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Antioxidant and Antimicrobial Activity of Basil, Thyme and Tarragon Used in Meat Products

Elisaveta Sandulachi[1], Artur Macari[1], Aliona Ghendov-Mosanu[1*], Daniela Cojocari[2*], Rodica Sturza[3]

[1]Department of Food Technology, Technical University of Moldova, Chisinau, Republic of Moldova
[2]Department of Preventive Medicine, "Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[3]Department of Oenology and Chemistry, Technical University of Moldova, Chisinau, Republic of Moldova
Email: *aliona.mosanu@tpa.utm.md, *daniela.cojocari@usmf.md

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Abstract

Currently, the food industry, including the meat industry, is paying close attention to the use of natural additives as preservatives. From ancient times, the plants are used to treat various diseases, to produce perfumes and as ingredients to improve the taste in food. This paper presents a bibliographic and experimental study of the antioxidant and microbial properties of basil, thyme and tarragon. International scientific papers on the use of these plants in the food industry, including the meat industry, are targeted. The role of basil, thyme and tarragon in the manufacture of functional and stable products in storage is mentioned. Percentage decrease in *Salmonella Abony* growth under the influence of basil constituted 84.4%, thyme 61.6% and tarragon 76.8% after 48 hours of action and respectively 97.2%, 90.2% and 95.3% after 72 hours of action. The interdependence between the percentage reduction of S. Abony infestation and the concentration of basil, mushrooms and tarragon was respectively: basil ($R^2 = 0.7725 \ldots 0.7916$), thyme ($R^2 = 0.7733 \ldots 0.7768$), tarragon ($R^2 = 0.7689 \ldots 0.8137$).

Keywords

Basil, Tarragon, Thyme, Antimicrobial Activity, Antioxidant Activity, Meat Products

1. Introduction

The European Commission has accepted various components of essential oil (EO) as food-friendly and safe. The FDA classifies these substances as GRAS (generally recognized as safe). This category includes thyme, cloves, cinnamon, oregano, mustard, nutmeg and basil [1] [2] [3] [4].

The use of new plant oil extraction technologies makes it possible to use them successfully in the manufacture of supplements and new products with high biological value [5] [6]. The authors of the studies [7] and [8] mention the great biological and structural diversity of the compounds found in different plants, noting their antifungal, antibacterial and antiparasitic properties. The antioxidant properties of thyme have been reported in studies [9] [10] [11], mentioning its use in the stability of meat lipids. Also of interest are the results of the study of thyme used for pork, reported by Tanabe H, *et al.* [12]; and for beef reported by Medina *et al.* [13].

The data reported in studies [13] [14] [15] [16] [17], regarding the antimicrobial properties of herbs and spices, would be of interest to food industry specialists.

Natural antioxidants are increasingly mentioned as an object of study in research worldwide [18] [19] [20] the most important being tocopherols, flavonoids and phenolic acids. Most often these substances are investigated as a potential to prevent or delay the oxidation of lipids [21] [22]. The content and activity of antioxidants in plants depends on several factors: climate genotype, temperature, light, soil type and other conditions (processing, storage after harvest), etc. [23].

2. General Characteristics of Thyme, Basil and Tarragon

Antioxidant and antimicrobial activity of thyme.

The role of thyme in increasing stability and reducing lipid oxidation during food storage is reported in papers [24] [25] [26]. The oxidative inhibitory capacity of thyme extracts is mainly due to the content of phenolic compounds [27]. The results presented by the study authors [28] [29] suggest the idea that essential oils from different Thymus species, having antimicrobial properties, can be used in the food industry.

Tepe *et al.* [30] studied the antioxidant properties of two varieties of *Thymus sipyleus*, reporting different constituents in their essential oil. Dorman *et al.* [31] established that there is no direct relationship between the antioxidant efficacy of an extract and its total phenolic compound content. Another study by Sun *et al.* [32] established that there is an apparent relationship between the antioxidant potential of *Thymus zygis* extracts and the total phenols they contain. Thyme phenols are characterized by redox properties and neutralize free radicals [1] [33]. Essential oils rich in phenolic compounds have antimicrobial properties [34]. Factors that influence the microbial activity of thyme oils are: low temperatures, anaerobic conditions and low pH. Gram-positive bacteria appear to be less sensitive to thyme action than gram-negative bacteria [2] [35]. Evans and Martin [36] reported antimicrobial activity of thyme on *Salmonella*, *Staphylococcus*, *Escherichia coli*, *Klebsiella*, *Pseudomonas and Enterococcus*.

Antioxidant and antimicrobial activity of basil.

Sweet basil (*Ocimum basilicum* L.) has relevant antioxidant and antimicrobial properties [37] [38] [39]. The authors of the studies [40] [41] report the antibacterial properties (Gram-negative and Gram-positive bacteria, yeast, and mold). Another study by Zheljazkov *et al.* includes oil content, composition and bioactivity of *Ocimum basilicum* L. [42]. Rezzoug M. *et al.* [43] also mentioned the antioxidant and antimicrobial activity of plants, and the possibility of using them as natural preservatives is discussed by Tiwari B.K *et al.* [44]. Also relevant are the studies: [45] [46], which report moderate antioxidant activity and strong antifungal and antibacterial activity of basil extracts, [47] the use of basil oil for the production of organic packaging is mentioned in the study, [48] cure fungal infections and stop the growth of *Aspergillus flavus* and aflatoxin B1 production. In their research, Gülten Ökmen *et al.* [49] noted that the antioxidant and antimicrobial properties of *Ocimum basilicum* L. depend on the solvent (methanol or water), a high antioxidant activity was obtained from water extracts (72%).

Antioxidant and antimicrobial activity of tarragon

Lipid oxidation [50] [51] and contamination with microorganisms diminish the quality and safety of meat products [52]. The results of the study by Chaleshtori *et al.* [53] in meat products have shown that Tarragon (*Artemisia dracunculus* L.) oil has antibacterial and flavoring properties. Behbahani *et al.* [54] reported the chemical composition of *Artemisia dracunculusas* L. essential oil, mentioning in detail the content of antioxidants, as well as antimicrobial activity against fungi with an average inhibitory area of 14.70 mm. The antibacterial potential of *Artemisia dracunculusas* L. plant oil has been reported in several studies: depending on the method of obtaining the oil [55] [56], the method of testing bacteriostatic and bactericidal activities [57], the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [58] [59].

The antibacterial activity of an aqueous infusion of tarragon against Gram-positive bacteria and Gram-negative bacteria was reported in the study by Majdan *et al.* [60].

Currently, the antioxidant and antimicrobial properties of thyme, basil and tarragon are being studied by many scientists. **Table 1** and **Table 2** are presented some results obtained at international level.

3. Materials and Methods

Materials.

Sausages obtained by the classical method (control test) and with the addition of lyophilized hydroalcoholic extracts 60% (v/v) of basil, thyme, and tarragon in concentrations of 0.1%; 0.2% and 0.3%. Sausages previously infected with reference strains: *Salmonella Abony* were investigated for the growth rate of pathogenic microorganisms within 24, 48 and 72 hours. The concentration of bacterial cell suspensions was 2×10^5 CFU/mL.

Table 1. The components that exhibit antibacterial properties.

Essential Oil	Plant source	Major components	Bibliographic sources
Thyme	*Thymus vulgaris*	Thymol, carvacrol, γ-terpinene, *p*-cymene, linalool.	[61]-[69]
Thyme	*Thymus vulgaris*	1,8-cineol (14.26%), γ-terpinen (12.06%), *p*-cimen (10.50%) and α-terpinen (9.22%).	[70]
Thyme sp		α-thujene, α-pinene, β-myrcene, phellandrene, α-terpinene, *p*-cymene, γ-terpinene, 4-terpineol, carvacrol, β-bisabolene, carvone.	[71] [72]
Thyme sp		Thyme contains monoterpene phenols, including carvacrol (isopropyl-*o*-cresol; 0.4% - 20.6%), thymol (2-isopropyl-5-methylphenol or isopropyl-*m*-cresol) and *p*-cymene, and other monoterpenes, such as α-pinene, 1,8-cineol, camphor, linalool and borneol.	[1] [3]
Basil	*Ocimum basilicum* L.	Geraniol, *p*-allylanisole, 1,8–cineole, *trans*–α–bergamotene and neryl acetate.	[73] [74]
Basil	*Ocimum basilicum* L.	Total amount of phenols. High activity of the extract was also demonstrated in the DPPH and ABTS tests.	[60]
Basil	*Ocimum basilicum* L.	Flavonoids and phenolic acids.	[58]
Basil	*Ocimum basilicum* L.	Methyl chavicol, gitoxigenin, trimethoquinol, β-guaiene, aciphyllene, alizarin, naphthaline, (−)-caryophyllene, and mequinol	[75] [76]
Basil	*Ocimum basilicum* L.	The 52 compounds were identified: linalool, 1,8-cineole, (Z)-isoeugenol, 1-epi-cubenol, α-transbergamotene, and (Z)-anethol. Further compounds, occurring in amounts between 2 and 3%, are *trans*-muurola-4-(14), 5-diene (2.8%), caryophyllene (2.4%), isobornylacetate (2.1%), whereas all the others are present in amounts lower than 2%.	[77]
Basil	*Ocimum basilicum* L.	Estragole, 1, 6-octadien-3-ol, 3,7-dimethyl.	[74]
Basil	*Ocimum basilicum* L.	Methyl chavicol, *trans*-ocimen, z-β-ocimen, limonene and α-pinene. Most of the compounds were monoterpene hydrocarbons and the lowest-sesquiter-feather hydrocarbons.	[60]
Tarragon	*Artemisia dracunculus* L.	Caffeoylquinic acids, quercetin, isorhamnetin, syringetin, apigenin, patuletin derivatives, davidigenin, sakuranetin, four phenolic acid derivatives and one coumarin.	[60]
Tarragon	*Artemisia dracunculus* L.	α-pinene, β-pinene, β-myrcene, limonene, z-β-ocimene, *trans*-ocimene, terpinene, linalool, ocimene (allo), methyl chavicol, geranial iso bornyl acetate eugenol, iso safrole (E), methyl eugenol, valencene, β-sesquiphellandrene cinnamaldehyde, spathulenol.	[78]
Tarragon	*Artemisia dracunculus* L.	Major phenolic compounds, chlorogenic, syringic, and caffeic acids, the predominant flavonoid-quercetin.	[79] [80]
French tarragon	*Artemisia dracunculus* L.	Anisaldehyde, paracymene, eugenol, limonene, linalool, menthol, cis-ocimene, α-phellandrene, α-pinene and β-pinene.	[81]

Table 2. Antimicrobial activity.

Essential Oil	Inhibited microorganisms	Bibliographic sources
Thyme	*B. ceruse, E. coli, L. monocytogenes, S. Typhimurium, S. typhi, S. aureus, Yersinia spp.*	[70]
Thyme	*Cl. perfringens, Shigella sonnei, Sarcina lutea, Brochothrix thermosphacta.*	[73] [82]
Thyme	*F. oxysporum, F. verticillioides, P. expansum, P. brevicompactum, A. flavus, A. fumigatus, Alternaria alternata.*	[83]
Thyme	*S. aureus, E. coli, P. aeruginosa as well as against Streptococcus pyogenes, Corynebacterium, Salmonella, Bacteroides and Candida albicans.*	[84]
Thyme sp	*S. aureus, E. coli.*	[72]
Thyme sp	*L. innocua* and *L. monocytogenes.*	[84] [85]
Basil	Gram-positive bacteria and moderate activity Gram-negative bacteria.	[83]
Basil	*B. cereus, B. subtilis, B. megaterium, S. aureus, L. monocytogenes, E. coli, Sh. boydii, Sh. dysenteriae, V. parahaemolyticus, V. mimicus, and S. typhi.*	[75]
Basil	*B. thermosphacta* 7R1, *B. thermosphacta* D274, *Carnobacterium maltaromaticum* 9P, *Carnobacterium maltaromaticum* D1203, *E. coli* 32, *E. faecalis* 226, *E. faecalis* E21, *Hafnia alvei* 53M, *Listeria innocua* 1770, *Serratia proteamaculans* 20P, *Streptococcus salivarius* GM, *Staphylococcus saprophyticus* 3S, *Staphylococcus xylosus* ES1.	[77]
Tarragon	*S. aureus* ATCC6538, *S. epidermidis* ATCC14990, and *S. aureus* MRSA (methicyllin-resistant *S. aureus*) ATCC43300.	[60]
Tarragon	*S. aureus* PTCC (Persian Type Culture Collection) 1189, *Al. faecalis* PTCC 1624, *Providencia rettgeri* PTCC 1512, *Serratia marcescens* PTCC 1621, *Sh. dysenteriae* PTCC 1188, *L. monocytogenes* PTCC 1163 and *Klebsiella oxytoca* 1402.	[78]
Tarragon	*E. coli, P. aeruginosa, S. aureus, Str, faecalis and Y. enterocolitica.*	[80]

Count bacteria.

Bacterial growth in the tested sausage samples was assessed by the standard method [86].

Growth rate of bacteria.

The specific growth rates of individual strains were calculated as:

$$\mu = \frac{\ln X - \ln X_0}{\Delta \tau} \tag{1}$$

where: X—the number of bacteria in the end of the exponential growth phase.

X_0—the number of bacteria in the beginning of the exponential growth phase.

$\Delta\tau$—the time interval between observations.

Statistical analysis.

The analysis of the variance of the results was performed by applying the Student test and the Microsoft Office Excel 2010 program (p value < 0.05). All tests were performed in triplicate. The experimental results are expressed as average ± SD.

4. Results and Discussions

The influence of various basil, thyme, and tarragon extracts on the development of *Salmonella Abony* after 24, 48 and 72 hours was studied, with which the sausage samples were inoculated. The results are presented in Figure 1. The control sample shows a much larger number of colonies of microorganisms compared to the other samples.

The growth rate of *Salmonella Abony* was calculated, the results are shown in Table 3. After 24 hours the growth rate of bacteria was 0.3 in all samples. Different results were obtained after 48 and 72 hours of incubation at 37˚C. In the control sample the growth rate of *Salmonella Abony* was 0.18 and respectively 0.12, in the samples with the addition of basil, thyme, and tarragon the rate for concentration of 0.3% was 0.14 - 0.15 and respectively 0.07 - 0.09.

Our experimental results confirm the antimicrobial properties of basil, thyme, and tarragon exposed by other authors presented above. Figure 2 shows the percentage decrease in *Salmonella Abony* growth under the influence of thyme, basil, and tarragon after 48 and 72 hours.

The most effective in reducing *Salmonella Abony* was basil, in the samples with an addition of 0.2%, the decrease in bacterial growth was 77.2%, and in

Table 3. Growth rate of *Salmonella Abony* bacteria on different nutritional substrates.

Name of the sample	Concentration, %	Incubation time, h						
		0	24		48		72	
		ln$X$	ln$X$	GM*, μ	ln$X$	GM, μ	ln$X$	GM, μ
Control		12.21	20.11	0.33 ± 0.01	20.72	0.18 ± 0.02	20.99	0.12 ± 0.01
Basil	0.1	12.21	19.87	0.32 ± 0.02	20.52	0.17 ± 0.01	19.30	0.10 ± 0.01
	0.2	12.21	19.76	0.31 ± 0.01	19.24	0.15 ± 0.02	19.03	0.09 ± 0.01
	0.3	12.21	10.82	0.32 ± 0.01	18.87	0.14 ± 0.01	18.40	0.09 ± 0.01
Thyme	0.1	12.21	19.20	0.29 ± 0.01	20.55	0.17 ± 0.01	19.37	0.10 ± 0.01
	0.2	12.21	19.23	0.29 ± 0.01	20.44	0.17 ± 0.01	18.79	0.09 ± 0.01
	0.3	12.21	19.04	0.28 ± 0.02	19.03	0.14± 0.02	17.18	0.07 ± 0.01
Tarragon	0.1	12.21	19.71	0.31 ± 0.01	20.70	0.18 ± 0.01	20.18	0.11 ± 0.01
	0.2	12.21	19.46	0.30 ± 0.02	20.51	0.17 ± 0.01	19.80	0.11 ± 0.01
	0.3	12.21	19.15	0.29 ± 0.01	19.26	0.15 ± 0.01	17.69	0.08 ± 0.01

*Note: GM—Growth Monitoring.

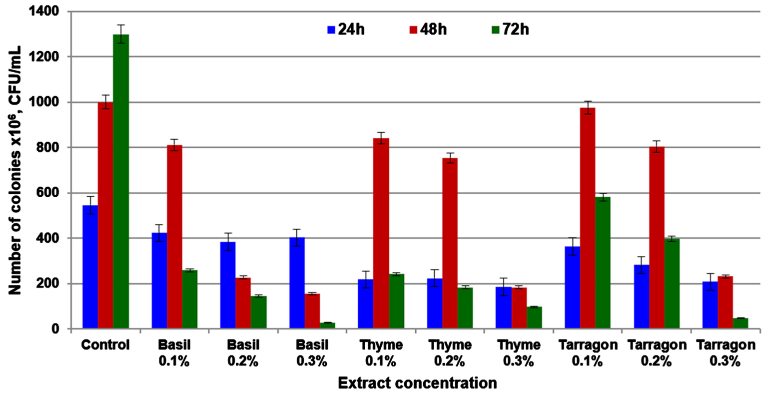

Figure 1. The impact of the addition of different concentrations of basil, thyme, and tarragon extracts on the growth of *Salmonella Abony* after 24, 48 and 72 hours (errors bars represent the standard deviation of three determinations).

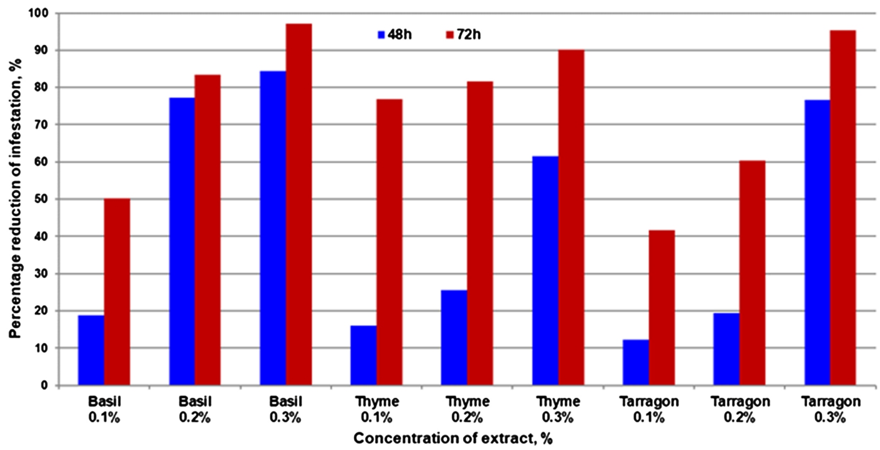

Figure 2. Percentage decrease in *Salmonella Abony* growth under the influence of basil, thyme, and tarragon extracts after 48 and 72 hours.

those with an addition of 0.3% the decrease in infestation was 84.4%. Approximately these values were also maintained in the samples with the addition of 0.3% tarragon (76.7%) and thyme (61.6%).

Analyzing the case study data after 72 hours, it was found that Basil showed the highest rate of stopping the growth of *S. abony* (97%) although at the concentration of 0.1%, the reduction rate was the average one (50.3%). The rate of decrease given by Tarragon was 95% (an average value among the samples of plants examined), although at concentration of 0.1%, the effect was the smallest (41.7%). Thyme showed the smallest decrease (90.2%), while at the concentration of 0.1%, the decrease was the highest (76.8%). Probably, the results were influenced by the chemical composition of the studied plants. Based on the bibliographic study we can see that the composition of plants is quite complex, it depends on several factors including climatic and regional.

The interdependence between the percentage reduction of *Salmonella Abony* infestation and the concentrations of basil, thyme and tarragon extracts is shown in **Figures 3-5**.

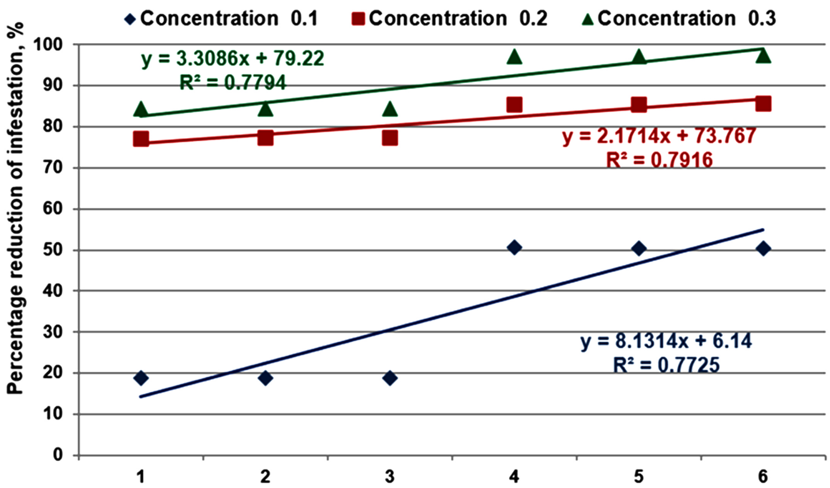

Figure 3. Interdependence between the percentage reduction of *Salmonella Abony* infestation and concentrations of basil extract.

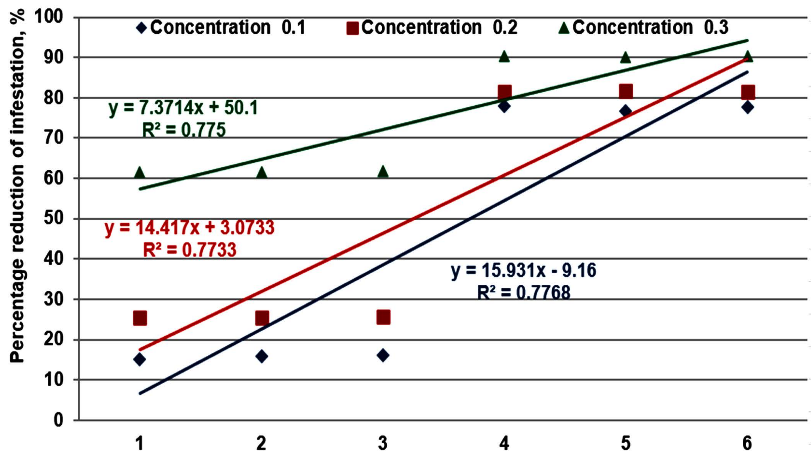

Figure 4. Interdependence between the percentage reduction of *Salmonella Abony* infestation and concentrations of thyme extract.

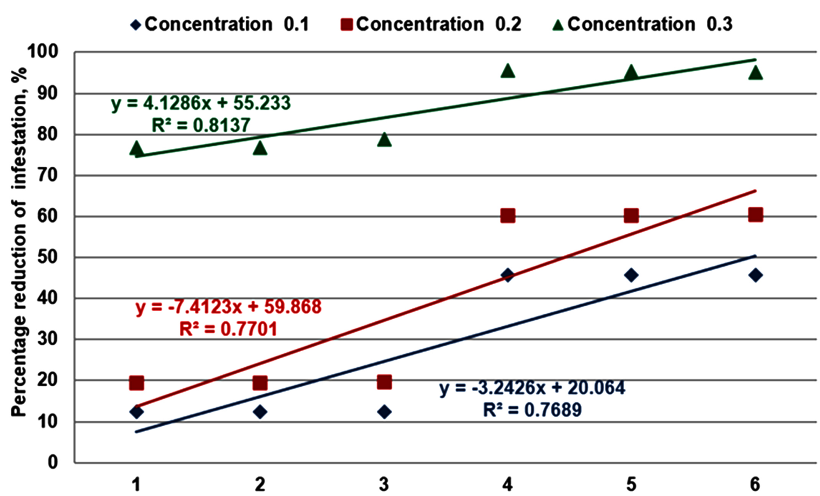

Figure 5. Interdependence between the percentage reduction of *Salmonella Abony* infestation and concentrations of tarragon extract.

The results in **Figures 3-5** show that the interdependence between the percentage reduction of *Salmonella Abony* infestation and the concentrations of basil, thyme and tarragon extracts was good.

5. Conclusion

Basil, thyme, tarragon have relevant antioxidant and antimicrobial properties. They are used as ingredients in products to control the infestation with pathogenic microorganisms. Reduction of *Salmonella Abony* infestation in sausage was 62% - 84%. The interdependence between the percentage reduction of *Salmonella Abony* infestation and the concentration of basil, thyme and tarragon was good: basil (R^2 = 0.7725 … 0.7916), thyme (R^2 = 0.7733 … 0.7768), tarragon (R^2 = 0.7689 … 0.8137). All plants showed the same antimicrobial effect on *Salmonella Abony*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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