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Antimicrobial Activity of the Essential Oil of Thyme and of Thymol against *Escherichia coli* Strains

Deise Flores Santurio[1], Francielli Pantella Kunz de Jesus[2], Régis Adriel Zanette[2], Karine Bizzi Schlemmer[2], Andressa Fraton[1] & Leadir Lucy Martins Fries[1]

ABSTRACT

***Background*:** The deterioration of food products, especially of those derived from meat, by pathogenic microorganisms is a major problem in industries. *Escherichia coli* is a facultative anaerobic bacteria of intestinal origin, and is a cause of concern in the meat industry. The use of essential oils as inhibitors of growth of spoilage and pathogenic microorganisms is a good choice for replacement of chemical additives in foods. This study was aimed at evaluating the in vitro activity of the essential oil of thyme (*Thymus vulgaris*) and thymol, against *E. coli* strains, by using a microdilution methodology based on the M31-A3 technique.

***Materials, Methods & Results*:** In this study the antimicrobial activities of the essential oil of *Thymus vulgaris* (thyme) and of the thymol compound were evaluated against 20 *E. coli* strains obtained from poultry and pigs. The strains are part of the collection of bacteria of the Laboratório de Pesquisas Micológicas of the Universidade Federal de Santa Maria (UFSM). The essential oil of thyme and its constituent, thymol, were commercially acquired (Sigma-Aldrich). Gas chromatography mass spectrometry (GC/MS) was realized on a Agilent chromatograph Model HP 6890 series CG, equipped with a mass selective detector 5973 with electron impact (CG-MS-EI) and identified p-cymene (23.71%), thymol (13.86%) and γ-terpinene (8.55%) as the major substances present in the thyme essential oil. The essential oil constituents were identified by comparing their mass spectra with those from the National Institute of Standards and Technology. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined for each isolate by using the broth microdilution technique based on the M31-A3 protocol. The geometric means of the MICs and MBCs against *E. coli* strains were of 627.7 $\mu g.mL^{-1}$ and 990.2 $\mu g.mL^{-1}$ for the thyme essential oil and of 2786 $\mu g.mL^{-1}$ and 2540 $\mu g.mL^{-1}$ for the thymol. These results show that the essential oil of thyme is a potential antimicrobial, and deserves further studies to be safely used as a preservative in foods.

***Discussion*:** The use of condiments, accompanied the process of civilization of humankind, which assures very low or no toxicity. These essential oils contain major components with significant antimicrobial activity. In this context, therefore, targeted studies are relevant to determine which essential oils are more active. Nonetheless, the inexistence of an international standardized technique for the evaluation of essential oils and vegetable extracts allows the use of different protocols, hampering the comparison of the results. The M31-A3 protocol used in our experiment is the most recommended among the techniques found in the literature. In this study, the essential oil of *T. vulgaris* and its major compound thymol showed bacteriostatic and bactericidal activities against *E. coli* strains *in vitro*. Nonetheless, the activity of the essential oil was superior to the compound alone. Such finding is explained by the fact that the high antimicrobial activity showed by some essential oils results from the synergism of the major components The use of essential oils is a viable and alternative option to replace chemical additives in food. Notwithstanding, more studies on the components of the essential oils are required to ensure their safety in food. The sensorial analysis is also an important item to be evaluated to estimate consumer acceptance of the product.

Keywords: *Escherichia coli*, thymol, thyme, *Thymus vulgaris*, antimicrobial activity, essential oil.

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[1]Departamento de Ciência e Tecnologia dos Alimentos, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. [2]Departamento de Microbiologia e Parasitologia, UFSM, Santa Maria. CORRESPONDENCE [deisesanturio@ibest.com.br - Tel.: 55 +(55) 3220-8906]. Campus UFSM, Prédio 42, Camobi. CEP 97105-900 Santa Maria, RS, Brazil.

INTRODUCTION

Escherichia coli is the predominant species among facultatively anaerobic intestinal bacteria. The ability to ferment glucose and lactose and to produce acid are amongst the characteristics of this gram negative bacillus of the family Enterobacteriaceae [9]. The conservation of meat products usually involves measures to retard or prevent microbiological, chemical and physical alterations, which would impact on organoleptic quality or make them inappropriate for consumption. Indeed, microbiological deterioration is the most important and often precedes other meat alterations [11]. On the other hand, consumers have demanded safer products, i.e., not only free of harmful biological agents but virtually free of chemical compounds [3].

In this context, the essential oils obtained from condiment and seasoning plants such as basil, cinnamon, oregano, rosemary, sage and thyme have been object of study, because besides their organoleptic properties these compounds have further functional properties. Since the major constituents of these essential oils are terpene hydrocarbons, alcohols, aldehydes, ketones, phenols, esters and organic acids, at different concentrations and with a major pharmacologically active component [15], they possess inhibitory activity against food-borne pathogens [1,3]. Among these substances, the phenolic compounds are the main responsible for the antimicrobial properties of the essential oils [6].

This study was aimed at evaluating the in vitro activity of the essential oil of thyme (*Thymus vulgaris*) and its major constituent, thymol, against *E. coli* strains, by using a microdilution methodology based on the M31-A3 technique [5].

MATERIALS AND METHODS

Microorganisms

Twenty *E.coli* enteric strains obtained from pigs and poultry were used. The strains had been previously identified by PCR and are part of the collection of bacteria of the Laboratório de Pesquisas Micológicas of the Universidade Federal de Santa Maria.

Gas chromatography mass spectrometry (GC/MS) analysis of the essential oil

The essential oil of thyme and its major constituent, thymol, were commercially acquired[1]. GC/MS analysis of the essential oil of thyme was determined on a Agilent chromatograph (Model HP 6890 series CG)[2], equipped with a mass selective detector 5973 with electron impact (CG-MS-EI) and a capillary column DB-5MS (30 m x 320 mm x 0.25 μm). Helium (carrier gas; 99.999%) flowed at a constant rate of 22.7 mL/min. The oven temperature program consisted of ramping up from 60°C to 325°C. Ionization was achieved by electron impact using an emission current of 70eV at an interface temperature of 310°C. The essential oil constituents were identified by comparing their mass spectra with those from the National Institute of Standards and Technology.

Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

One gram of the thyme oil and of thymol were diluted first in methanol to the concentration of 640 mg mL^{-1} (solution I) and then in Mueller-Hinton (MH) broth3 to the concentration of 6400 μg mL^{-1} (solution II). Thereafter, 100 μL of the solution II were added by serial dilution to 96-well plates containing 100 μL of MH broth, according to the CLSI M31-A3 guidance [5]. The concentration for both substances ranged from 3200 to 100 μg mL^{-1}. The strains of *E. coli* grown in MH agar were suspended in 0.85% saline to achieve 0.5 McFarland (1 x 10^8 $CFU.mL^{-1}$). Then 10 μL (1x10^5 $CFU.mL^{-1}$) of this inoculum were added to each well of the microdilution plates, which were incubated at 35ºC for 24h. The MIC was defined as the lowest concentration of compound at which no growth was evident compared to positive control (broth only). Immediately after the MICs were determined, the MBCs were assayed by transferring 10 μL from each culture with a compound concentration equal to or greater than the established MIC to MH agar plates. The MBC was defined as the lowest drug concentration at which no growth could be observed after 24 h of incubation at 35°C. All of the assays were performed in duplicate.

Statistical analysis

The non-parametric Mann-Whitney test was used to compare data between the two groups ($P < 0.05$).

RESULTS

Chromatographic analysis identified p-cymene (23.71%), thymol (13.86%) and γ-terpinene (8.55%) as the major substances present in the thyme essential oil (Table 1).

Table 1. Chemical composition and yield percentage of the essential oil of thyme.

Compound	Percentage
p-Cymene	23.71%
Thymol	18.39%
γ-Terpinene	8.55%
Linalool	6.5%
1-R-α-pinene	5.04%
Camphor	3.24%
Borneol	2.15%
Carvacrol	2.15%
Caryophyllene	3.51%

The geometric means (GMs) of the MICs and MBCs against *E. coli* strains were of 627.7 μg.mL-1 and 990.2 μg.mL^{-1} for the thyme essential oil and of 2786 μg.mL^{-1} and 2540 μg.mL^{-1} for the thymol compound, respectively (Table 2). The significant lower MIC and MBC GMs ($P < 0.05$) showed that the essential oil of thyme had better activity than its major component thymol used alone.

Table 2. Antimicrobial activity (μg.mL^{-1}) of thymol and of the essential oil of thyme against 20 *Escherichia coli* strains.

	MBC		MIC			
	Range	GM	MIC50	MIC90	Range	GM
Thymol	3200-1600	2786^{A}	3200	3200	6400-1600	2540^{A}
Thyme	3200-400	627.7^{B}	800	800	6400-400	990.2^{B}

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MIC50: lowest concentration able to inhibit 50% of the isolates; MIC90: lowest concentration able to inhibit 90% of the isolates; GM: geometric mean of MIC. Different superscript letters in a column indicates statistically difference ($P < 0.05$; Mann-Whitney test).

DISCUSSION

The inexistence of an international standardized technique for the evaluation of essential oils and vegetable extracts allows the use of different protocols, hampering the comparison of the results [16,17]. The M31-A3 protocol [5] used in our experiment is the most recommended among the techniques found in the literature.

In this study, the essential oil of *T. vulgaris* and its major compound thymol showed bacteriostatic and bactericidal activities against *E. coli* strains *in vitro*. Nonetheless, the activity of the essential oil was superior to the compound alone. Such finding is explained by the fact that the high antimicrobial activity showed by some essential oils results from the synergism of the major components [8].

The antimicrobial activity of the thyme essential oil and of thymol has been evaluated in other studies. Ivanovic *et al*. [10] reported significant activity of the extract and essential oil of thyme against *E. coli* and *Salmonella* strains, with MIC of 640 μg mL^{-1}. Such activity was attributed to the high concentration of thymol in the extract (39.7%) and in the essential oil (48.49%). It was also reported antimicrobial activity of the essential oil of thyme against *E. col*i 5% (V/V) and other food borne bacteria [14]. An important role of bacteriostatic and bactericidal activity of the essential oils of thyme and oregano against *E. coli* O157:H7 isolated from bovine feces has also been observed [4]. The phenolic compounds carvacrol and thymol are responsible for the activity of these oils.

The essential oil of thyme has also been reported to show activity against yeasts susceptible and resistant to antifungal drugs [13]. Interestingly, Klaric *et al*. [12] found three-times stronger inhibition of pure thymol against different mould species than the thyme oil, which was constituted mainly of p-cymene (36.5%), thymol (33%) and 1,8-cineole (11.3%). The results differ from our study, where the activity of the essential oil of thyme was superior to its major compound thymol. In another study, Baskaran *et al*.

[2] investigated the antimicrobial activity of plants containing trans-cinnamaldehyde, eugenol, carvacrol and thymol, and found trans-cinnamaldehyde as the compound with the best activity against the main pathogens of mastitis, including *E. coli*.

CONCLUSION

It was showed that the essential oil of thyme and the compound thymol have antimicrobial activity *in vitro* against *E. coli* strains. The MIC and MBC values obtained showed that the essential oil was superior to the compound alone. This finding also highlights the potential use of the essential oil of thyme as a substitute for artificial inhibitors of food spoilage and pathogenic microorganisms.

MANUFACTURERS

[1]Sigma-Aldrich, Saint Louis, MO, USA.

[2]Hewllet Packard, Palo Alto, CA, USA.

[3]Merck, Darmstadt, Germany.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Bakkali F., Averbeck S., Averbeck D. & Idaomar M. 2008.** Biological effects of essential oils - a review. *Food Chemistry Toxicology*. 46(2): 446-475.

2 **Baskaran A.S., Kazme G.W., Hinckley L., Andrew S.M. & Venkitanarayanan K. 2009.** Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens *in vitro*. *Journal of Dairy Science*. 92(4): 1423-1429.

3 **Burt S. 2004.** Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology*. 94(3): 223-253.

4 **Burt S.A. & Reinders R.D. 2003.** Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology*. 36(3): 162-167.

5 **Clinical and Laboratory Standards Institute (CLSI). 2008.** Antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved Standard - 3rd edn. *CLSI document M31-A3 Clinical and Laboratory Standards Institute*. Wayne: CLSI, 112p.

6 **Cosentino S., Tuberoso C.I., Pisano B., Satta M., Mascia V., Arzedi E. & Palmas F. 1999.** In vitro antimicrobial activity and chemical composition of *Sardinian Thymus* essential oils. *Letters in Applied Microbiology*. 29(3): 130-135.

7 **Griffin P. & Tauxe R.V. 1991.** The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *Escherichia coli*, and the associated hemolytic uremic syndrome. *Epidemiologic Review*. 13(1): 60-98.

8 **Höferl M., Buchbauer G., Jirovetz L., Schmidt E., Stoyanova A., Denkova Z., Slavchev A. & Geissler M. 2009.** Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. *Journal of Essential Oil Research*. 21(5): 459-464.

9 **Ito N.M.K., Miyaji C.I. & Miyaji S.O. 2007.** *Diagnóstico diferencial das enfermidades bacterianas, fúngicas e parasitárias que acometem os frangos de corte*. Cascavel: Coluna do Saber, 160p.

10 **Ivanovic J., Misic D., Zizovic I. & Ristic M. 2012.** *In vitro* control of multiplication of some food-associated bacteria by thyme, rosemary and sage isolates. *Food Control*. 25(1): 110-116

11 **Kinsman D.M., Kotula A.W. & Breidenstein B.C. 1994.** *Muscle Foods, Meat, Poultry and Seafood Technology*. New York: Chapman & Hall, 573p.

12 **Klaric M., Kosalec I., Mastelic J., Pieckova E. & Pepeljnak S. 2007.** Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. *Letters in Applied Microbiology*. 44(1): 36-42

13 **Pozzatti P., Loreto E.S., Lopes, P.G., Athayde M.L., Santurio J.M. & Alves S.A. 2010.** Comparison of the susceptibilities of clinical isolates of *Candida albicans* and *Candida dubliniensis* to essential oils. *Mycoses*. 53(1): 12-15.

14 **Silva N., Alves S., Gonçalves A., Amaral J.S. & Poeta P. 2013.** Antimicrobial activity of essential oils from mediterranean aromatic plants against several foodborne and spoilage bacteria. *Food Science and Technology International*. 19(6): 503-510.

15 **Simões C.M.O. & Spitzer V. 2004.** Óleos voláteis. In: Simões C.M.O., Schenkel E.P., Gosmann G., Mello J.C.P., Mentz L.A. & Petrovick P.R. (Eds). *Farmacognosia: da planta ao medicamento.* PortoAlegre/Florianópolis: UFRGS/UFSC, 475p.

16 **Smith-Palmer A., Stewart J. & Fyfe L. 1998.** Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*. 26(2): 118-122.

17 **Viuda-Martos M., Navajas R., López F. & Álvarez P. 2008.** Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *International Journal of Food Science and Technology*. 43(3): 526-531.