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The Effect of *Thymus vulgaris* on the Respiratory Activity of Uropathogenic *Escherichia coli*

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Abstract: *Essential oils are organic compounds that have demonstrated antimicrobial properties. Some essential oils are potent bactericidal agents; such is the case of Thymus vulgaris. It has been reported that the essential oil of T. vulgaris is a bacterial growth inhibitor; it has potent bactericidal properties including efficacy against antibiotic-resistant strains. Some mechanisms from antibacterial activity of essential oils have been reported. In this work, the effect of T. vulgaris on the respiratory activity of uropathogenic E. coli was studied.*

Keywords: *Essential oil, bacteria, E. coli, antibiotic, Thymus vulgaris, respiratory activity*

1. INTRODUCTION

Antibiotic resistance has led to the search for new alternatives to combat infectious diseases (Michael et al., 2014). In this context, some substances of plant origin have presented favorable results eliminating pathogenic bacteria, viruses, fungi or parasites for humans (Górniak et al., 2019; Nascimento et al., 2000). Among the substances with these antimicrobial properties, essential oils have been studied, which have been used for different purposes since ancient times: in food preservation, pharmaceutical, agronomic industries (Saranra and Devi, 2017). In regard to its antibacterial properties, essential oils have demostrated a significant antibacterial activity against Gram-negative and Gram-positive bacteria (Boskovica et al., 2015). It has been reported that the essential oil of *T. vulgaris* is a bacterial growth inhibitor; it has potent bactericidal properties including efficacy against antibiotic-resistant strains (Al-Shuneigat et al., 2014; Flores-Encarnación el al., 2019). There have been a number of reports validating the in vitro antibacterial and antifungal activities of this essential oil on some human pathogens, including *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Candida albicans, Mycobacterium smegmatis, Proteus mirabilis, and Salmonella* sp. (de Lira-Mota et al., 2012; Imelouane et al., 2009). The essential oil of *T. vulgaris* was found to be able to interfere with bacterial colonization and the formation of biofilm on surfaces for uropathogenic *E. coli* (Flores-Encarnación et al., 2018). On the other hand, some mechanisms from antibacterial activity of essential oils have been reported. One of the reported mechanisms of action is the the ability to alter and to penetrate the lipid membrane of bacteria, making it more permeable and causing leaking ions and cytoplasm (bacterial lysis and death) (O'Bryan et al., 2015). There are few reports about the bactericidal mechanism of the essential oil of *T. vulgaris*. It is believed that this essential oil alters the permeability of membrane as do other oils (Hussein et al., 2018). For this, in the present work and trying to bring out a new mechanism

of action of the essential oil, the effect of *T. vulgaris* on the respiratory activity of uropathogenic *E. coli* was studied.

2. Materials and Methods

2.1. Source of Material

In this study a commercial essential oil of *T. vulgaris* was used. It was obtained from a flavour and fragrance company at Puebla, México.

2.2. Biological Material

The strain of uropathogenic *E. coli* CFT073 was used. Bacterial strain was stored in cryovials at -40°C until analysis.

2.3. Culture and Antimicrobial Activity of *T. vulgaris*

The uropathogenic *E. coli* strain was cultured at 37°C for 18 to 24 h in trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md). The antimicrobial activity was determined using the technique of diffusion in agar using paper discs. For it, tripticasein soy agar plates (containing 20 mL of medium) were prepared. Sterile Petri dishes (150 mm) were used. Plates were inoculated by crossstriation with uropathogenic *E. coli*. Each inoculum contained approximately 10^6 CFU mL^{-1}. Then, sterile filter paper disks (5 mm diameter) were placed on the surface of tripticasein soy agar plates. Different concentrations (0.66 to 13.2 mg mL^{-1}) of the essential oil were used. The agar plates were incubated at 37°C for 24 h. The diameters of the inhibition halos formed were measured. The analyses were conducted in triplicate.

2.4. Respiratory Activity

Respiratory activity was measured polarographically with a Clark oxygen electrode. For it, both complete and permeated cells of uropathogenic *E. coli* were used. The reaction mixture (final volumen= 5 mL) contained 20 mM phosphate buffer (pH 7.0) and the cell suspension at optical density (OD_{560nm})= 2-4. The reactions were initiated adding the following substrates: 100 mM glucose, 100 mM succinate (pH 7.0) and the oxygen consumption kinetics were recorded for 15 min. The temperature was kept constant at 37°C. In all tests, the respiratory activity of uropathogenic *E. coli* was reported as consumed nmol O_2 min^{-1} OD^{-1}. The analyses were conducted in triplicate. The effect of *T. vulgaris* on respiratory activity of uropathogenic *E. coli* was determinated adding the different concentrations of essential oil: 260, 780, 1300 and 2600 µg mL^{-1}.

2.5. Cell Permeation

The uropathogenic *E. coli* cells were permeated according to modified procedure described by Serrano *et al*., (1973). For this, 1 mL of an *E. coli* 24-hour preculture at 37ºC was taken, washed twice with 20 mM phosphate buffer (pH 7.0). To permeate the cells, 450 µL of 20 mM phosphate buffer (pH 7.0), 10 µL of Triton X100 and 40 uL of etanol were added. The cell suspension was vigorously mixed for 20 min. The permeated cells were washed twice with 500 µL of 20 mM phosphate buffer (pH 7.0). Finally, the pellet was resuspended in 1mL of 20 mM phosphate buffer (pH 7.0) and the respiratory activity was measured as described above.

3. Results

The antibacterial activity of *T. vulgaris* was determined using the agar diffusion test. So, tripticasein soy agar plates were inoculated with uropathogenic *E. coli* and different concentrations of the essential oil were added: 0.66, 1.3, 2.6, 6.6 and 13.2 mg. The results were shown in Fig. 1. The Fig. 1A shows the strong inhibitory effect on the growth of uropathogenic *E. coli*, where trypticasein soy agar surface lacked bacterial growth and the surface of the agar acquired a bright appearance. These results were obtained after the tripticasein soy agar plates were incubated for 24 hours at 37°C.

On the other hand, in order to determine the effect of *T. vulgaris* essential oil in a shorter time, in complete cells of uropathogenic *E. coli* the respiratory activity was measured as was described in Materials and Methods. In each essay, the reactions were initiated adding 100 mM glucose or 100 mM succinate (pH 7.0) and the oxygen consumption kinetics were recorded for 15 min (data not shown). The specific respiratory activity was reported as consumed nmol O_2 min^{-1} OD^{-1}. The results obtained showed that in complete cells of uropathogenic *E. coli*, the respiratory rates were 88 and 115 nmol O_2

min^{-1} OD^{-1}, using glucose and succinate as substrates, respectively. Later, the effect of *T. vulgaris* on respiratory activity of uropathogenic *E. coli* was determinated, adding 260, 780, 1300 and 2600 µg mL^{-1} of essential oil. Those concentrations of *T. vulgaris* were sublethal in complete cells of uropathogenic *E. coli* at the beginning of each assay. The results were shown as the relative respiratory activity, using as reference the specific respiratory activity recorded in the absence of *T. vulgaris* (Table 1). As shown in Table 1, the respiratory activity in complete cells of *E. coli* was inhibited in the presence of *T. vulgaris*. Using glucose as substrate, the respiratory activity of uropathogenic *E. coli* was decreasing as the concentration of *T. vulgaris* was increased. At 260 µg mL^{-1} of *T. vulgaris* the respiratory activity decreased by 20%, while with 1300 µg mL-1 the respiratory activity decreased by up to 70%. At 2600 µg mL^{-1} of *T. vulgaris*, respiratory activity was not registered because it decreased by up to 100%. Using succinate as substrate, respiratory activity was completely inhibited at low concentrations of the essential oil of *T. vulgaris*. At 260 µg mL^{-1} and higher concentrations of *T. vulgaris*, the respiratory activity decreased by 100%.

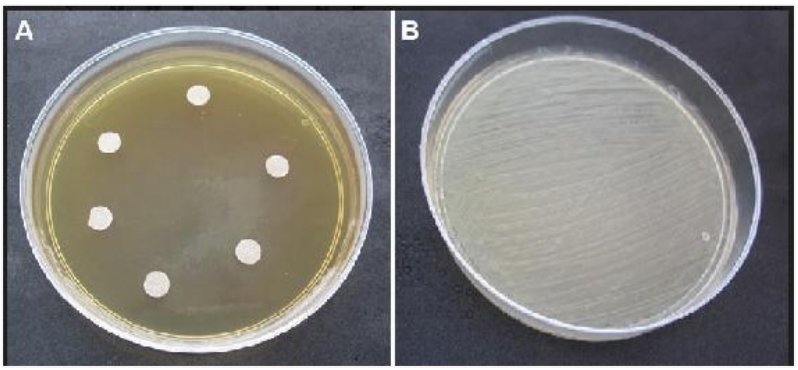

Fig1. *The effect of essential oil of T. vulgaris on the growth of uropathogenic E. coli. A. The growth was completely inhibited by T. vulgaris using 0.66, 1.3, 2.6, 6.6 and 13 mg of essential oil, which was placed on the paper disk counterclockwise starting with the paper disk located at the top; B. Control condition.*

Table1. *The effect of T. vulgaris on respiratory activity of uropathogenic E. coli.*

Relative respiratory activity (%)				
Concentration (µg mL^{-1})	Complete cells		Permeated cells	
	Glucose	Succinate	Glucose	Succinate
0	100%	100%	100%	100%
260	80%	0%	0%	0%
780	80%	0%	0%	0%
1300	30%	0%	0%	0%
2600	0%	0%	0%	0%

To determine the direct effect of *T. vulgaris* on the components of respiratory chain, the cells the *E. coli* were permeated as described in Materials and Methods. As expected, the respiratory activities in permeated cells was lower compared to recorded in complete cells of *E. coli*. The respiratory activity rates were 18.4 and 22.5 nmol O_2 min^{-1} OD^{-1}, using glucose and succinate as susbtrates, respectively. In this case, the addition of *T. vulgaris* in permeated cells of uropathogenic *E. coli* inhibited completely the respiratory activity, using glucose and succinate as substrates. By virtue of this, it was proposed that *T. vulgaris* affected some other component involved in the respiration of *E. coli*. Probably, some components of the respiratory chain of bacterium. In relation to this, the spectral analysis of cytochromes was done using complete cells of uropathogenic *E. coli* in the presence and absence of essential oil of *T. vulgaris*. The cells of *E. coli* were reduced with sodium dithionite and oxidized with ammonium persulfate. The difference spectra at room temperatura were recorded. Reduced minus-oxidized spectra of complete cells of uropathogenic *E. coli* in absence of *T. vulgaris* showed *b* and *d*-type cytochromes (data not shown). However, the presence of the essential oil caused a loss of the reduction of cytochromes, so it was not possible to register the cytochromic components seen above. *T. vulgaris* possibly affected some component of the respiratory chain of uropathogenic *E. coli*.

4. Discussion

Due to antibiotic resistance and failure of chemotherapy by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity (Hammer *et al.*, 1999; Inayatullah

et al., 2017). In that context, several essential oils that have shown antimicrobial properties have been investigated. Essential oils are plant secondary metabolites and are extracted from different parts of plants: leaves, barks, flowers, seeds, buds, twigs and fruits (Zaouali *et al*., 2010). Many different essential oils are important sources of natural antioxidants, so they have been used in pharmacology, medicine and food and cosmetic industries (Tural and Turhan, 2017; Zaouali *et al*., 2010).

In the present work, the effect of *T. vulgaris* on the respiratory activity of uropathogenic *E. coli* was studied. At the beginning, the antimicrobial activity of *T. vulgaris* was determined using the technique of diffusion in agar. The results showed a strong inhibitory effect on the growth of uropathogenic *E. coli* at the concentrations of *T. vulgaris* tested. The trypticasein soy agar surface lacked bacterial growth and the surface of the agar acquired a bright appearance (Fig. 1). The antimicrobial activity of *T. vulgaris* is has been reported against Gram negative bacteria such as *Salmonella enteritidis, S. choleraesuis, S. typhimurium, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Vibrio cholera, E. coli*, and Gram positive bacteria such as *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, S. epidermidis* (Kon and Rai, 2012; Lević *et al*., 2011; Mohsenipour and Hassanshahian, 2015; Rattanachaikunsopon and Phumkhachorn, 2010; Soković *et al*. 2010; Tohidpour *et al*. 2010). It has been reported that quantitatively thymol and carvacrol are the major components of essential oil of *T. vulgaris* and that they are largely responsible of antimicrobial properties (Lee *et al*., 2005; Tural and Turhan, 2017). On the other hand, some mechanisms of antibacterial action of essential oils have been reported. It has been known that phenolic components of essential oils alter and to penetrate the lipid membrane of bacteria, making it more permeable and causing leaking ions and cytoplasm (bacterial lysis and death) (O'Bryan *et al*., 2015; Swamy *et al*., 2016). However, little is known about mechanism of action of *T. vulgaris*. It is believed that it must also alter the permeability of the cell membrane. For this, in the present work the effect of *T. vulgaris* on the respiratory activity of uropathogenic *E. coli* was studied. The assays were performed on complete and permeated cells of uropathogenic *E. coli* in short periods of time (up to 15 min). The results showed that in the absence of *T. vulgaris*, the complete cells of uropathogenic *E. coli* had respiratory rates of 88 and 115 nmol O_2 min^{-1} OD^{-1}, using glucose and succinate as substrates, respectively. In the presence of *T. vulgaris* (260, 780, 1300 and 2600 µg mL^{-1}), the respiratory activity of complete cells of *E. coli* was inhibited (Table 1). The relative respiratory activity decreased by 20% at 260 µg mL^{-1} of *T. vulgaris* and by up to 70% using 1300 µg mL^{-1}. At 2600 µg mL^{-1} of *T. vulgaris*, respiratory activity decreased by up to 100%. Using succinate as substrate, respiratory activity was completely inhibited at low concentrations of essential oil of *T. vulgaris*. At 260 µg mL^{-1} and higher concentrations of *T. vulgaris*, the respiratory activity decreased by 100%. It has been reported that essential oils destabilize the cellular architecture, leading to the breakdown of membrane integrity and increased permeability, which disrupts many cellular activities, including energy production (membrane-coupled), membrane transport, and other metabolic regulatory functions (Oussalah *et al*., 2006; Raut and Karuppayil, 2014; Saad *et al*., 2013; Swamy *et al*., 2016). In the present work, the respiratory activity was completely inhibited at concentrations of *T. vulgaris* used, however as it could be seen, the results were different using glucose and succinate as substrates. In the case of the respiration inhibition of uropathogenic *E. coli* using glucose as substrate, the loss of activity was progressive, not so for the succinate oxidase activity. To explain these results, it was proposed that in complete cells the glucose oxidase activity involves the participation of several metabolic pathways, whereas the oxidation of succinate is directly linked to the respiratory chain of uropathogenic *E. coli*. Therefore, the essential oil of *T. vulgaris* could alter the permeability of cell membrane and thereby release the cytoplasmic components involved in glucose oxidation. As the succinate dehydrogenase is located in the cytoplasmic membrane of the bacterium, the direct action of *T. vulgaris* inhibited at a lower concentration the oxidation of succinate (as shown in the results). To confirm this, the cells the uropathogenic *E. coli* were permeated, thus respiratory activities (using glucose and succinate as substrates) were lower that respiratory activities of complete cells (18.4 and 22.5 nmol O_2 min^{-1} OD^{-1}, respectively). Subsequently, it was observed that the addition of *T. vulgaris* in permeated cells inhibited completely the respiratory activity. The disruption of the cell membrane by essential oils may assist various vital processes, such as energy conversion processes and nutrient processing (Nazzaro *et al*., 2013; Oussalah *et al*., 2006; Raut and Karuppayil, 2014). Essential oils are easily penetrable through the bacterial cell membranes owing to their lipophilic nature (Saad *et al*., 2013). Thus, in this study, *T. vulgaris* could have rapidly affected one of the components of the *E. coli* respiratory chain: the succinate dehydrogenase. It has been reported that carvacrol is a hydrophobic compound that influences cell membranes by altering the composition of fatty acids, which then affects the membrane fluidity and

permeability (Rudramurthy *et al*., 2016). However, its exact mechanism of action is still unclear. It was reported that carvacrol significantly depleted the internal ATP pool of bacterial cells and induced the leakage and loss of ATP from bacterial cells (Rudramurthy *et al*., 2016; Swamy *et al*., 2016; Ultee *et al*., 2002). In the present study, the inhibition assays of respiratory activity, specially made using succinate as a substrate, led to suspect another possible mode of action of the essential oil of *T. vulgaris*, which consists of the blockage of the respiratory chain of the bacterium and that could explain the loss of ATP from bacterial cells reported by other authors. Finally, other evidence supporting the respiratory inhibition as a new mode of action by *T. vulgaris* (not described so far), was that *T. vulgaris* caused a loss of the reduction of cytochromes in complete cells of uropathogenic *E. coli*.

5. Conclusion

Essential oils represent an alternative to combat infectious diseases. However, it is important to conduct more studies that show its broad antimicrobial properties, especially its mechanisms of action. In this study, some evidence was provided for a new mode of action of the essential oil of *T. vulgaris*, which is related to the inhibition of respiratory activity using as model to uropathogenic *E. coli*.

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References

[1] Al-Shuneigat J., Al-Sarayreh S., Al-Saraireh Y., Al-Qudah M., Al-Tarawneh I. (2014). Effects of wild *Thymus vulgaris* essential oil on clinical isolates biofilm-forming bacteria. IOSR J. Dental Med. Sci. 13: 62-66.

[2] Boskovica M., Zdravkovica N., Ivanovica J., Janjica J., Djordjevica J., Starcevica M. and Baltica M.Z. (2015). Antimicrobial activity of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms. Procedia Food Sci. 5:18-21.

[3] de Lira-Mota K.S., de Oliveira-Pereira F., Araújo-de Oliveira W., Oliveira-Lima I. and de Oliveira-Lima E. (2012). Antifungal activity of *Thymus vulgaris* L. essential oil and its constituent phytochemicals against *Rhizopus oryzae*: interaction with ergosterol. Mol. 17:14418-14433.

[4] Flores-Encarnación M., Acosta-Báez, L.A., Aguilar-Gutiérrez G.R., Cabrera-Maldonado C., Gallardo-Soto H.M. and Sánchez-Herrera A.J. (2019). The effect of essential oil of *Thymus vulgaris* on the growth of bacterial environmental and clinical isolates. Inter. J. Res. Stud. Biosci. 7:1-8.

[5] Flores-Encarnación M., Nava-Nolazco R.M., Aguilar-Gutiérrez G.R., Carreño-López R. and García-García S. C. (2018). The effect of *Thymus vulgaris* on growth and biofilm formation of uropathogenic Escherichia coli. Afr. J. Microbiol. Res. 12:237-242.

[6] Górniak I., Bartoszewski R. and Króliczewski J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem. Rev. 18:241-272.

[7] Hammer K.A., Carson C. and Riley T. (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985-990.

[8] Hussein H.J., Hadi M.Y. and Hameed I.H. (2018). Cytotoxic activity of *Thymus vulgaris*: antibacterial and antifungal activity. Inter. J. Pharm. Quality Assurance. 9:166-169.

[9] Imelouane B., Amhamdi H., Wathelet J.P., Ankit M., Khedid K. and El Bachiri A. (2009). Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from eastern Morocco. Int. J. Agric. Biol. 11:205-208.

[10] Inayatullah S., Shafee M., Shafiq M., Asif M., Parveen S., Kakar K., Khan Y.H., Amir M., Qadir A., Yakoob M. and Baloch A. (2017). Antimicrobial activity of thyme (*Thymus vulgaris*) essential oil cultivated in Quetta, Balochistan, Pakistan. International Journal of Biosciences. 10:105-110.

[11] Kon K. and Rai M. (2012). Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with other essential oils. Nusantara Biosci. 4:50-56.

[12] Lee S.J., Umano K., Shibamoto T. and Lee K.G. (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. 91:131-137.

[13] Lević J., Čabarkapa I., Todorović G., Pavkov S., Sredanović S., CoghillGalonja T. and Kostadinović L. (2011). In vitro antibacterial activity of essential oils from plant family *Lamiaceae*. Roman Biotechnol. Let. 16:6034-6041.

[14] Michael C.A., Dominey-Howes D. and Labbate M. (2014). The antimicrobial resistance crisis: causes, consequences, and management. Front. Pub. Health. 2:1-8.

[15] Mohsenipour Z. and Hassanshahian M. (2015). The inhibitory effect of *Thymus vulgaris* extracts on the planktonic form and biofilm structures of six human pathogenic bacteria. 2015. Avicenna J. Phytomed. 5:309-318.

[16] Nascimento G.G.F., Locatelli J., Freitas P.C. and Silva G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibioticresistant bacteria. Braz. J. Microbiol. 31:247-256.

[17] Nazzaro F., Fratianni F., De Martino L., Coppola R. and De Feo V. (2013). Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 6: 1451-1474.

[18] O'Bryan C.A., Pendleton S.J., Crandall P.G. and Ricke S.C. (2015). Potential of plant essential oils and their components in animal agriculture- in vitro studies on antibacterial mode of action. Frontiers Vet. Sci. 2:1-8.

[19] Oussalah M., Caillet S. and Lacroix M. (2006). Mechanism of action of spanish oregano, chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Jo. Food Protect. 69:1046-1055.

[20] Rattanachaikunsopon P. and Phumkhachorn P. (2010). Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. J. Biosci. Bioeng. 10:614-619.

[21] Raut J.S. and Karuppayil S.M. (2014). A status review on the medicinal properties of essential oils. Ind. Crops and Prod. 62:250-264.

[22] Rudramurthy G.R., Swamy M.K., Sinniah U.R. and Ghasemzadeh A. (2016). Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules. 21:836-866.

[23] Saad N.Y., Muller C.D. and Lobstein A. (2013). Major bioactivities and mechanism of action of essential oils and their components. Flavour Fragr. J. 28:269-279.

[24] Saranra P. and Devi V.D. (2017). Essential oils and its antibacterial properties- a review. Life Sci. Arch. 3:994-1011.

[25] Serrano R., Gancedo J.M. and Gance C. (1973). Assay of yeast enzymes *in situ* a potential tool in regulation studies. Eur. J. Biochem. 34:479-482.

[26] Soković M., Glamočlija J., Marin P.D., Brkić D. and van Griensven L.J.L.D. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules. 15:7532-7546.

[27] Swamy M.K., Akhtar M.S. and Sinniah U.R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Compl. Altern. Med. 2016:1-21.

[28] Tohidpour A., Sattari M., Omidbaigi R., Yadegar A. and Nazemi J. (2010). Antibacterial effect of essential oils from two medicinal plants against Methicillin-resistant *Staphylococcus aureus* (MRSA). Phytomed. 17:142-145.

[29] Tural S. and Turhan S. (2017). Antimicrobial and antioxidant properties of thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.) and laurel (*Lauris nobilis* L.) essential oils and their mixtures. GIDA The J. Food. 42:588-596.

[30] Ultee A., Bennik M.H.J. and Moezelaar R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68:1561-1568.

[31] Zaouali Y., Bouzaine T. and Boussaid M. (2010). Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem. Toxicol. 48:3144-3152.

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