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Efficacy assessment of various natural and organic antimicrobials against *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*

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Abstract

Background: Foodborne illness is a public health alarm with a deleterious effect on human health and the economy all over the world. Searching for possible solutions to beat foodborne pathogens is still a demanding concern. The scope of this study is to evaluate the antimicrobial activity of some natural and organic compounds against important pathogens including *Escherichia coli* O157:H7 C9490, *Listeria monocytogenes* Lm2 Scott A 4b, and *Salmonella enteritidis* 8-9-99.

Results: The bactericidal effect of eight compounds and their concentrations were evaluated by the tube dilution assay against the tested bacterial strains. Thymol was found to be superior to all tested compounds. Antimicrobial activities found to be highly influenced by varying pH values. Low pH 4.5 found to report higher inhibition when compared with pH 7.1. For instance, minimum inhibitory concentration (MIC) occurred at pH 7.1 with 25 ppm of thymol against *Escherichia coli* O157:H7 and *Salmonella enteritidis*, while 200 ppm against *Listeria monocytogenes*. However, MIC occurred at pH 4.5 with 25 ppm of thymol against all tested bacterial strains.

Conclusions: Thymol is the most active antimicrobial recorded in our study at low concentrations. Our results indicated thymol, benzoic acid, sodium benzoate, salicylic acid, 3-*t*-butyl-4-Hydroxyanisole, and acetylsalicylic acid have promising potential applications in controlling tested foodborne pathogens.

Keywords: *E. coli* O157:H7, *L. monocytogenes*, *S. enteritidis*, Food preservation, Antimicrobials

Background

Foodborne illness has been a great public health concern and arduous challenge all over the world. The World Health Organization (WHO) indicated that 31 foodborne hazards were estimated to cause 600 million cases of foodborne disease and 420,000 deaths annually, worldwide (WHO 2015). The US Center for Disease Control and Prevention estimates that each year 48 million people get sick, 128,000 are hospitalized, and 3000 dies of foodborne pathogens (Scallan et al. 2011; Oliver 2019). In July 2016, an outbreak of Shiga toxin-producing *Escherichia coli* O157 PT34 was notified to WHO in England and Wales. In 2016, WHO has been reported four multistate outbreaks of human Salmonella infections by the National International Health Regulations Focal Point of the USA (Hassan et al. 2019). It was a case reported that some tourists in Egypt and Turkey had endured from foodborne illnesses of unknown origin (Todd 2016). Based on the evidence, different studies declared the prevalence of foodborne pathogens and their possible cause of human infection in Egypt (El-Sharkawy et al. 2017; Sallam et al. 2013; Ombarak et al. 2016; Helmy et al. 2017). Health Protection Scotland

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agency reported Egypt as the second most common country associated with possible overseas outbreaks mainly due to *Salmonella* sp. (Smith-Palmer and Cowden 2009). WHO reported *Salmonella* species, *Listeria* species, and *E. coli* as hazardous foodborne pathogens (WHO 2015). Therefore, there is still an essential need for the assessment of new safe methods to reduce or eliminate foodborne pathogens.

Some bacterial strains such as *Salmonella* sp., *E. coli* and *Listeria monocytogenes* are a major cause of foodborne illness due to their ability to grow and tolerate different conditions. *Salmonella* sp. and *E. coli* are rod-shaped, motile, non-spore-forming, Gram-negative bacteria and can grow at a wide range of temperatures (5–47 °C at pH range 4.2–9.5) and (7–46 °C at pH range 4.4–9), respectively (Dodd et al. 2017; Leyer et al. 1995), while *L. monocytogenes* is a rod-shaped, non-spore-forming, Gram-positive bacterium, motile and can grow at a wide range of temperatures (0–45 °C at pH range 4.4–9.4) (Zhao 2005). Most microorganisms are known to form biofilms, which serve as protective shells (Lemon et al. 2007). So, searching for different strategies and improved methodologies to control these pathogens is still a prime concern. One such possibility is to evaluate the efficacy of natural and organic antibacterial additives.

Most foodstuffs require microbial spoilage protection during storage. This drives the hunt for gentle preservation techniques by food authorities and scholars to improve microbial quality and health without affecting the nutritional and sensory values of food (Quinto et al. 2019). The danger of bacterial food poisoning and food spoilage is minimized by various food protection methods including heat treatment and the use of chemical preservatives (Periago and Moezelaar 2001). Consumer demands should also be considered fresh, additive-free, and more natural food degustation, thus preserving microbiological health and stability (Gould 1996). One of the main natural compounds is essential oils obtained from plants, enzymes, organic acids, and polymers which occur naturally. They are gaining a wide interest in the food industry because of their potential as decontaminating agents, as the US Food and Drugs Administration generally recognizes them as healthy (GRAS) status (Walsh et al. 2003; Gutierrez et al. 2009; Lens-Lisbonne et al. 1987). Some natural and organic compounds are reported as an antioxidant (Yanishlieva et al. 1999), anti-inflammatory (Riella et al. 2012), a local anesthetic (Haeseler et al. 2002), antibacterial (Burt and Reinders 2003; Tuncel and Nergiz 1993; Ding 2017), and anticancer (Kang et al. 2016) activities. Recently, it was evolved in the treatment of periodontal diseases (Patole et al. 2019). However, their potential as a novel source of food preservatives has yet to be fully exploited.

The objective of the present study is to evaluate the bactericidal efficiency of some natural and organic compounds against *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* at different pH values. We used the tube dilution method as an in vitro test of different concentrations against these significant microbes. Minimum inhibitory concentration (MIC value) is known as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth (Pfaller et al. 2004). This data highlights a clear image of the degree of inhibition of different natural organic compounds against the selected pathogens and supports further utilization in the pharmaceutical and food industries.

Methods

Bacterial strains and routine cultivation

In this study, microorganisms (*Escherichia coli* O157:H7 C9490, *Listeria monocytogenes* Lm2 Scott A 4b, and *Salmonella enteritidis* 8-9-99) were kindly provided as a gift from Dr. Levin's laboratory at the University of Massachusetts, Amherst, USA. Each culture was routinely cultivated overnight in (100 ml) tryptic soy broth in (250 ml) Elmer flasks (TSB; Difco, Sparks, MD) at 37 °C using an orbital shaker at 200 rpm. After 12 h, 0.1 ml culture aliquot was transferred to 10 ml TSB in a test tube and incubated for 4 h at 37 °C to reach the log phase. Cell densities were measured at 600 nm using a DU730 spectrophotometer (Beckman Coulter, Pasadena, CA) along with CFU using plate count of serial dilutions.

Preparation of antimicrobial compounds

Different natural and organic compounds were prepared until completely dissolved using different solutions. A volume of 10 ml of 5% thymol was prepared in 50% ethanol. Benzoic acid was prepared at a concentration of 5% in 40% ethanol. Sodium benzoate was prepared as 10% in distilled H_2O and then passed through 0.2 µl filter sterilization. Salicylic acid (5%) was prepared in 47.5% ethanol. Acetyl salicylic acid was prepared as 5% in 40% ethanol. Ibuprofin was prepared as 5% in distilled H_2O. 4-acetamidophenol was prepared as 5% in 15% ethanol. 3-*t*-Butyl-4-Hydroxyanisol was prepared as 4% in 47% ethanol. All experiments were conducted with different concentrations of the above compounds in 10 ml TSB media inoculated with each culture at 200 rpm for 24 h. Absorbance was measured at 600 nm using the digital spectrum, and pH values were adjusted using 1 mol l^{-1} of HCl or NaOH. All chemicals were purchased from Sigma-Aldrich (St. Louis, USA).

Tube dilution assay

The bactericidal effect of bacteria strains was determined by tube dilution assay (Ismaiel and Pierson 1990).

Bacterial culture media were harvested at the lag phase, and then, cell suspensions were adjusted at A_{600} (1.818 *E. coli* O157:H7, 1.718 *L. monocytogenes*, and 1.718 *S. enteritidis*). Different volumes of each prepared antimicrobial compound were added to 10 ml TSB media tubes to obtain a concentration range (0–500 ppm) and then spelled into two groups (pH 4.5 and 7.1). An aliquot of 50 µL cell suspension was inoculated into each tube and incubated at 37 °C for 24 h in an orbital shaker at 200 rpm. The growth was assessed at A_{600}, and graphs were plotted using Origin 6.0 software, OriginLab Corporation, Northampton, MA, USA (Deschenes and David A. Vanden BoutUniversity of Texas 2000). Negative controls were conducted with TSB only and positive control with TSB plus bacteria.

Statistical analysis

Data were statistically analyzed by using the two-way variance of analysis (ANOVA) with less significant difference (L.S.D.) at ($P < 0.05$). Growth curve results were recorded in duplicates, and standard error was calculated for each treatment.

Results

Influence of different pH values on bactericidal activity of thymol against *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes*

Foodborne illness is a global problem that threatens all communities. These bacterial strains showed different antimicrobial sensitivity against tested antimicrobial compounds. Thymol found superior with the highest antibacterial activity over other tested compounds. The bactericidal activity of thymol was mainly pH-independent. At pH 7.1, a gradual decrease in the growth of *L. monocytogenes* was recorded, followed by a sharp decrease with a minimum inhibitory concentration of 200 ppm, and then a steady growth pattern. A sharp decrease in the growth of both *E. coli* O157:H7 and *S. enteritidis* was recorded at an MIC of 25 ppm, followed by a steady inhibition. At pH 4.5, a sudden decrease in the growth of *L. monocytogenes*, *E. coli* O157:H7and *S. enteritidis* occurred at a minimum inhibition concentration of 25 ppm, followed by a steady decrease in growth (Fig. 1).

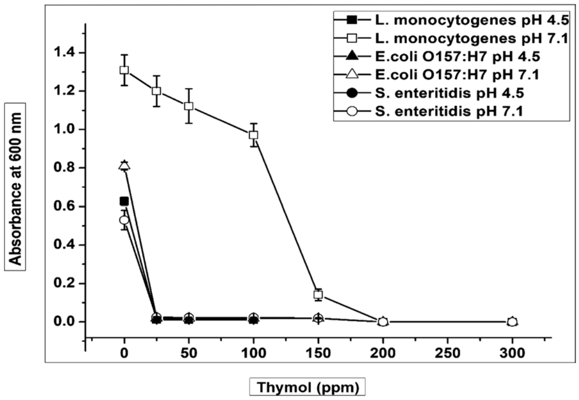

Fig. 1 The Influence of different pH values on bactericidal activity of Thymol against *E.coli* O157: H7, *S. enteritidis*, and *L. monocytogenes*. Thymol concentrations (0, 50, 100, 150, 200, 250 and 300) ppm were each added to 10 ml tryptone soya broth tubes, and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data are shown as a mean of two independent experiments with standard error ($P < 0.05$)

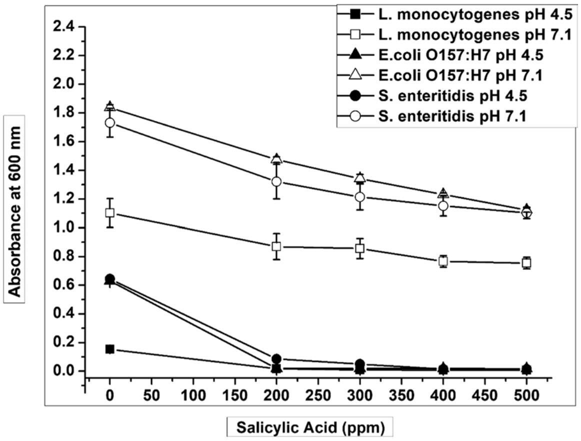

Fig. 2 The Influence of different pH values on bactericidal activity of salicylic acid against *E.coli* O157: H7, *S. enteritidis*, and *L. monocytogenes*. Salicylic acid concentrations (0, 20, 40, 60, 80, and 100) ppm were each added to 10 ml tryptone soya broth tubes, and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data are shown as a mean of two independent experiments with standard error ($P < 0.05$)

Influence of different pH values on bactericidal activity of salicylic acid against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

At pH 7.1, salicylic acid produced a gradual linear decrease in the growth of *L. monocytogenes*, *E. coli* O157:H7, and *S. enteritidis* with a minimum inhibition concentration of 500 ppm. At pH 4.5, a rapid decline in the growth of *L. monocytogenes*, *E. coli* O157:H7, and *S. enteritidis* at 200 ppm occurred along with an unchanging level of the growth. Antimicrobial activities of salicylic acid against *E. coli* O157:H7 were higher than *S. enteritidis* at pH 4.5 (Fig. 2).

Influence of different pH values on bactericidal activity of acetylsalicylic acid against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

A proportional decline in the growth of *L. monocytogenes*, *E. coli* O157:H7, and *S. enteritidis* occurred at pH 7.1 with a minimum inhibition concentration of 500 ppm acetylsalicylic acid. At pH 4.5, a slight gradual reduction in the growth of *E. coli* O157:H7 and *S. enteritidis* was recorded with the increase in the concentration of acetylsalicylic acid. Acetylsalicylic acid was more inhibitive against *S. enteritidis* than *E. coli* O157:H7 (Fig. 3).

Influence of different pH values on bactericidal activity of benzoic acid against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

A remarked decline in the growth of *L. monocytogenes* and *S. enteritidis* occurred at pH 7.1 with an MIC of 200 ppm benzoic acid. *E. coli* O157:H7 recorded a proportional decrease in the growth pattern with an MIC of 300 ppm. At pH 4.5, *S. enteritidis* and *L. monocytogenes* showed an inhibitory effect at an MIC of 150 ppm and 50 ppm benzoic acid, respectively. *E. coli* O157:H7 recorded a rapid decline in growth level with an MIC of 300 ppm. The inhibitory effect of benzoic acid against *L. monocytogenes* was higher than *S. enteritidis* and *E. coli* O157:H7 at pH 4.5 (Fig. 4).

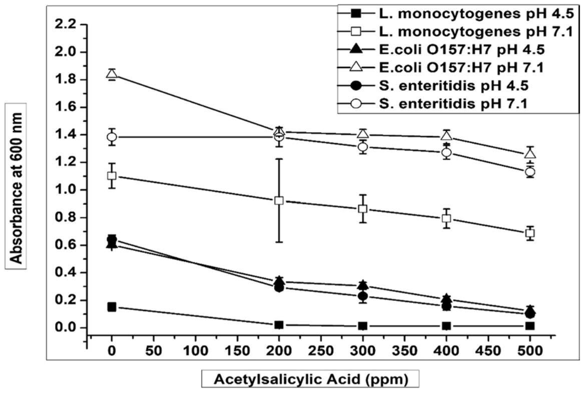

Fig. 3 Influence of different pH values on bactericidal activity of acetylsalicylic acid against *E.coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*. Acetylsalicylic acid concentrations (0, 5, 10, 15, 20, and 25) ppm were each added to 10-ml tryptone soya broth tubes and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data shown as a mean of two independent experiments with standard error ($P < 0.05$)

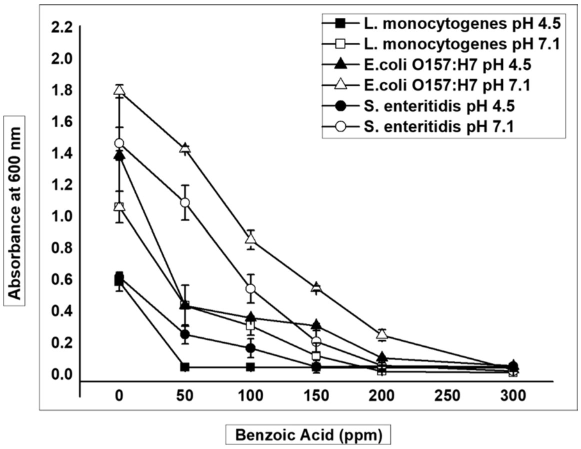

Fig. 4 Influence of different pH values on bactericidal activity of benzoic acid against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*. Benzoic acid concentrations (0, 5, 10, 15, 20, and 25) ppm were each added to 10-ml tryptone soya broth tubes and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data shown as a mean of two independent experiments with standard error ($P < 0.05$)

Influence of different pH values on bactericidal activity of sodium benzoate against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

A linear relationship in the inhibition of *L. monocytogenes*, *E. coli* O157:H7, and *S. enteritidis* occurred and reporting a minimum inhibition concentration of 300 ppm sodium benzoate at pH 7.1. At pH 4.5, a rapid proportional decrease occurred in the growth of *L. monocytogenes* with a minimum inhibition concentration of 50 ppm. On the other hand, *E. coli* O157:H7 and *S. enteritidis* showed higher inhibition with minimum inhibition concentration of 300 ppm and 200 ppm, respectively (Fig. 5).

Influence of different pH values on bactericidal activity of 3-*t*-butyl-4-Hydroxyanisole against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

At pH 7.1, a proportional inhibitory relationship occurred between 3-*t*-butyl-4-Hydroxyanisole concentrations and the growth of *E. coli* O157:H7, and *S. enteritidis* reporting an MIC of 300 ppm. However, a dramatic decrease of *L. monocytogenes* occurred with an MIC of 200 ppm. The effect of *t*-butyl-4-Hydroxyanisole was greater on *S. enteritidis* than *E. coli* O157:H7. At pH 4.5, the initial inhibition was gradually decreased with *E. coli* O157:H7, and

S. enteritidis followed by a steady pattern at 300 ppm. In the case of *L. monocytogenes*, a gradual reduction in the growth occurred at a minimum inhibition concentration of 200 ppm (Fig. 6).

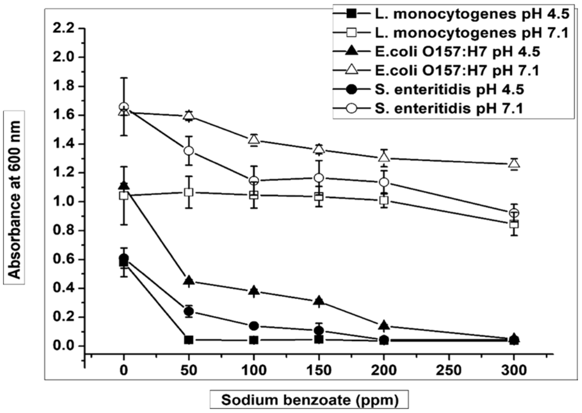

Fig. 5 Influence of different pH values on bactericidal activity of sodium benzoate against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*. Sodium benzoate concentrations (0, 5, 10, 15, 20, and 25) ppm were each added to 10-ml tryptone soya broth tubes and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data shown as a mean of two independent experiments with standard error ($P < 0.05$)

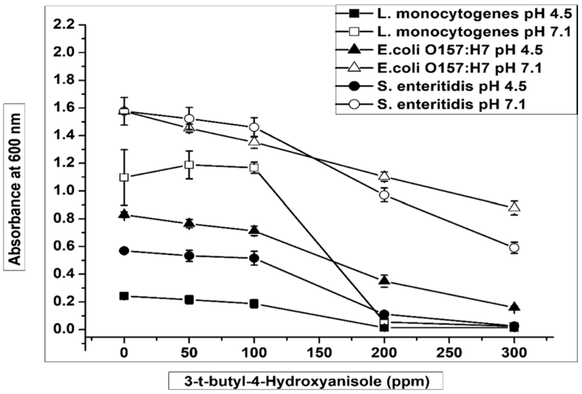

Fig. 6 Influence of different pH values on bactericidal activity of 3-*t*-butyl-4-Hydroxyanisole against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*. 3-*t*-butyl-4-Hydroxyanisole concentrations (0, 5, 10, 15, 20, and 25) ppm were each added to 10-ml tryptone soya broth tubes and then inoculated (50 µl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data shown as a mean of two independent experiments with standard error ($P < 0.05$)

Influence of different pH values on bactericidal activity of ibuprofen and 4-Acetamidophenol against *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes*

Both Ibuprofin and 4-Acetamidophenol did not show significant inhibition in the growth of *E. coli* O157:H7, and *S. enteritidis* and *L. monocytogenes*. Our findings confirmed that 4-Acetamidophenol did not inhibit both *E. coli* O157:H7 and *L. monocytogenes*. At pH 4.5, a steady growth curve was noticed then a decrease in the growth of *S. enteritidis* was recorded at 300 ppm (Fig. 7).

Our findings stated that as the pH of the media decreases, thymol becomes more inhibitive and recorded low MIC against the tested bacterial strains. The inhibitory effect of all antimicrobials was higher against *L. monocytogenes* at pH 4.5 than pH 7.1. Some antimicrobials such as salicylic acid, benzoic acid, and sodium benzoate showed lower MIC against either *E. coli* O157:H7 or *S. enteritidis*. Generally, the MIC of all antimicrobials recorded the lowest value with *L. monocytogenes* as shown in Tables 1 and 2.

Discussion

Our study is an attempt to search for alternative solutions to inhibit foodborne pathogens. This paper highlights the antibacterial activity of natural and organic compounds against the following significant bacterial pathogens: *L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* at pH 4.5 and pH 7.1. Thymol was the most inhibitive with an MIC of 25 ppm against all bacterial strains at pH 4.5. A study conducted by Mathela et al. reported a higher MIC (250 ppm) of thymol against *E. coli* (Mathela et al. 2010). Other studies reported an MIC 2.5 mM of thymol against both *E. coli* and *S. typhimurium* (Palaniappan and Holley 2010). Trombetta et al. reported an MIC of 5.00 mg/ml against *E. coli* ATCC 15221 using the microdilution method (Trombetta et al. 2005). In 2014, a study documented the antimicrobial properties and mechanism of action of thymol against *S. typhimurium* with an MIC value of 750 mg/L thymol (Chauhan and Kang 2014). It is of interest to mention that our results showed a lower MIC range of thymol against bacterial pathogens in comparison to the above-mentioned studies. The higher concentration of thymol might be toxic, and therefore, more studies need to be conducted to reach the critical concentration of non-toxic and antimicrobial activity (Burt 2004). Our findings represent the lower concentration of thymol for potential safe utilization in food preservation.

Our results indicated that salicylic acid recorded an inhibitory effect against the tested pathogens. Many plants part produces salicylic acid as a defense mechanism against microbial attack. Many medical and food industries rely on salicylic acid as it harbors anti-infective

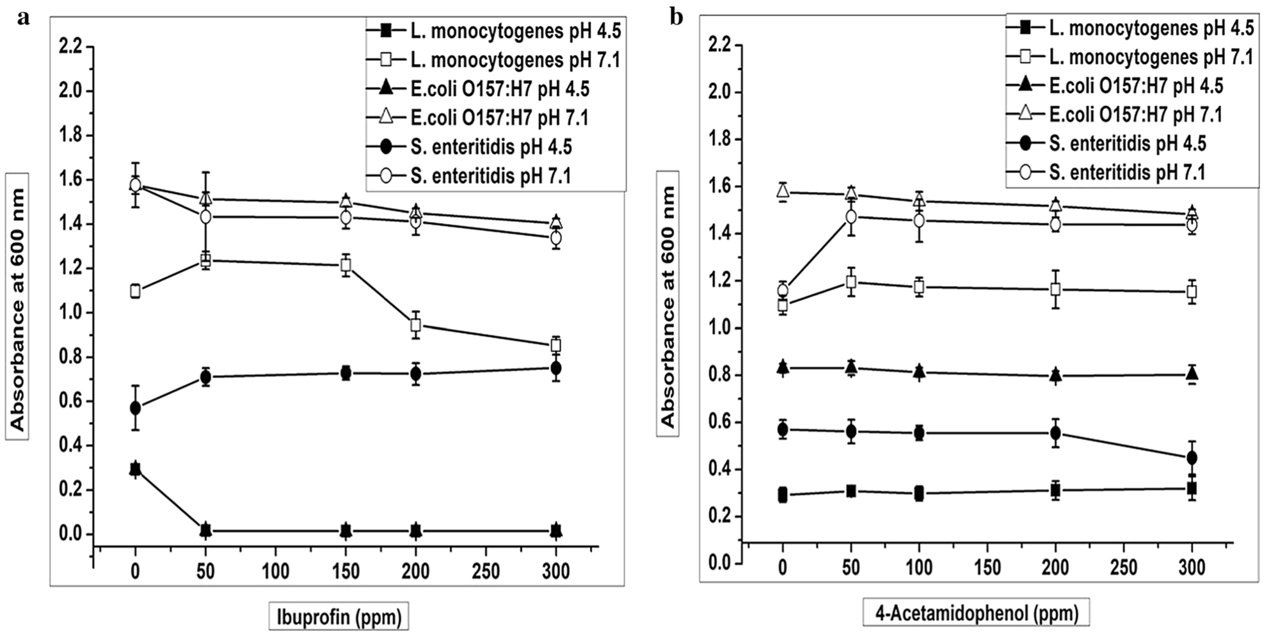

Fig. 7 Influence of pH on bactericidal activity of each antimicrobial against *E. coli* O157:H7, *S. enteritidis,* and *Listeria monocytogenes.* **a** Ibuprofen, **b** 4-Acetamidophenol. Ibuprofin and 4-Acetamidophenol concentrations (0, 5, 10, 15, 20, and 25) ppm were each added to 10-ml tryptone soya broth tubes and then inoculated (50 μl) of an overnight culture of bacterial suspension (O.D 600 = 1.818). Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Data shown as a mean of two independent experiments with standard error ($P < 0.05$)

Table 1 Minimum inhibitory concentration of the natural and organic antimicrobials against *L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* at pH 4.5

Tested compound	Tested range	Minimum inhibitory concentration in ppm		
		L. monocytogenes	***E. coli* O157:H7**	***S. enteritidis***
		pH 4.5	**pH 4.5**	**pH 4.5**
Thymol	0–300	25 ppm	25 ppm	25 ppm
Salicylic acid	0–500	200 ppm	200 ppm	200 ppm
Acetylsalicylic acid	0–500	200 ppm	500 ppm	500 ppm
Benzoic acid	0–300	50 ppm	300 ppm	150 ppm
Sodium benzoate	0–300	50 ppm	300 ppm	200 ppm
3-*t*-butyl-4-Hydroxyanisole	0–300	200 ppm	300 ppm	300 ppm
Ibuprofin	0–300	50 ppm	50 ppm	NA
4-Acetamidophenol	0–300	NA	NA	300 ppm

Different concentrations of each antimicrobial were each added to 10-ml tryptone soya broth tubes and then inoculated (50 μl) of an overnight culture of the bacterial suspension. Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Minimum inhibitory concentration is the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth

NA not applicable

activities rather than antimicrobial activity. Consequently, no antibiotic resistance was evolved with salicylic acid like other antimicrobials (Gilbert et al. 2002; Prithiviraj et al. 2005). Rosenberg et al. (2008) reported that salicylate-based poly(anhydride esters) prevented biofilm formation of *Salmonella enterica* serovar *Typhimurium* by affecting surface attachment. Lemos et al. (2014) reported that 500 μg/ml salicylic acid inhibited *Bacillus cereus* and *Pseudomonas fluorescens.* Another study reported an MIC of salicylic acid (450 and 400 μg/ml) against *E. coli* O157:H7 and *Salmonella Typhimurium*, respectively (Tuncel and Nergiz 1993).

Table 2 Minimum inhibitory concentration of the natural and organic antimicrobials against *L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* at pH 7.1

Tested compound	Tested range	Minimum inhibitory concentration in ppm		
		L. monocytogenes	*E. coli* O157:H7	*S. enteritidis*
		pH 7.1	pH 7.1	pH 7.1
Thymol	0–300	200 ppm	25 ppm	25 ppm
Salicylic acid	0–500	500 ppm	500 ppm	500 ppm
Acetylsalicylic acid	0–500	500 ppm	500 ppm	500 ppm
Benzoic acid	0–300	200 ppm	300 ppm	200 ppm
Sodium benzoate	0–300	300 ppm	300 ppm	300 ppm
3-*t*-butyl-4-Hydroxyanisole	0–300	300 ppm	300 ppm	300 ppm
Ibuprofin	0–300	50 ppm	50 ppm	NA
4-Acetamidophenol	0–300	NA	NA	NA

Different concentrations of each antimicrobial were each added to 10-ml tryptone soya broth tubes and then inoculated (50 μl) of an overnight culture of the bacterial suspension. Tubes were incubated at 37 °C for 24 h. Negative and positive controls were performed. The optical density of each tube was measured using a spectrophotometer. Minimum inhibitory concentration is the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth

NA not applicable

To the best of our knowledge, this is the first study to highlight the inhibitory effect of *L. monocytogenes* and *E. coli* O157:H7 with very low MIC of 200 ppm salicylic acid at pH 4.5. Our results disagreed with another study that clarified the null inhibitory effect of acetylsalicylic acid against *Escherichia coli, L. monocytogenes,* and *Salmonella enterica* (Friedman et al. 2003). More reports recorded the efficiency of acetylsalicylic acid against fungal and bacterial pathogens. El-Metwally et al. (2015) emphasized that the treatment of seeds with (15 mM) of acetylsalicylic acid followed by H_2O_2 (0.50 mM) can be used as a fungicide substitutes for controlling lupine root rot disease, improve growth and production. Additionally, Almoneafy et al. declared the synergism of acetylsalicylic acid and DL-Beta-aminobutyric acid on biocontrol effectiveness against *Bacillus* strains causing tomato bacterial wilt (Almoneafy et al. 2013). Our results contradict another study of benzoic acid against *Escherichia coli, L. monocytogenes,* and *Salmonella enterica* (Friedman et al. 2003). In 2017, a synergistic study of UV-A light and benzoic acid was conducted against *E. coli* O157:H7 and enhanced antimicrobial activity (Ding 2017).

Our findings confirmed that sodium benzoate showed higher antimicrobial activities on *L. monocytogenes* than *S. enteritidis* and *E. coli* O157:H7 at pH 4.5. Additionally, sodium benzoate was more effective on *S. enteritidis* than *E. coli* O157:H7. Similarly, 0.05 or 0.1% sodium benzoate was found to highly inactivate *L. monocytogenes* at pH 5.0, at 35 °C (EL-shenawy and Marth 1988). Some synergistic studies were conducted and inhibited some foodborne pathogens. Kumar et al. (2017) reported that sodium benzoate-functionalized silver nanoparticles showed strong antimicrobial activities against *Escherichia coli* and *Salmonella typhimurium* type 2. Ceylan et al. (2004) declared that sodium benzoate (0.1%) with cinnamon (0.3%) showed antimicrobial activity against *E. coli* O157:H7 in apple juice.

The highest inhibitory effect of *t*-butyl-4-Hydroxyanisole occurred with *L. monocytogenes* at pH 4.5 and pH 7.1. Synergistic antimicrobial activity of butylated hydroxyl anisole was enhanced when mixed with sucrose laurate and ethylene diamine tetraacetate against *L. monocytogenes* and *S. typhimurium* (Sikes and Ehioba 1999).

Ibuprofin was more inhibitive against *S. enteritidis* than *E. coli* O157:H7. Lowering the pH signifies the effect of ibuprofen on *L. monocytogenes* and *E. coli* O157:H7. A recent study declared an enhanced effect of ibuprofen accompanied by sodium chloride (Muñoz et al. 2018). Another study confirmed the anti-biofilm effect of ibuprofen against *E. coli* (Baldiris et al. 2016). Controversy, the antimicrobial effect of 4-acetamidophenol against *E. coli* O157:H7 was reported (Kang and An 2010). Other studies affirmed the microbial degradation of 4-acetamidophenol when used as sole carbon and energy (Hu et al. 2013; De Gusseme et al. 2011). These findings can explain the recorded promoted bacterial growth of *S. enteritidis* with 4-Acetamidophenol.

Our results are in agreement with previous studies, reporting that Gram-positive bacteria are more susceptible than Gram-negative to natural antimicrobials (Harpaz et al. 2003; Pintore et al. 2002). It is probably due to the lipopolysaccharide outer membrane surrounding the cell wall which prevents diffusion of hydrophobic compounds (Vaara 1992). However, some studies recorded no evidence for a difference in sensitivity between

Gram-negative and Gram-positive. (Wan et al. 1998; Wilkinson et al. 2003). Others suggested that the inhibitory effect was more often extended to 48 h with Gram-negative rather than Gram-positive bacteria (Ouattara et al. 1997). The present findings are in agreement with studies, demonstrating that thymol possesses higher antibacterial toward a large range of species forming biofilm at low pH values of 4.33–5.32 (Gutierrez et al. 2008; Zheng et al. 2013). Additionally, the present study is in agreement with previous reports that demonstrate the effect of pH on the inhibitive activity of thymol against *E. coli* O157:H7 and *L. monocytogenes* (Shah et al. 2012; Zheng et al. 2013) since thymol molecule becomes more hydrophobic at low pH. It may bind and dissolve to hydrophobic areas of proteins properly and resulted in more bactericidal activity (Juven et al. 1994). Our work confirmed a different degree of inhibition against tested strains. In 2018, a recent study supported this opinion describing that different physical, chemical factors, and their interaction influence the inhibitive effect of the natural antimicrobial compound against *salmonella Typhimurium* PT4 and *E. coli* O157:H7 (Carvalho et al. 2018).

Conclusion

This research highlights the utilization of various natural and organic antimicrobials in the inhibition of foodborne pathogens. Thymol found to be superior to other antimicrobial agents as follows: Ibuprofin, benzoic acid, sodium benzoate, salicylic acid, 3-*t*-butyl-4-Hydroxyanisole, and acetylsalicylic acid. We suggest future investigations in vivo studies on the bactericidal effect at low pH should be for food safety and public health applications. Our findings will support pharmaceutical and food industries, decrease foodborne pathogen outbreaks, and hence improve public health in Egypt and on a global scale.

Abbreviations

%: Percentage; °C: Degree celsius; μl: Microliter; CFU: Colony forming unit; g: Gram; GRAS: Generally recognized as safe; h: Hour; MIC: Minimum inhibition concentration; min: Minute; ml: Milliliter; mol l^{-1}: Mol per liter; N/A: Not applicable; nm: Nanometer; O.D: Optical density; ppm: Part per million; rpm: Revolution per min; TSB: Tryptone soya broth; WHO: World Health Organization.

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Authors' contributions

AS managed the paper work, DHA and AS wrote the first draft of manuscript, agreed with the manuscript's results and conclusions. Both authors approved the final manuscript.

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Competing interests

The authors DHA and AS declare that they have no competing interests.

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