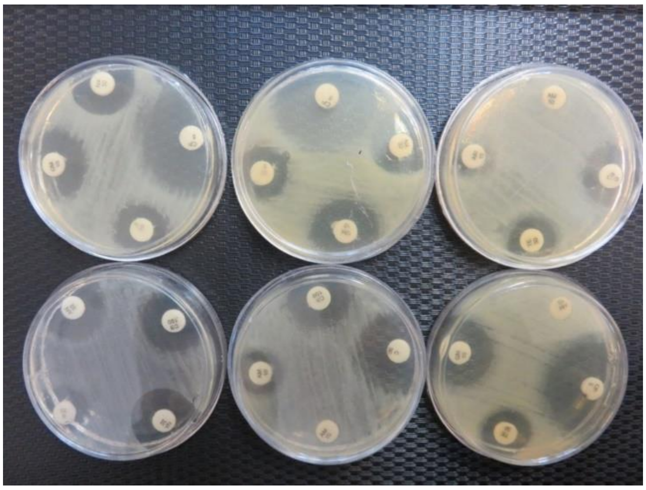

Fig. 9 Diameter of the growth inhibition zone of *Cronobacter sakazakii* strains when using the plants and antibiotics.

DISCUSSION

Cronobacter species are opportunistic pathogens with a resulting mortality rate estimated to be between 40 and 80 percent. These pathogens can cause a wide range of deadly diseases, such as meningitis, septicemia, neonatal enterocolitis, and brain abscess. Although *Cronobacter* can cause illness in adults and children, infections in infants, often caused by contaminated infant formula and baby food, are more commonly observed. Since the first *Cronobacter* infection outbreak was reported in 1958, and infant formula was recognized as the main source, numerous infant formula batches were collected from pharmacies and analyzed. The contamination of infant formula has thus become a significant problem, making the monitoring of infant formula and baby food for the presence of these bacteria a necessity.

In a part of the study, the antibiotic resistance pattern of *C. sakazakii* isolates separated from baby food and infant formula has been assessed against commonly used antibiotics. The results showed that the *C. sakazakii* isolates separated from baby food and infant formula had the largest diameter of the growth inhibition zone when using antibiotics of Ciprofloxacine (20.38), meropenem (19.83), and imipenem (19.63). *C. sakazaki* shows more sensitivity to these antibiotics and reduces the growth of bacteria. *C. sakazakii* isolates had the lowest diameter of the growth inhibition zone when using antibiotics of amoxicillin, ampicillin, Tyjsyklin, Tykarcillin, aztreonam, and ceftazidime. These bacteria had the highest resistance to these antibiotics. The growth inhibition zone diameter of *C. sakazakii* isolates was moderate compared to other antibiotics.

The study conducted by Sharma and Prakash [16] showed that 300 *C. sakazakii* isolates were separated from the total 55 samples of milk and dairy products, and 5 isolates were identified biochemically as *C. sakazakii*. *The* sensitivity *of C. sakazakii* isolates to antibiotics of ciprofloxacin, rifampin, gentamicin, spectinomycin, vancomycin, tobramycin, amoxicillin, Mymosaykllin, seftirakson, and ceftazidime was reported as 100, 0, 100, 100, 0, 80, 40, 40, 100, and 60 percent, respectively. The above-named researchers also evaluated the effects of plant essential oils on *C. sakazakii* isolates. Their study results showed that the essential oils of cinnamon, orange, mango, tropical plants, ginger, garlic, and clove had lethal and inhibitory effects on the *C. sakazakii* isolates [16].

Our study results showed that *C. sakazakii* isolates separated from baby food and infant formula had the most sensitivity to *T. daenensis*, *S. bachtiarica* and *C. carvi* at the concentration of 1000 mg per cl, respectively. Lethal effects of *T. daenensis* essential oil on *C. sakazakii* have also been reported by Martins *et al*. [10]. Nabulsi *et al*. [17] investigated the growth inhibitory effect of medicinal plants essential oils on *C. sakazakii* isolates. Their study results showed that the essential oil of fennel, cinnamon, chamomile, eucalyptus, fenugreek and rosemary had significant growth inhibitory effect on the isolates [17].

Unfortunately, few studies have been conducted in this area. The main reason for the higher antimicrobial effects of *T. daenensis* essential oil than *S. bachtiarica* and *C. carvi* on *C. sakazakii* isolates separated from baby food and infant formula is probably due to the presence of higher levels of phenolic compounds, which have high antimicrobial properties. *T. daenensis* contains tannins, bitter primary materials, saponins, and plant disinfectant. Phenols (20 to 80 percent) and monoterpenes hydrocarbons (e.g., p-cymene and y-terpinen) form the bulk of *T. daenensis* essential oil. Normally, thymol is the main phenolic constituent of *T. daenensis,* and carvacrol is also its minor part, so all the materials have antimicrobial properties [18, 19].

CONCLUSION

Overall results of the study are as follows: *C. sakazakii* isolates separated from baby food and infant formula had the highest diameter of the growth inhibition zone when using antibiotics of ciprofloxacin, meropenem, and imipenem. *C. sakazakii* isolates separated from baby food and infant formula had the highest sensitivity to *T. daenensis*, *S. bachtiarica,* and *C. carvi* in a concentration of 1000 mg/ml, respectively.

ACKNOWLEDGEMENTS

The authors would like to thank A. Mousakhani-Ganjeh for his great assistance and cooperation during this study.

REFERENCES

1. Estuningsih S., Kress C., Hassan A.A., Akineden O., Schneider E., Usleber E. Enterobacteriaceae in dehydrated powdered infant formula manufactured in

Indonesia and Malaysia. Journal of Food Protection. 2006;69:3013-3017.
2. Arroyo C., Condon S., Pagon R. Thermobacteriological characterization of *Enterobacter sakazakii*. International Journal of Food Microbiology. 2009;136(1):110-118.
3. Bar-Oz B., Preminger A., Peleg O., Block C., Arad I. *Enterobacter sakazakii* infection in the newborn. Acta Paediatr. 2007;90(3):356-358.
4. Biering G., Karlsson S., Clark N.C., Jonsdottir K.E., Ludvigsson P., Steingrimsson O. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. Journal of Clinical Microbiology. 1989;27(9): 2054-2056.
5. Drudy D., Mullane N., Quinn T., Wall P.G., Fanning S. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. Clinical Infectious Diseases. 2006;42:996-1002.
6. Dumen E. *Cronobacter sakazakii* (*Enterobacter sakazakii*): Only an Infant Problem. Kafkas Univ. Vet. Fak. Derg. Review. 2010; 16 (Suppl-A):S171-S178.
7. Sagdic O., Aksoy A., Ozkan G., Ekici L., Albayrak S. Biological activities of the extracts of two endemic Sideritis species in Turkey. Innovative Food Science & Emerging Technologies. 2008;9:80-84.
8. Zampini I.C., Vattuone M.A., Isla M.I. Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. Journal of Ethnopharmacology. 2005; 102:450-456.
9. Morton J.F. Major medicinal plants: botany, culture and uses. Springfield. Ill.: Charles C. Thomas Publisher. 1977.
10. Martins N., Barros L., Santos-Buelga C., Silva S., Henriques M., Ferreira I.C. Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. Food Chemistry. 2015;167:131-137.
11. Gorran A., Farzaneh M., Shivazad M., Rezaeian M., Ghassempour A. Aflatoxin B1-reduction of Aspergillus flavus by three medicinal plants (Lamiaceae). Food Control. 2013;31(1):218-223.
12. Sefidkon F., Jamzad Z. Essential oil of *Satureja bachtiarica* Bunge. Journal of Essential Oil Research. 2000;12(5):545-546.
13. Iacobellis N.S., Lo Cantore P., Capasso F., Senatore F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. Journal of Agricultural and Food Chemistry. 2005;53(1):57-61.
14. Iversen C., Forsythe S. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. Food Microbiology. 2004;21(6):771-777.
15. CLSI. Clinical and Laboratory Standards Institute. Standards for antimicrobial. susceptibility testing; Twenty-first international supplement. M100-S21. 2011;31:1.
16. Sharma G., Prakash A. Susceptibility of *Cronobacter sakazakii* to plant products, antibiotics, and to lactic acid bacteria. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2013; 3:263.
17. Al-Nabulsi A.A., Awaisheh S.S., Osaili T.M., Olaimat A.N., Rahahaleh R.J., Al-Dabbas F.M., Al-Kharabsheh L.A., Gyawali R., Ibrahim S.A. Inactivation of *Cronobacter sakazakii* in reconstituted infant milk formula by plant essential oils. Journal of Applied Botany and Food Quality. 2015;88:97-101.
18. Lai K.K. *Enterobacter sakazakii* infections among neonates, infants, children, and adults: case reports and a review of the literature. Medicine. 2001;80:113-122.
19. Mardaneh J., Dallal M.M.S. Isolation, identification and antimicrobial susceptibility of Pantoea (Enterobacter) agglomerans isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. Iranian Journal of Microbiology. 2013;5:263.