

# ARTISANEFOOD

Innovative Bio-interventions and Risk Modelling  
Approaches for Ensuring Microbial Safety and  
Quality of Mediterranean Artisanal Fermented

**Report on Minimum Inhibitory Concentration  
(MIC) and Minimum Bactericidal Concentration  
(MBC) Values of Natural Antimicrobials against  
Indicator Microorganisms**



**Partner: AUA**



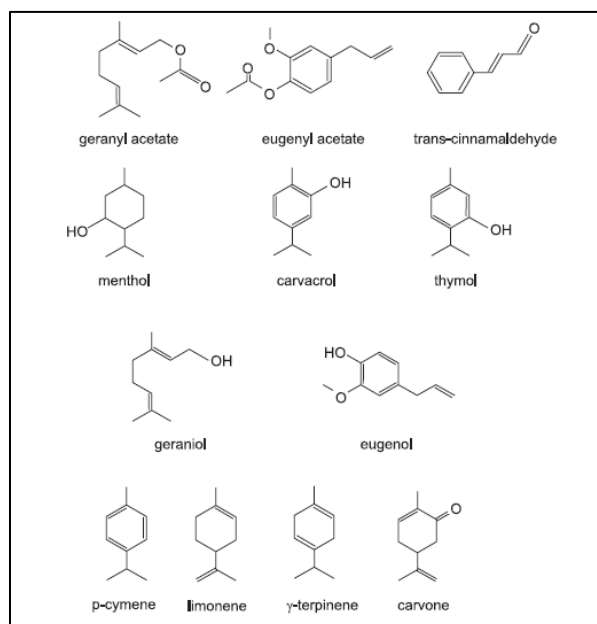
## 1. Introduction

The use of antimicrobials is a common practice for preservation of foods. Incorporation, in a food recipe, of chemical antimicrobials towards inhibition of spoilage and pathogenic micro-organisms results in the compositional modification of food. This treatment is nowadays undesirable for the consumer, who likes natural products. Scientific community reflecting consumers demand for natural antimicrobials has made efforts to investigate the possibility to use natural antimicrobials such as essential oils of plant origin to inhibit microbial growth. In addition, to the compositional modification of a food, antimicrobials are also used for a food surface treatment or for incorporation in the packaging material. This is especially important for cooked meat products, to decontaminate them from post-thermal processing cross-contamination. Antimicrobial substances are also used in certain stages of food process corresponding to critical control points; their presence contributes to the safety design of a food with other existing hurdles of microbial growth.

This report is mainly focused on natural antimicrobials and specially the use of essential oils as food additives, providing in parallel basic information on their mode of action and their antimicrobial activity against selected foodborne pathogens.

### 1.1. Essential oils as natural antimicrobials

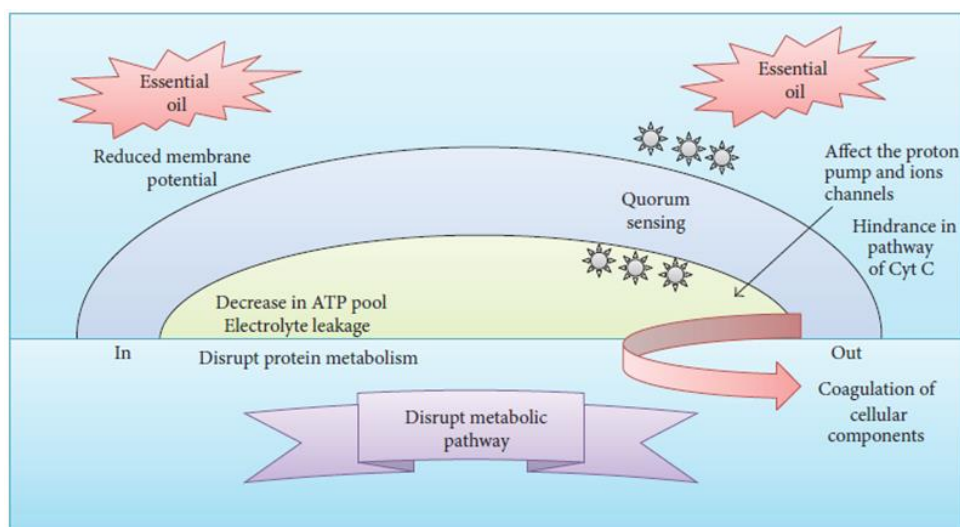
Since ancient times (with the earliest report in 1550 BC), spices and herbs have been used for their perfume and flavor as seasoning additives and as preservatives due to their strong antimicrobial and antioxidant properties (Tassou, Lambropoulou, & Nychas, 2004; Coma, 2008). The antimicrobial activity of spices and herbs is primarily attributed to the phenolic component of their essential oil fraction (phyto-phenols; Davidson & Naidu, 2000). Although EOs usually contains more than sixty individual components, the phenolic components are chiefly responsible for their antibacterial properties (Cosentino et al., 1999). More specifically, essential oils mainly consist of terpenes (e.g. mono terpenes, sesquiterpenes), terpenoides and other aromatic compounds (e.g. simple phenols, such as eugenol and thymol, aldehydes, esters and alcohols) (**Figure 1**) (Burt, 2004; Davidson, 1997; Smid & Gorris, 1999; Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Other plant extracts include isothiocyanate derivatives (e.g., found in cabbage, horseradish, mustard, broccoli) and phenolic compounds, such as di- or tri-phenols, phenolic acids, such as hydroxycinnamic acid, and flavonoids (Davidson, 1997). Based on toxicological studies, the majority of active components of herbs and spices are considered as food-grade or generally recognized as safe (GRAS) (Smid & Gorris, 1999).



**Figure 1:** Structural formulae of selected components of EOs (*Adapted from Burt, 2004*)

### 1.1.1. Mode of Action of EOs

The mechanisms of action of essential oils have not been clearly identified but it seems to be related with their hydrophobic nature (Nazzaro et al., 2013; Khorshidian et al., 2018; Swamy et al., 2016). In fact, the lipophilic nature of the hydrocarbon skeleton and hydrophilic nature of functional groups of EOs (**Figure 1**) has been suggested of having substantial role in the antimicrobial effects of these compounds.



**Figure 2:** Antimicrobial mechanisms of essential oils (*Adapted from Swamy et al., 2016*)

**Table 1** illustrates some examples of different mechanisms of action found to be used by different EOs and their compounds.

**Table 1:** Different mechanisms of the antimicrobial action of some essential oils and their active compounds

<b>Essential Oil- Active Compound</b>	<b>Mechanism of Antimicrobial Action</b>
<b>Tea tree oil</b>	Alter cell permeability, increase the leakage of intracellular K <sup>+</sup> ions and disrupt cell respiration.
<b>Thymol, menthol, and linalyl acetate</b>	Cause perturbation of the lipid fractions of bacterial plasma membranes
<b>Carvacrol</b>	<ul style="list-style-type: none"> <li>• Alter the composition of fatty acids which then affects the membrane fluidity and permeability</li> <li>• Deplete the internal ATP</li> <li>• Induced the leakage and loss of ATP from bacterial cells</li> <li>• Abolish the enterotoxin production of <i>S.aureus</i></li> <li>• Disturb the insertion and folding of proteins such as DnaK and GroEL</li> </ul>
<b>Methyl carvacrol, menthol, citronellol, and thymol</b>	Cause an enlargement of the cell membrane that leads to passive diffusion of ions between the expanded phospholipids
<b>Trans-cinnamaldehyde</b>	Enters the periplasm of the cell and disrupts cellular functions
<b>p-cymene</b>	Disturb the membrane integrity
<b>Phenylpropene, eugenol,</b>	Modify the fatty acid outline to alter the cytoplasmic membrane of different bacteria. In addition, they can destroy various bacterial enzymes such as ATPase, amylase, histidine carboxylase, and proteases
<b>Vanillin</b>	Obstruct the pathways of bacterial respiration and disrupting the flux of K <sup>+</sup> ions and pH gradient

Generally, the ability of EOs to damage structural and functional properties of bacterial membranes is probably the main mechanism of inhibition (Nazzaro et al., 2013). It has been suggested that EOs disrupt the proton pumps, the arrangement of dissimilar fatty acids, phospholipids bilayers and polysaccharides molecules (Swamy et al., 2016). EOs deplete ATP, may cause cascade effect, and finally lead to the coagulation of inner cellular components in the cytoplasm and ultimately cell-death. Additionally, some EOs have been found to inhibit the quorum sensing communication network between cells which is mediated by different bacterial signal molecules (**Figure 2**) (Swamy et al., 2016).

Moreover, recent research has shown that essential oils may not cause cell death but instead may cause damage to cells by turning them into a **Viable But Non Culturable** (VBNC) state, a microbial response to stress conditions (Paparella et al., 2008). This was also shown in a recent research where *Origanum vulgare L.* and *Rosmarinus officinalis L.* essential oils caused loss of culturability in *Listeria monocytogenes* cells in Phosphate buffer solution (PBS) and meat broth, forcing *L. monocytogenes* to enter a VBNC state (Barbosa et al., 2020).

Although the results of most in vitro assays suggest that essential oils have a substantial antimicrobial effectiveness, several factors may limit their commercial application in foods. More specifically, studies have shown that factors such as

- i) the intrinsic properties of foods (such as fat, pH, salt, water and proteins, which determine the solubility of EOs in the water phase) ;
- ii) the structure and viscosity of the foods (solid vs. liquid foods) (Skandamis et al., 2000);
- iii) the decomposition of some EOs constituents (e.g. allyl isothiocyanate) in aqueous phase and/or their interaction with certain hydrophilic substances, such as thiols, and sulphhydryl or terminal amino groups of proteins (Ward, Delaquis, Holley, & Mazza, 1998); and
- iv) factors affecting the physiology of the micro-organisms, such as composition of bacterial membranes, availability of nutrients, oxygen tension and incubation temperature (Kabara, 1991; Juven et al., 1994; Smid & Gorris, 1999; Gill, Delaquis, Russo, & Holley, 2002; Nychas & Skandamis, 2005),

may affect the antimicrobial efficacy of EO in foods.

To overcome the above-mentioned limitations on the application of EOs in foods, researchers have focused on the inclusion of EOs in formulations (emulsions, powders) which will protect essential oil. In such formulations the EO (core material) is being encapsulated in a carrier material (wall material) (Sagalowicz & Leser, 2010) to form a colloidal dispersion. Commercially colloidal dispersions (nanoemulsions and microemulsions) are produced to encapsulate lipophilic compounds to be dispersed into an aqueous medium, the so-called **Oil-in-Water** (O/W) type system. Reverse type systems, **Water-in-oil** (W/O) type systems, may also be applied in certain

industries (McClements, 2012). Droplet size of these encapsulation systems are of importance as it affects the antimicrobial activity of the system. Specifically, the strength of the net attractive forces acting between droplets usually decreases with decreasing droplet diameters, reducing aggregation phenomena in nanoemulsions. As droplets' size decreases, the antimicrobial activity of encapsulated lipophilic compound increases because the ratio surface area/volume increases. That improves the reactivity of the compound with the microbe as it enhances the transport of active molecules (Donsì and Ferrari, 2016). Droplet size is highly related to the delivery system (carrier) (**Table 2**) and the method of encapsulation applied (such as molecular inclusion, spray drying, liposome encapsulation etc) (Sagalowicz & Leser, 2010).

**Table 2:** Size of Different Oil-in-Water Systems

<b>Delivery System</b>	<b>Size</b>
<b>Powder particles</b> (glass encapsulation, core-shell capsules, matrix capsules)	10µm-1mm
<b>Emulsions</b> (ordinary emulsions, multilayered emulsions, double emulsions, nanoemulsions, Solid Lipid Nanoparticles -SLNS)	100nm-10µm
<b>Liposomes, Vesicles</b>	20nm-100µm
<b>Microemulsions</b>	5-100nm
<b>Dispersed reversed surfactant systems</b> (Cybosomes, hexosomes, Dispersed reversed Microemulsions, Micellosomes)	100nm-1µm

Liposomes and nanoliposomes are attractive encapsulation systems for the delivery of both lipophilic and hydrophilic functional compounds. They are spherical-shell structures consisting of a phospholipid bilayer (or more such bilayers) enclosing a liquid core. They can be categorized according to their lamellarity and their size. Small Unilamellar Vesicle (SUV) is between 20-100nm, Large Unilamellar Vesicles (LUV) are >100nm and the size of Giant Unilamellar Vesicle (GUV) is >1000nm. As it has already been mentioned, essential oils are able to be entrapped in liposomes. There are many methods which are used to formulate these liposomes, such as the thin-film hydration, Extrusion, Sonication, Melting, Freezing- thawing etc. (Emami et al., 2016).

## 1.2. *In vitro* tests of antibacterial activity of EOs

Several methods can be applied to determine the antibacterial activity of compound; however, for EOs most commonly applied methods are:

- a) The agar diffusion methods (disk diffusion or agar well) (qualitative method)
- b) The dilution methods (agar dilution method or broth dilution) (quantitative method) (Kalemba & Kunicka, 2003; Burt, 2004).

Both type of methods aim to determine the Minimum Inhibitory Concentration (MIC) as a measure of the antibacterial performance of EO. However, as there is no standardized method or definition for MIC, variations between the applied methodologies exist. Moreover, Minimum Bactericidal Concentration (MBC) or the bacteriostatic concentration is also stated in many research studies, both terms agreeing closely with the MIC (Burt, 2004).

To avoid confusion, for the purposes of the current review the following definitions (defined by EUCAST) will be adapted:

“**Minimal Inhibitory Concentration (MIC)** is the lowest concentration of an antimicrobial agent that completely inhibits the growth of the organism (EUCAST, 2000) whereas,

“**Minimum Bactericidal Concentration (MBC)** is the lowest concentration of a compound that under defined *in vitro* conditions reduces by 99.9% (3 log units) the number of organisms in a medium containing a defined inoculum of bacteria, within a defined period of time”.

### a) **Antimicrobial Activity of Essential Oils and Active Compounds against Selected Pathogens - Literature Review**

During the past decades, extensive documentation on the antimicrobial activity of different essential oils and their constituents against foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus* has been carried out (**Table 3a, 3b, 4, 5, 6, 7 & 8**). Due to the use of many different microbiological methods for susceptibility testing and different definitions of antimicrobial activity, the comparability of studies on essential oils is often critical as variation in the method applied may result in variation in the expression of results (mg/ml, µg/ml, %v/v, %w/w, etc.). For ease of comparison, in the current review, all the results have been converted to %v/v. More specifically, several studies have been focused on the determination of antimicrobial activity of essential oils against foodborne pathogens. It is generally shown that Gram-positive bacteria are more sensitive to essential oils or antibacterial compounds than Gram-negative bacteria (**Table 3a, 3b**). This resistance is usually ascribed to the structure of the cellular walls of Gram-negative bacteria, mainly with regards to the presence of lipoproteins and lipopolysaccharides that form a barrier to restrict entry of hydrophobic compounds. Differences between the antimicrobial activity of different EOs can also be attributed to differences on their structure and polarity (Budiati et al., 2020, Takayama et al., 2016, Carolina et al., 2003).

**Table 3a:** MIC and MBC of different essential oils against selected foodborne pathogens

Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
<i>Orange peel (Citrus sinensis)</i>	<i>L. monocytogenes</i>	3.13	nd	Broth Dilution	Budiati et al., 2020
<i>Lemon oil (Cymbopogon citratus)</i>	<i>L. monocytogenes</i>	0.32	nd	Broth Dilution	Budiati et al., 2020
	<i>Salmonella ATCC 14030</i>	6.25	nd	Broth Method	Budiati et al. 2018
<i>Lemon peel (Citrus lemon)</i>	<i>Staphylococcus aureus</i>	2.5			
	<i>Escherichia coli</i>	2.5	nd	Micro-dilution	Otang and Afolaya, 2016
	<i>Bacillus cereus</i>	0.02			
<i>Juniperus communis</i>	<i>L. monocytogenes ATCC 19111</i>				
	<i>L. monocytogenes isolated from salmon (LMS)</i>	0.5	2		
	<i>L. monocytogenes from slaughterhouse water drainage tunnel (LMT)</i>	0.5	1	Micro-dilution	Nikolic et al., 2019
	<i>L. monocytogenes from beef carcass (LMB)</i>	1	4		
<i>Tarragon</i>	<i>E. coli ATCC 25922</i>				
	<i>St. aureus ATCC 6538</i>	0.00008			
	<i>S. typhimurium ATCC 14028</i>		0.00001	Broth Dilution	Akarca et al., 2019
	<i>L. monocytogenes ATCC 51774</i>	0.00004			
<i>Salvia</i>	<i>St. aureus ATCC 12600</i>				
	<i>L. monocytogenes ATCC 13932</i>	0.05120	nd	Micro-dilution	Bhavya et al., 2020
<i>Mentha</i>	<i>St. aureus ATCC 29213</i>				
	<i>B. cereus</i>	0.25	0.25		
	<i>E. coli ATCC 25922</i>				
	<i>S. typhi</i>	0.5	1	Micro-dilution	Chraibi et al (2017)
	<i>Pseudomonas aeruginosa ATCC 27853</i>	8	>8		
	<i>Listeria monocytogenes</i>	0.5	0.5	Micro-dilution	Bouyahya et al., 2017
<i>Lavandula mairei</i>	<i>Listeria monocytogenes</i>	15.3	nd	Micro-dilution	Teixeira et al. 2012
	<i>L. monocytogenes CECT 4032</i>	0.008	0.01		
	<i>S. aureus CECT 976</i>	0.012	0.012	Macro-dilution	A. El Hamdaoui et al., 2018

<sup>‡</sup> For ease of comparison different MIC and MBC units (i.e., ppm, mg ml<sup>-1</sup>, µl l<sup>-1</sup> and µg ml<sup>-1</sup>) have been converted to % (v/v) values



**Table 3b:** MIC and MBC of different essential oils against selected foodborne pathogens

Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
<i>Date (Phoenix dactylifera L.)</i>	<i>B. cereus</i> ATCC 11778	1.56	3.13	Broth Dilution	El Arem et al., 2013
	<i>St. aureus</i> ATCC 25923	0.78	1.56		
	<i>L. monocytogenes</i> ATCC 19115	0.09	0.19		
	<i>E. coli</i> ATCC 35218	12.50	25		
<i>Sesame seeds (Sesamum indicum)</i>	<i>Bacillus subtilis</i>	0.092	nd	Streak method	Sandeep et al., 2014
	<i>E. coli</i>	0.095	nd		
<i>Oleuropein (olive leaves)</i>	<i>P. aeruginosa</i>	0,10	nd	Micro-dilution	Djenane et al., 2012
	<i>St aureus</i>	0.05	nd		
	<i>S. enterica</i>	0.1	nd		
<i>Hesperidin (orange byproduct)</i>	<i>Aeromonas hydrophila</i> ATCC 7966	3125	12500	Agar diffusion	Abass et al., 2014
	<i>B.subtilis</i>	45	nd		
	<i>E. coli</i>	75	nd		
	<i>S. typhi</i>	175	nd		
	<i>St. aureus</i>	175	nd		
<i>Garlic</i>	<i>St.aureus</i>	0.00016	0.00064	Tube-dilution	Elsom et al., 2000
	<i>E/.coli</i>	0.0032	0.0032		
	<i>Salmonella</i>	1 - 1.25	1.3 - 1.5	Broth Dilution	Belguith et al., 2010
	<i>L. monocytogenes</i> CECT 4032	0.01	nd	Tube Dilution	Somrani et al., 2020
	<i>L. monocytogenes</i>	0.4	>0.8	Micro-dilution	S. Pedrós-Garrido et al., 2020
	<i>S. enterica</i> ATCC 25957	0.2	nd	Broth Dilution	H. Al-Talib et al., 2015
	<i>E. coli</i> ATCC 43889	0.1	nd		
	<i>St. aureus</i>	0.8	nd		

<b>Fennel seeds (<i>Foeniculum vulgare</i>)</b>	<i>L. monocytogenes</i>	15.3	nd	Broth Dilution	Sayed Ahmad et al., 2017
	<i>St. aureus</i>	0.13	0.13		
<b>Corriander (EO)</b>	<i>L. monocytogenes</i>	<0.01	nd	Broth Dilution	Delaquis et al., 2004
	<i>E. coli</i> 25922	0.2	0.2	Broth Dilution	Casetti et al., 2012
	<i>St. aureus</i> ATCC 25923	0.2	1.6	Broth Dilution	Silva et al., 2011
	<i>Salmonella</i> ATCC 13311	0.4	0.8	Broth Dilution	Silva et al., 2012
<b>Red pepper</b>	<i>L. monocytogenes</i>	0.40	nd	Micro-dilution	Omolo et al., 2014
	<i>E.coli</i>	0.06	nd		

¥ For ease of comparison different MIC and MBC units (i.e., ppm, mg ml<sup>-1</sup>, µl l<sup>-1</sup> and µg ml<sup>-1</sup>) have been converted to % (v/v) values

**Table 4:** MIC and MBC values (% v/v) of the active compound 'Carvacrol' against different pathogenic microorganisms

Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
Carvacrol	<i>L. monocytogenes</i> 10403S				
	<i>L. monocytogenes</i> DMST17303				
	<i>L. monocytogenes</i> CM2-BM-HF-Black				
	<i>L. monocytogenes</i> CM8-ISO-HF-Black				
	<i>L. monocytogenes</i> CM9-ISO-HF-Black	0.025	0.05	Micro-dilution	Churklam et al., 2019
	<i>L. monocytogenes</i> CM11-ISO-HF-Black				
	<i>L. monocytogenes</i> CM12-ISO-HF-Black				
	<i>L. monocytogenes</i> CM13-ISO-HF-Black				
	<i>L. monocytogenes</i> CM15-ISO-HF-Black				
	<i>L. monocytogenes</i> CECT 4032	0.01	nd	Tube-dilution	Somrani et al., 2020
	<i>Salmonella</i> SP 11	0.03			
	<i>Salmonella</i> SP 28	0.06			
	<i>Salmonella</i> CECT 443		nd	Micro-dilution	Gomez-Garcia et al. 2019
	<i>Salmonella</i> CECT 700	0.03			
	<i>Salmonella</i> CECT 915				
	<i>Salmonella</i> CECT 4300				
	<i>S. Enteritidis</i> SE86				
	<i>S. Typhimurium</i> isolated from pork feces	0.0331	nd	Micro-dilution	Both Engel et.al., 2017
	<i>S. Newport</i>				
	<i>S. Saint Paul</i> isolated from meat				
	<i>Escherichia coli</i> 60	0.03	nd		
	<i>Escherichia coli</i> 61	0.03	nd		
	<i>Escherichia coli</i> 67	0.06	nd	microdilution	Gomez-Garcia et al., 2019
	<i>Escherichia coli</i> 96	0.015	nd		
	<i>Escherichia coli</i> 107	0.06	nd		
	<i>Escherichia coli</i> 115	0.03	nd		
	<i>E. coli</i> O157:H7 (ATCC 35150)	0.25	nd	microdilution	Yuan et al., 2019
	<i>Staphylococcus aureus</i> 4668/03	0.0662	nd		
<i>Staphylococcus aureus</i> S6	0.0662	nd	Micro-dilution	Both Engel et.al., 2017	
<i>Staphylococcus aureus</i> S8	0.0662	nd			
<i>Staphylococcus aureus</i> ATCC 2998	0.0662	nd			
<i>Staphylococcus aureus</i> isolated from meat	0.2	0.4	Micro-dilution	Gochev&Girova, 2009	

**Table 5:** MIC and MBC values (% v/v) of 'Oregano essential oil' against different pathogenic microorganisms

Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
<i>Oregano oil</i>	<i>L. monocytogenes ATCC 13932</i>	0.000052	nd	Micro-dilution	Hulánková and Bořilová, 2011
	<i>L. monocytogenes ATCC 13932</i>				
	<i>L. monocytogenes ATCC 7644</i>	0.000125	nd	Tube-dilution	Azeredo et.al., 2011
	<i>L. monocytogenes ATCC 7644</i>				
	<i>L. monocytogenes ATCC 19112</i>	0.0025	nd	Micro-dilution	Barbosa et al., 2020
	<i>L. monocytogenes ATCC 19117</i>				
	<i>S. Typhimurium</i>	0.04	0.04	Micro-dilution	Mogan et al, 2020
	<i>Salmonella SP 11</i>	0.06			
	<i>Salmonella SP 28</i>	0.12			
	<i>Salmonella CECT 443</i>	0.06			
	<i>Salmonella CECT 700</i>	0.06	nd	Micro-dilution	Gomez-Garcia et al. 2019
	<i>Salmonella CECT 915</i>	0.06			
	<i>Salmonella CECT 4300</i>	0.06			
	<i>Salmonella ATCC 13076</i>	0.0005	nd	Micro-dilution	Hulánková&Bořilová, 2011
	<i>Salmonella entericakasenyi</i>	0.001			
	<i>Salmonella enterica Veneziana</i>	0.0005			
	<i>Salmonella enterica Derby</i>	0.0002	nd	Micro-dilution	Rossi et al. 2019
	<i>Salmonella enterica Thomson</i>	0.0005			
	<i>Salmonella enterica Napoli</i>	0.0005			
	<i>Escherichia coli 60</i>	0.06	nd		
	<i>Escherichia coli 61</i>	0.03	nd		
	<i>Escherichia coli 67</i>	0.06	nd		
	<i>Escherichia coli 96</i>	0.03	nd	Micro-dilution	Gomez-Garcia et al., 2019
<i>Escherichia coli 107</i>	0.12	nd			
<i>Escherichia coli 115</i>	0.03	nd			
<i>Escherichia coli O157:H7 ATCC 700728</i>	0.000051	nd	Micro-dilution	Hulánková&Bořilová, 2011	
<i>Staphylococcus aureus ATCC 25923</i>	0.05%	nd			
<i>Staphylococcus aureus isolated from meat</i>	0.05	0.05	Micro-dilution	Gochev&Girova, 2009	

**Table 6:** MIC and MBC values (% v/v) of 'Cinnamon essential oil' against different pathogenic microorganisms

Essential oil or active substance	Strain Identification	MIC (% v/v)¥	MBC (% v/v)¥	Method Applied	Reference	
Cinnamon oil ( <i>C. cassia</i> , EOC)	<i>L. monocytogenes</i> train- FSL	0.078	0.078	Micro-dilution	Paudel et al., 2019	
	<i>L. monocytogenes</i> N1-017, LMI					
	<i>L. monocytogenes</i> strain- FSL J2-064, LMII					
	<i>L. monocytogenes</i> strain- FSL N3-165, LMIII					
	<i>Salmonella entericakasenyi</i>	0.0002	nd	Micro-dilution	Rossi et al. 2019	
	<i>Salmonella enterica</i> Veneziana	0.0001				
	<i>Salmonella enterica</i> Derby	0.0001				
	<i>Salmonella enterica</i> Thomson	0.000125				
		<i>Salmonella enterica</i> Napoli	0.0001			
		<i>S. Typhimurium</i> T123	0.1	0.2	Micro-dilution	Al-Nabulsi et al., 2020
		<i>S. Aberdeen</i> T069,				
		<i>S. Cubana</i> T109				
		<i>S. Paratyphi A</i> T193				
		<i>SalII</i> ( <i>Salmonella enterica</i> )	0.039	0.078	Micro-dilution	Paudel et al. 2019
	<i>SalIII</i> ( <i>Salmonella enterica</i> subspecies <i>enterica</i> serovar <i>Newport</i> )					
	<i>SalIII</i> ( <i>Salmonella choleraesuis</i> subsp. <i>Choleraesuis</i> )					

**Table 7:** MIC and MBC values (% v/v) of 'Rosemary essential oil' against different pathogenic microorganisms

Essential oil or active substance	Strain Identification	MIC (% v/v)¥	MBC (% v/v)¥	Method Applied	Reference
<b>Rosemary oil</b>	<i>L. monocytogenes</i>	3.13	nd	Broth Dilution	Budiati et al., 2020
	<i>L. monocytogenes</i> ATCC 7644	0.002	nd	Tube-dilution	Azeredo et.al., 2011
	<i>L. monocytogenes</i> ATCC 7644				
	<i>L. monocytogenes</i> ATCC 19112	0.0005	nd	Micro-dilution	Barbosa et al., 2020
	<i>L. monocytogenes</i> ATCC 19117				
	<i>Salmonella entericakasenyei</i>				
	<i>Salmonella enterica</i> Veneziana				
	<i>Salmonella enterica</i> Derby	>0.004	nd	Micro-dilution	Rossi et al. 2019
	<i>Salmonella enterica</i> Thomson				
	<i>Salmonella enterica</i> Napoli				
<i>Esherichia coli</i> ATCC 8739	0.30	0.50	Microdilution	Jiang et.al., 2011	
<i>Staphylococcus aureus</i> MRSA 53	0.03	0.1	Micro-dilution	Jiang et.al., 2011	

**Table 8:** MIC and MBC values (%v/v) of Thyme essential oil & Thymol against different pathogenic microorganisms

Essential oil (EO) or active substance (AS)	Application	Strain Identification	MIC % (v/v)	MBC % (v/v)	Method Applied	Reference				
Thyme	EO	<i>E. coli</i> ATCC 25922	4.0	4.0	Agar Dilution	Moghimi et al., 2016				
		<i>Staphylococcus aureus</i> isolated from meat	0.1	0.2	Micro-dilution	Gochev&Girova, 2010				
		<i>E. coli</i> O157:H7 (NCCP 11090)	0.01	0.03	Microbroth diltion	Sadekuzzaman et al., 2018				
		<i>S. Typhimurium</i> T123	0.1	0.2	Micro-dilution	Al-Nabulsi et al., 2020				
		<i>S. Aberdeen</i> T069,								
		<i>S. Cubana</i> T109								
		<i>S. Paratyphi A</i> T193								
		<i>S. ser Enteritidis</i> ATCC13076	0.03	0.07	Microbroth dilution	Sadekuzzaman et al., 2018				
		<i>L.monocytogenes</i> ATCC19113	0.06	0.12	Microbroth diltion	Sadekuzzaman et al., 2018				
		Thymol	AS	<i>Salmonella</i> SP 11	0.06	nd	Micro-dilution	Gomez-Garcia et al. 2019		
<i>Salmonella</i> SP 28	0.12									
<i>Salmonella</i> CECT 443	0.03									
<i>Salmonella</i> CECT 700										
<i>Salmonella</i> CECT 915										
<i>Salmonella</i> CECT 4300										
<i>S. Enteritidis</i> SE86	0.0331			nd	Micro-dilution				Both Engel et.al., 2017	
<i>S. Typhimurium</i> isolated from pork feces										
<i>S. Newport</i>										
<i>S. Saint Paul</i> isolated from meat	0.0662			nd	Micro-dilution	Both Engel et.al., 2017				
<i>Staphylococcus aureus</i> 4668/03										
<i>Staphylococcus aureus</i> S6										
<i>Staphylococcus aureus</i> S8										
<i>Staphylococcus aureus</i> ATCC 2998										
<i>Staphylococcus aureus</i> isolated from meat							0.2	0.4	Micro-dilution	Gochev&Girova, 2009
<i>Escherichia coli</i> 60							0.12	nd	Broth microdilution	Gomez-Garcia et al., 2019
<i>Escherichia coli</i> 61	0.06			nd						
<i>Escherichia coli</i> 67	0.06	nd								
<i>Escherichia coli</i> 96	0.06	nd								
<i>Escherichia coli</i> 107	0.12	nd								
<i>Escherichia coli</i> 115	0.06	nd								
<i>E. coli</i> O157:H7 (ATCC 35150)	0.063	nd	microdilution	Yuan et al., 2019						

However, other experimental factors such as the choice of bacterial strains and their sensitivity, volume of inoculum, incubation time, and temperature may also be related to the variation in the antimicrobial activity presented among studies.

Essential oils of cinnamon, oregano, thyme and rosemary are found to exhibit promising antimicrobial effects against foodborne pathogens (**Table 5, 6, 7, 8**), that can be attributed to the presence of their principle bioactive constituents, such as carvacrol (**Table 4**) and thymol (**Table 8**). As such it has been suggested that the MICs of oregano EOs can be set as the benchmark for other Eos (Thielmann et al., 2019).

However well EOs perform in antibacterial assays in vitro, it has generally been found that a greater concentration of EO is needed to achieve the same effect in foods (Burt 2004). The later forced researchers to focus on the antimicrobial effects of encapsulated essential oils.

#### **b) Antimicrobial Activity of Essential Oils and Active Compounds against Selected Pathogens- *In-Vitro* Screening of natural extracts**

The ***In-Vitro* screening of the MIC** values of selected antimicrobials was determined against number of indicator (spoilage and pathogenic) organisms, such as *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, *S. aureus*. From the stock solution of each antimicrobial, nine principal dilutions (i.e. fractional dilutions from 1 to 0.2) were prepared in TSB and 300 µl of each dilution was added into the wells of the first row of a microplate. The remaining wells were filled with 150 µl of TSB. Two-fold dilutions follow, from the 1st to the 4th row, transferring 150 µl from one well to the next, across the same column. Culture suspension (50 µl) was added to each well, containing 10<sup>5</sup>CFU/ml of the target strain. Negative control wells contained only TSB. Microplates were incubated in Bioscreen C at the optimum temperature (from 25 to 37 °C) and incubation period per indicator organism. OD600 nm measurements were recorded every 30 min and MIC was determined as the minimum concentration of the antimicrobial solution at which no increase in OD was observed. For **MBC determination**, aliquots of 100 µl culture suspension (CFU/ml) were added into each microplate well, except for the negative control wells, which contain only TSB. Microplates were incubated under static conditions at optimum conditions per microorganism. The wells of a separate microplate were filled with various concentrations of each antimicrobial according to fractional and twofold dilutions. Dilutions of the antimicrobial took place in TSB or in dH<sub>2</sub>O: ethanol mixture at ratio 70:30. An aliquot of 200 µl antimicrobial from each concentration was transferred to the respective well of the microplate with the culture. The antimicrobial solution was left in contact with cells for 5 min at 25°C. Then, cells were transferred to Eppendorf tubes with 300 µl of neutralizing broth (Dey-Engley neutralizing broth, Fluka Analytical) and incubated at 25°C for 30 s. Finally, 400 µl TSB were added to each well and the treated microplate was incubated at the optimum conditions per organism. Control wells were not be subjected to the antimicrobial treatments. After incubation, turbidity (OD) of TSB was be



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measured at A =600 nm. MBC was determined as the antimicrobial concentration for which no regrowth is observed. Tables 9, 10 and 11 show the in vitro screening of MIC & MBC values of selected essential oils against foodborne pathogens.

**Table 9:** MIC and MBC values (%v/v) of extracts against different pathogenic microorganisms, tested by partners  
UCO and UIZ

Partner	Essential oil or active substance	Strain Identification	MIC (% w/v) <sup>‡</sup>	MBC (% w/v) <sup>‡</sup>
<b>UCO (Spain)</b>	<i>Thymus vulgaris</i> (thyme)	<i>Salmonella</i> Enteritidis ATCC13076	0.0625	nd
	<i>Cymbopogon martinii</i> (palm rose)	<i>Salmonella</i> Enteritidis ATCC13077	0.125	nd
	<i>Cymbopogon citratus</i> (lemongrass)	<i>Salmonella</i> Enteritidis ATCC13078	0.25	nd
	<i>Ocimum gratissimum</i> (african basil)	<i>Salmonella</i> Enteritidis ATCC13079	1	nd
<b>UIZ (Morocco)</b>	Thymus satureioides-E.O.-	<i>L. monocytogenes</i> CECT 4032	0.032	0.032
		<i>L. innocua</i> CECT 4031	0.032	0.032
		<i>S. aureus</i> CECT 976	0.04	0.125
		<i>B. subtilis</i> DSM 6633	0.25	0.25
		<i>P. aeruginosa</i> CECT 118	1	>1
		<i>P. vulgaris</i> CECT 484	>1	>1
	Thymus satureioides-Eth.Ext.-	<i>L. monocytogenes</i> CECT 4032	0.5	1
		<i>L. innocua</i> CECT 4031	0.5	>1
		<i>S. aureus</i> CECT 976	>1	>1
		<i>B. subtilis</i> DSM 6633	>1	>1
		<i>P. aeruginosa</i> CECT 118	>1	>1
		<i>P. vulgaris</i> CECT 484	0.5	0.5

**Table 10:** MIC and MBC values (%v/v) of extracts against different pathogenic microorganisms, tested by partner IPB

Partner	Essential oil or active substance	Strain Identification	MIC (% w/v) <sup>‡</sup>	MBC (% w/v) <sup>‡</sup>
<b>IPB (Portugal)</b>	Rosemary	<i>L. monocytogenes</i> WDCM 00019	2	nd
	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Basil	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Tarragon	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Salvia	<i>L. monocytogenes</i> WDCM 00019	2	nd
	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Rosemary	<i>L. monocytogenes</i> WDCM 00019	1	nd
	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Basil	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Tarragon	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Salvia	<i>L. monocytogenes</i> WDCM 00019	1	nd
	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	1	nd
	Rosemary	<i>L. monocytogenes</i> WDCM 00019	2	nd
	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd
	Basil	<i>L. monocytogenes</i> WDCM 00019	0.5	nd
	Tarragon	<i>L. monocytogenes</i> WDCM 00019	1	nd
	Salvia	<i>L. monocytogenes</i> WDCM 00019	0.125	nd
	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	1	nd
	Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	2	nd
	Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
	Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
	Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium	> 2	nd

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	ATCC 43971		
Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	2	nd
Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serotype Typhimurium ATCC 43971	> 2	nd
Rosemary	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Lemon balm	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Basil	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Tarragon	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Salvia	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Mentha spicata	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Rosemary	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Lemon balm	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Basil	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Tarragon	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Salvia	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Mentha spicata	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Rosemary	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Lemon balm	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Basil	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Tarragon	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Salvia	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd
Mentha spicata	<i>S. aureus</i> WDCM 00032 (=ATCC 6538)	> 2	nd

Specifically, Partner **UCO (Spain)** determined the MIC of thyme, palm rose, lemongrass and African basil essential oils against *Salmonella* Enteritidis ATCC3076. Partner **UIZ (Morocco)** tested the antimicrobial activity (MIC & MBC values) of thyme essential oil and thyme in hydroalcoholic extraction against *L. monocytogenes*, *L. innocua*, *S. aureus*, *B. subtilis*, *Pseudomonas aeruginosa* and *P. vulgaris*. Partner **IPB (Portugal)** determined activity (MIC values) of rosemary, lemon balm, basil, tarragon, salvia and *Mentha spicata* essential oils against *L. monocytogenes* WDCM 00019, *Salmonella enterica* subsp. *Enterica* serotype Typhimurium ATCC 43971 and *S. aureus* WDCM 00032 (=ATCC 6538).

Table 11: MIC and MBC values (%v/v) of extracts against different pathogenic microorganisms, tested by AUA

Partner	Essential oil or active substance	Strain Identification	MIC (% w/v) <sup>‡</sup>	MBC (% w/v) <sup>‡</sup>
AUA (Greece)	Carvacrol	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.04	0.08
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	0.06	0.05
		<i>Escherichia coli</i> O157:H7 NCTC 12079	0.08	0.15
		<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538	0.035	0.08
	Cinnamon oil (C. cassia, EOC)	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.115	0.3
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	0.225	0.225
	Oregano oil	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.13	0.37
		<i>L. monocytogenes</i> 6179 (1/2a)	0.21	0.45
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	0.15	0.225
		<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agona 23	0.3	0.3
		<i>Escherichia coli</i> O157:H7 NCTC 12079	0.115	0.3
		<i>Escherichia coli</i> O157:H7 NCTC 13127	0.3	0.3
		<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538	0.15	0.26
		<i>Staphylococcus aureus</i> NCBF 1499	0.15	0.3
	Rosemary oil	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.9	2.35
		<i>L. monocytogenes</i> 6179 (1/2a)	0.9	3.5
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	0.6	1.2
		<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agona 23	0.6	1.2
		<i>Escherichia coli</i> O157:H7 NCTC 12079	0.9	0.9
		<i>Escherichia coli</i> O157:H7 NCTC 13127	0.45	2.05
		<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538	1.2	3.5
		<i>Staphylococcus aureus</i> NCBF 1499	1.2	1.2
	Orange peel ( <i>Citrus sinensis</i> )	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.6	4.25
		<i>L. monocytogenes</i> 6179 (1/2a)	0.9	3.5
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	0.9	1.475
		<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agona 23	0.9	3.5
		<i>Escherichia coli</i> O157:H7 NCTC 12079	1.2	1.475
		<i>Escherichia coli</i> O157:H7 NCTC 13127	0.45	1.2
		<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538	1.2	2.125
		<i>Staphylococcus aureus</i> NCBF 1499	1.2	4.2
	Lemon oil ( <i>Cymbopogon citratus</i> )	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	1.475	4.2
		<i>L. monocytogenes</i> 6179 (1/2a)	0.9	5
		<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74	1.2	5
		<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Agona 23	1.2	>5
		<i>Escherichia coli</i> O157:H7 NCTC 12079	1.65	>5
		<i>Escherichia coli</i> O157:H7 NCTC 13127	0.9	5
		<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538	3	4.25
		<i>Staphylococcus aureus</i> NCBF 1499	3.5	3.5
	Aloe vera oil	<i>L. monocytogenes</i> C5 (4b, surface isolate from dairy farm environment)	0.525	1.05
<i>Salmonella enterica</i> subsp. <i>enterica</i> (ex Kauffmann and Edwards) Le Minor and Poppof serovar Typhimurium 4/74		1.05	1.2	
<i>Escherichia coli</i> O157:H7 NCTC 12079		2.1	2.1	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538		2.1	4.2	

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Finally, partner AUA (Greece) tested the antimicrobial activity (MIC & MBC values) of the essential oils of Cinnamon, Oregano, Rosemary, Orange peel, Lemon, and aloe vera as well as the active compound of Carvacrol against 2 strains of *L. monocytogenes*, *Escherichia coli O157:H7*, *Salmonella enterica subsp. Enterica* and *St. aureus*. Task 4.2 has been completed by IPB, UCO and AUA. Based on the screening results from both the *in-vitro* testing and literature review, the aforementioned partners selected the natural extracts (or essential oils), according to region (Table 12). The extracts currently studied by UIZ and ISBST/UMA – and listed in Table 12 – are subject to confirmation when in-vitro testing is completed by April 2021. The in-situ antimicrobial capacity of the extracts or essential oils shown in Table 23 will be assessed in the target artisanal foods.

Table 12: Natural extracts selected to be assessed in the target artisanal foods (\*: to be confirmed)

Country	Extracts	Type	To be tested in
IPB	Lemon balm	Extraction in water	Alheira sausage
	<i>Bixa orellana</i>	Extraction in oil	Alheira sausage
	Spearmint	Hydroalcoholic extraction	Goat milk cheese
	Salvia	Hydroalcoholic extraction	Goat milk cheese
UCO	Thyme	Extraction in water	Goat milk cheese
	Thyme	Extraction in water	Salchichón sausage
	Palm rose	Extraction in water	Salchichón sausage
	Lemongrass	Extraction in water	Salchichón sausage
	African basil	Extraction in water	Salchichón sausage
AUA	Oregano essential oil	Pure essential oil	Katiki
	Oregano essential oil	Encapsulated in liposomes	Katiki
	Oregano essential oil	Encapsulated in $\beta$ -cyclodextrins	Katiki
	Rosemary essential oil	Pure essential oil	Noumboulo sausage
	Rosemary essential oil	Encapsulated in liposomes	Noumboulo sausage
	Rosemary essential oil	Encapsulated in $\beta$ -cyclodextrins	Noumboulo sausage
UIZ*	Thyme	Hydroalcoholic Extraction	Jben cheese
	Oregano	Essential oil	Jben cheese
	Rosemary	Hydroalcoholic Extraction	Merguez sausage
	Oregano	Essential oil	Merguez sausage
ISBST/UMA*	Lemon peel	Extraction in hydroethanolic solution	Leben milk
	Date	Extraction in hydroacetone solution	Leben milk
	Sesame seeds	Extraction in hydroethanolic solution	Leben milk
	Hesperidin	Extraction in hydroethanolic solution	Leben milk
	Fennel seeds	Extraction in hydroethanolic solution	Dry merguez sausage
	Mint	Extraction in hydroethanolic solution	Dry merguez sausage
	Red pepper	Extraction in hydroethanolic solution	Dry merguez sausage
	Coriander	Extraction in hydroethanolic solution	Dry merguez sausage
	Oleuropein	Extraction in hydromethanolic solution	Dry merguez sausage
	Garlic	Extraction in water	Kaddid sheep meat
	Mint	Pure essential oil / extraction in methanolic solution	Kaddid sheep meat

## References

- Al-Nabulsi, A. A., Osaili, T. M., Olaimat, A. N., Almasri, W. E., Ayyash, M., Al-Holy, M. A., Jaradat, Z. W., Obaid, R. S., & Holley, R. A. (2020). Inactivation of Salmonella spp. in tahini using plant essential oil extracts. *Food Microbiology*, 86(September 2019), 103338. <https://doi.org/10.1016/j.fm.2019.103338>
- Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46, 446–475.
- Bouyahya, A., Lagrouh, F., El Omari, N., Bourais, I., El Jemli, M., Marmouzi, I., Salhi, N., Faouzi, M. E. A., Belmehdi, O., Dakka, N., & Bakri, Y. (2020). Essential oils of Mentha viridis rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. *Biocatalysis and Agricultural Biotechnology*, 23(December 2019), 101471. <https://doi.org/10.1016/j.bcab.2019.101471>
- Braca, A., Siciliano, T., D'Arrigo, M., & Germanò, M. P. (2008). Chemical composition and antimicrobial activity of Momordica charantia seed essential oil. *Fitoterapia*, 79(2), 123–125. <https://doi.org/10.1016/j.fitote.2007.11.002>
- Budiati, T., Suryaningsih, W., Umaroh, S., Poerwanto, B., Bakri, A., & Kurniawati, E. (2018). Antimicrobial activity of essential oil from Indonesian medicinal plants against food-borne pathogens. *IOP Conference Series: Earth and Environmental Science*, 207(1). <https://doi.org/10.1088/1755-1315/207/1/012036>
- Budiati, T., Wibisono, Y., Pambayun, R. A., Fahrezy, M. F., Ariyani, R., Kurniawati, E., Suryaningsih, W., Yudiastuti, S. O. N., & Bakri, A. (2020). Inhibition of Listeria monocytogenes by natural antimicrobial. *IOP Conference Series: Earth and Environmental Science*, 411(1). <https://doi.org/10.1088/1755-1315/411/1/012042>
- Bukvički, D., Stojković, D., Soković, M., Vannini, L., Montanari, C., Pejin, B., Savić, A., Veljić, M., Grujić, S., & Marin, P. D. (2014). Satureja horvatii essential oil: In vitro antimicrobial and antiradical properties and in situ control of Listeria monocytogenes in pork meat. *Meat Science*, 96(3), 1355–1360. <https://doi.org/10.1016/j.meatsci.2013.11.024>
- Burt, S. (2004). *Essential oils : their antibacterial properties and potential applications in foods — a review*. 94, 223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- Chaves, T. P., Pinheiro, R. E. E., Melo, E. S., Soares, M. J. dos S., Souza, J. S. N., Andrade, T. B. de, Lemos, T. L. G. de, & Coutinho, H. D. M. (2018). Essential oil of Eucalyptus camaldulensis Dehn potentiates  $\beta$ -lactam activity against Staphylococcus aureus and Escherichia coli resistant strains. *Industrial Crops and Products*, 112(June 2017), 70–74. <https://doi.org/10.1016/j.indcrop.2017.10.048>
- Chen, P., Ference, C., Sun, X., Lin, Y., Tan, L., & Zhong, T. (2020). Antimicrobial Efficacy of Liposome-Encapsulated Citral and Its Effect on the Shelf Life of Shatangju Mandarin. *Journal of Food Protection*, 83(8), 1315–1322. <https://doi.org/10.4315/JFP-20-115>
- Churklam, W., Chaturongakul, S., Ngamwongsatit, B., & Aunpad, R. (2020). The mechanisms of action of carvacrol and its synergism with nisin against Listeria monocytogenes on sliced bologna sausage. *Food Control*, 108(September 2019), 106864. <https://doi.org/10.1016/j.foodcont.2019.106864>
- Coma, V. (2008). Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science*, 78, 90–103. Davidson & Naidu, 2000.
- Davidson, P. M. (1997). Chemical preservatives and natural antimicrobial compounds. In M. P. Doyle, L. R. Beuchat, & T. J. Montville (Eds.), *Food microbiology fundamentals and frontiers* (pp. 520–556). New York: ASM Press.

- De Azeredo, G. A., Stamford, T. L. M., Nunes, P. C., Gomes Neto, N. J., De Oliveira, M. E. G., & De Souza, E. L. (2011). Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Research International*, 44(5), 1541–1548. <https://doi.org/10.1016/j.foodres.2011.04.012>
- de Medeiros Barbosa, I., da Cruz Almeida, É. T., Gomes, A. C. A., & de Souza, E. L. (2020). Evidence on the induction of viable but non-culturable state in *Listeria monocytogenes* by *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in a meat-based broth. *Innovative Food Science and Emerging Technologies*, 62(December 2019), 102351. <https://doi.org/10.1016/j.ifset.2020.102351>
- Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of Biotechnology*, 233, 106–120. <https://doi.org/10.1016/j.jbiotec.2016.07.005>
- Ehsani, A., Rezaeiyan, A., Hashemi, M., Aminzare, M., Jannat, B., & Afshari, A. (2019). Antibacterial activity and sensory properties of *Heracleum persicum* essential oil, nisin, and *Lactobacillus acidophilus* against *Listeria monocytogenes* in cheese. *Veterinary World*, 12(1), 90–96. <https://doi.org/10.14202/vetworld.2019.90-96>
- Emami, S., Azadmard-Damirchi, S., Peighambaroust, S. H., Valizadeh, H., & Hesari, J. (2016). Liposomes as carrier vehicles for functional compounds in food sector. *Journal of Experimental Nanoscience*, 11(9), 737–759. <https://doi.org/10.1080/17458080.2016.1148273>
- Engel, J. B., Heckler, C., Tondo, E. C., Daroit, D. J., & da Silva Malheiros, P. (2017). Antimicrobial activity of free and liposome-encapsulated thymol and carvacrol against *Salmonella* and *Staphylococcus aureus* adhered to stainless steel. *International Journal of Food Microbiology*, 252(April), 18–23. <https://doi.org/10.1016/j.ijfoodmicro.2017.04.003>
- Fetsch, A., & Jöhler, S. (2018). *Staphylococcus aureus* as a Foodborne Pathogen. *Current Clinical Microbiology Reports*, 5(2), 88–96. <https://doi.org/10.1007/s40588-018-0094-x>
- Ghasemi Pirbalouti, A., Hashemi, M., & Ghahfarokhi, F. T. (2013). Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. *Industrial Crops and Products*, 48, 43–48. <https://doi.org/10.1016/j.indcrop.2013.04.004>
- Gochev, V. K., & Girova, T. D. (2009). Antimicrobial activity of various essential oils against spoilage and pathogenic microorganisms isolated from meat products. *Biotechnology and Biotechnological Equipment*, 23, 900–904. <https://doi.org/10.1080/13102818.2009.10818568>
- Gómez-García, M., Sol, C., De Nova, P. J. G., Puyalto, M., Mesas, L., Puente, H., Mencía-Ares, Ó., Miranda, R., Argüello, H., Rubio, P., & Carvajal, A. (2019). Antimicrobial activity of a selection of organic acids, their salts and essential oils against swine enteropathogenic bacteria. *Porcine Health Management*, 5(1), 1–8. <https://doi.org/10.1186/s40813-019-0139-4>
- Guandalini Cunha, B., Duque, C., Sampaio Caiaffa, K., Massunari, L., Araguê Catanoze, I., dos Santos, D. M., de Oliveira, S. H. P., & Guiotti, A. M. (2020). Cytotoxicity and antimicrobial effects of citronella oil (*Cymbopogon nardus*) and commercial mouthwashes on *S. aureus* and *C. albicans* biofilms in prosthetic materials. *Archives of Oral Biology*, 109(September 2019). <https://doi.org/10.1016/j.archoralbio.2019.104577>
- Habeeb, F., Shakir, E., Bradbury, F., Cameron, P., Taravati, M. R., Drummond, A. J., Gray, A. I., & Ferro, V. A. (2007). Screening methods used to determine the anti-microbial properties of Aloe vera inner gel. *Methods*, 42(4), 315–320. <https://doi.org/10.1016/j.ymeth.2007.03.004>

- Hassanshahian, M., Bayat, Z., Saeidi, S., & Shiri, Y. (2014). Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial. *International Journal of Advanced Biological and Biomedical Research*, 2(1), 18–24. <https://doi.org/10.1051/0004-6361/200911869>
- Hulánková, R., & Bořilová, G. (2011). In vitro combined effect of oregano essential oil and caprylic acid against salmonella serovars, escherichia coli O157:H7, staphylococcus aureus and listeria monocytogenes. *Acta Veterinaria Brno*, 80(4), 343–348. <https://doi.org/10.2754/avb201180040343>
- Hyltdgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, 3(JAN), 1–24. <https://doi.org/10.3389/fmicb.2012.00012>
- Jiang, Y., Wu, N., Fu, Y. J., Wang, W., Luo, M., Zhao, C. J., Zu, Y. G., & Liu, X. L. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental Toxicology and Pharmacology*, 32(1), 63–68. <https://doi.org/10.1016/j.etap.2011.03.011>
- Kang, J., Jin, W., Wang, J., Sun, Y., Wu, X., & Liu, L. (2019). Antibacterial and anti-biofilm activities of peppermint essential oil against *Staphylococcus aureus*. *Lwt*, 101(August 2018), 639–645. <https://doi.org/10.1016/j.lwt.2018.11.093>
- Khorshidian, N., Yousefi, M., Khanniri, E., & Mortazavian, A. M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science and Emerging Technologies*, 45(March 2017), 62–72. <https://doi.org/10.1016/j.ifset.2017.09.020>
- Knobloch, K., Pauli, A., Iberl, B., Weigand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, 1(3), 119–128. <https://doi.org/10.1080/10412905.1989.9697767>
- Mallmann, C. A., Brugnari, T., Abreu Filho, B. A. de, Mikcha, J. M. G., & Machinski, M. (2017). Curcuma longa L. essential oil composition, antioxidant effect, and effect on *Fusarium verticillioides* and fumonisin production. *Food Control*, 73, 806–813. <https://doi.org/10.1016/j.foodcont.2016.09.032>
- Manzan, A. C. C. M., Toniolo, F. S., Bredow, E., & Povh, N. P. (2003). Extraction of Essential Oil and Pigments from *Curcuma longa* [L.] by Steam Distillation and Extraction with Volatile Solvents. *Journal of Agricultural and Food Chemistry*, 51(23), 6802–6807. <https://doi.org/10.1021/jf030161x>
- McClements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter*, 8(6), 1719–1729. <https://doi.org/10.1039/c2sm06903b>
- Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016). Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chemistry*, 194, 410–415. <https://doi.org/10.1016/j.foodchem.2015.07.139>
- Mohan, A., & Purohit, A. S. (2020). Anti-Salmonella activity of pyruvic and succinic acid in combination with oregano essential oil. *Food Control*, 110(October 2019), 106960. <https://doi.org/10.1016/j.foodcont.2019.106960>
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451–1474. <https://doi.org/10.3390/ph6121451>
- Nikolic, B., Vasilijevic, B., & Mitic-Culafic, D. (2019). Antilisterial effect of juniper (*Juniperus communis*) and its mixed application with winter savory (*Satureja montana*) in beef protection. *IOP Conference Series: Earth and Environmental Science*, 333(1). <https://doi.org/10.1088/1755-1315/333/1/012019>

- Paparella, A., Taccogna, L., Aguzzi, I., Chaves-López, C., Serio, A., Marsilio, F., & Suzzi, G. (2008). Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*. *Food Control*, *19*(12), 1174–1182. <https://doi.org/10.1016/j.foodcont.2008.01.002>
- Paudel, S. K., Bhargava, K., & Kotturi, H. (2019). Antimicrobial activity of cinnamon oil nanoemulsion against *Listeria monocytogenes* and *Salmonella* spp. on melons. *Lwt*, *111*(October 2018), 682–687. <https://doi.org/10.1016/j.lwt.2019.05.087>
- Ricke, S. C., Kundinger, M. M., Miller, D. R., & Keeton, J. T. (2002). Alternatives to Antibiotics: Chemical and Physical Antimicrobial Interventions and Foodborne Pathogen Response. *Poultry Science*, *84*(4), 667–675. <https://doi.org/10.1093/ps/84.4.667>
- Rossi, C., Chaves-López, C., Možina, S. S., Di Mattia, C., Scuota, S., Luzzi, I., Jenič, T., Paparella, A., & Serio, A. (2019). *Salmonella enterica* adhesion: Effect of *Cinnamomum zeylanicum* essential oil on lettuce. *Lwt*, *111*(February), 16–22. <https://doi.org/10.1016/j.lwt.2019.05.026>
- Sadekuzzaman, M., Mizan, M. F. R., Kim, H. S., Yang, S., & Ha, S. Do. (2018). Activity of thyme and tea tree essential oils against selected foodborne pathogens in biofilms on abiotic surfaces. *LWT - Food Science and Technology*, *89*(April 2017), 134–139. <https://doi.org/10.1016/j.lwt.2017.10.042>
- Sagalowicz, L., & Leser, M. E. (2010). Delivery systems for liquid food products. *Current Opinion in Colloid and Interface Science*, *15*(1–2), 61–72. <https://doi.org/10.1016/j.cocis.2009.12.003>
- Shahrezaee, M., Soleimani-Zad, S., Soltanizadeh, N., & Akbari-Alavijeh, S. (2018). Use of Aloe vera gel powder to enhance the shelf life of chicken nugget during refrigeration storage. *Lwt*, *95*(September 2017), 380–386. <https://doi.org/10.1016/j.lwt.2018.04.066>
- Smid, E. J., & Gorris, L. G. M. (1999). Natural antimicrobials for food preservation. In M. S. Rahman (Ed.), *Handbook of food preservation* (pp. 285–308). New York: Marcel Dekker Inc.
- Somrani, M., Inglés, M. C., Debbabi, H., Abidi, F., & Palop, A. (2020). Garlic, onion, and cinnamon essential oil anti-biofilms' effect against *Listeria monocytogenes*. *Foods*, *9*(5), 1–12. <https://doi.org/10.3390/foods9050567>
- Stanley, M. C., Ifeanyi, O. E., & Eziokwu, O. G. (2014). *Original Research Article Antimicrobial effects of Aloe vera on some human pathogens*. *3*(3), 1022–1028.
- Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. *Evidence-Based Complementary and Alternative Medicine*, 2016. <https://doi.org/10.1155/2016/3012462>
- Tanhaeian, A., Sekhavati, M. H., & Moghaddam, M. (2020). Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. *Chemical and Biological Technologies in Agriculture*, *7*(1), 1–11. <https://doi.org/10.1186/s40538-020-00181-9>
- Tassou, C. C., Lambropoulou, K., & Nychas, G.-J. E. (2004). Effect of prestorage treatments and storage conditions on the survival of *Salmonella enteritidis* PT4 and *Listeria monocytogenes* on fresh marine and freshwater aquaculture fish. *Journal of Food Protection*, *67*, 193–198.
- Yuan, W., Teo, C. H. M., & Yuk, H. G. (2019). Combined antibacterial activities of essential oil compounds against *Escherichia coli* O157:H7 and their application potential on fresh-cut lettuce. *Food Control*, *96*(September 2018), 112–118. <https://doi.org/10.1016/j.foodcont.2018.09.005>



**Annexes**

**MIC and MBC values of plant-based extracts or essential oils pre-selected by partners IPB, UCO, AUA, UIZ and ISBST/UMA**

**ArtiSaneFood – D4.1 MIC and MBC of natural antimicrobials**

<b>Partner</b>	<b>Essential oil or active substance</b>	<b>Strain Identification</b>	<b>MIC (% w/v)<sup>‡</sup></b>	<b>MBC (% w/v)<sup>‡</sup></b>	<b>Method Applied</b>	<b>Reference</b>
IPB	Rosemary	<i>L. monocytogenes</i> WDCM 00019	2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>L. monocytogenes</i> WDCM 00019	2	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>L. monocytogenes</i> WDCM 00019	1	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>L. monocytogenes</i> WDCM 00019	1	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	1	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>L. monocytogenes</i> WDCM 00019	2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>L. monocytogenes</i> WDCM 00019	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>L. monocytogenes</i> WDCM 00019	0.5	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>L. monocytogenes</i> WDCM 00019	1	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>L. monocytogenes</i> WDCM 00019	0.125	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>L. monocytogenes</i> WDCM 00019	1	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Mentha spicata	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	> 2	nd	Micro-dilution	Own experiments
IPB	Rosemary	<i>S. aureus</i> WDCM 00032 (=ATCC 65	> 2	nd	Micro-dilution	Own experiments
IPB	Lemon balm	<i>S. aureus</i> WDCM 00032 (=ATCC 65	> 2	nd	Micro-dilution	Own experiments
IPB	Basil	<i>S. aureus</i> WDCM 00032 (=ATCC 65	> 2	nd	Micro-dilution	Own experiments
IPB	Tarragon	<i>S. aureus</i> WDCM 00032 (=ATCC 65	> 2	nd	Micro-dilution	Own experiments
IPB	Salvia	<i>S. aureus</i> WDCM 00032 (=ATCC 65	> 2	nd	Micro-dilution	Own experiments

## ArtiSaneFood – D4.1 MIC and MBC of natural antimicrobials

Partner	Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Salmonella</i> Enteritidis ATCC13076	0.0625	nd	Micro-dilution	Own experiments
UCO	<i>Cymbopogon martinii</i> (palm rose)	<i>Salmonella</i> Enteritidis ATCC13077	0.125	nd	Micro-dilution	Own experiments
UCO	<i>Cymbopogon citratus</i> (lemongrass)	<i>Salmonella</i> Enteritidis ATCC13078	0.25	nd	Micro-dilution	Own experiments
UCO	<i>Ocimum gratissimum</i> (african basil)	<i>Salmonella</i> Enteritidis ATCC13079	1	nd	Micro-dilution	Own experiments
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Staphylococcus aureus</i> ATCC6538	0.3013	nd	Micro-dilution	Zhang (2019)
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Staphylococcus aureus</i> ATCC25923	0.062	0.062	Micro-dilution	Veloso (2019)
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Staphylococcus aureus</i> STA32	0.25	nd	Micro-dilution	Pellegrini (2018)
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Staphylococcus aureus</i> STA47	0.125	nd	Micro-dilution	Pellegrini (2018)
UCO	<i>Thymus vulgaris</i> (thyme)	<i>Staphylococcus aureus</i> STA39	0.125	nd	Micro-dilution	Pellegrini (2018)

**ArtiSaneFood – D4.1 MIC and MBC of natural antimicrobials**

Partner	Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
AUA	Carvacrol	<i>L. monocytogenes</i> 10403S	0.025	0.05	Micro-dilution	Churklam et al., 2019
		<i>L. monocytogenes</i> DMST17303				
		<i>L. monocytogenes</i> CM2-BM-HF-Black				
		<i>L. monocytogenes</i> CM8-ISO-HF-Blac				
		<i>L. monocytogenes</i> CM9-ISO-HF-Black				
		<i>L. monocytogenes</i> CM11-ISO-HF-Black				
		<i>L. monocytogenes</i> CM12-ISO-HF-Black				
		<i>L. monocytogenes</i> CM13-ISO-HF-Black				
		<i>L. monocytogenes</i> CM15-ISO-HF-Black				
		<i>L. monocytogenes</i> CECT 4032	0.01	nd	Tube Dilution	Somrani et al., 2020
AUA	Cinnamon oil nanoemulsion	<i>L. monocytogenes</i> train- FSL	0.078	0.078	Micro-dilution	Paudel et al., 2019
		<i>L. monocytogenes</i> N1-017, LMI				
		<i>L. monocytogenes</i> strain- FSL J2-064, LMII				
		<i>L. monocytogenes</i> strain- FSL N3-165, LMIII				
AUA	Galangal ( <i>Alpinia galanga</i> )	<i>L. monocytogenes</i>	2.6	nd	Broth Dilution	Budiati et al., 2020
AUA	Garlic	<i>L. monocytogenes</i> CECT 4032	0.01	nd	Tube Dilution	Somrani et al., 2020
AUA	Ginger ( <i>Zingiber officinale</i> )	<i>L. monocytogenes</i>	1.56	nd	Broth Dilution	Budiati et al., 2020
AUA	Heracleum persicum	<i>L. monocytogenes</i> PTCC1165	0.00025	0.00025	Micro-dilution	Eshani et al., 2019
AUA	<i>Juniperus communis</i>	<i>L. monocytogenes</i> ATCC 19111	0.5	2	Micro-dilution	Nikolic et al., 2019
		<i>L. monocytogenes</i> isolated from salmon (LMS)		1		
		<i>L. monocytogenes</i> from slaughterhouse water drainage tunnel (LMT)		4		
		<i>L. monocytogenes</i> from beef carcass (LMB)				
AUA	Lemongrass ( <i>Cymbopogon citratus</i> )	<i>L. monocytogenes</i>	0.32	nd	Broth Dilution	Budiati et al., 2020
AUA	<i>Mentha viridis</i>	<i>L. monocytogenes</i> 4b CECT 4032	0.25	0.25	Micro-dilution	Bouyahya et.al., 2020
AUA	Onion	<i>L. monocytogenes</i> CECT 4032	0.0025	nd	Tube Dilution	Somrani et al., 2020
AUA	Orange peel ( <i>Citrus sinensis</i> )	<i>L. monocytogenes</i>	3.13	nd	Broth Dilution	Budiati et al., 2020
AUA	<i>Oreganum vulgare</i>	<i>L. monocytogenes</i> ATCC 13932	0.000052	nd	Micro-dilution	Hulánková and Bořilová, 2011
		<i>L. monocytogenes</i> ATCC 13932				
		<i>L. monocytogenes</i> ATCC 7644	0.000125	nd	Tube Dilution	Azeredo et.al., 2011
		<i>L. monocytogenes</i> ATCC 7644				
		<i>L. monocytogenes</i> ATCC 19112				
		<i>L. monocytogenes</i> ATCC 19117	0.0025	nd	Micro-dilution	Barbosa et al., 2020
AUA	<i>Rosmarinus officinalis</i> L.	<i>L. monocytogenes</i>	3.13	nd	Broth Dilution	Budiati et al., 2020
		<i>L. monocytogenes</i> ATCC 7644	0.002	nd	Tube Dilution	Azeredo et.al., 2011
		<i>L. monocytogenes</i> ATCC 7644	0.0005	nd	Micro-dilution	Barbosa et al., 2020
		<i>L. monocytogenes</i> ATCC 19112				
		<i>L. monocytogenes</i> ATCC 19117				
AUA	<i>Satureja montana</i>	<i>L. monocytogenes</i> ATCC 19111	0.5	1	Micro-dilution	Nikolic et al., 2019
		<i>L. monocytogenes</i> isolated from salmon (LMS)	1			
		<i>L. monocytogenes</i> from slaughterhouse water drainage tunnel (LMT)				
		<i>L. monocytogenes</i> from beef carcass (LMB)				
AUA	Turmeric ( <i>Curcuma longa</i> )	<i>L. monocytogenes</i>	12.5	nd	Broth Dilution	Budiati et al., 2020

**ArtiSaneFood – D4.1 MIC and MBC of natural antimicrobials**

Partner	Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>¶</sup>	MBC (% v/v) <sup>¶</sup>	Method Applied	Reference
UIZ	<b>Thymus satureioides-E.O.-</b>	L. monocytogenes CECT 4032	0.032	0.032	Micro-dilution	Own experiments
		L. innocua CECT 4031	0.032	0.032	Micro-dilution	
		S. aureus CECT 976	0.04	0.125	Micro-dilution	
		B. subtilis DSM 6633	0.25	0.25	Micro-dilution	
		P. aeruginosa CECT 118	1	>1	Micro-dilution	
		P. vulgaris CECT 484	>1	>1	Micro-dilution	
	<b>Thymus satureioides-Eth.Ext.-</b>	L. monocytogenes CECT 4032	0.5	1	Micro-dilution	Own experiments
		L. innocua CECT 4031	0.5	>1	Micro-dilution	
		S. aureus CECT 976	>1	>1	Micro-dilution	
		B. subtilis DSM 6633	>1	>1	Micro-dilution	
		P. aeruginosa CECT 118	>1	>1	Micro-dilution	
		P. vulgaris CECT 484	0.5	0.5	Micro-dilution	
	<b>Thyme</b>	L. monocytogenes NCTC 11994	0.05	0.05	Broth Dilution	H. Mith et al., 2014
		S. typhimurium ATCC 14028	0.1	0.1	Broth Dilution	
		E. coli ATCC 35150	0.05	0.1	Broth Dilution	M. Carvalho et al., 2018
		S. aureus ATCC 25923	0.78	1.56	Broth Dilution	
	<b>Oreganum compactum</b>	S. aureus MBLA	0.125	0.125	Micro-dilution	Bouyahya et al (2017)
		S. aureus CECT 976	0.25	0.25	Micro-dilution	
		S. aureus CECT 994	0.125	0.125	Micro-dilution	
		P. aeruginosa IH	2	2	Micro-dilution	
		L. monocytogenes CECT 4032	0.5	0.5	Micro-dilution	
		E. coli K12	0.25	0.25	Micro-dilution	
	<b>Rosmarinus officinalis</b>	S. aureus CECT 239	1.5	>3	Tube Dilution	Ait-Ouazzou et al (2011)
		L. monocytogenes EGD-e	0.5	1	Tube Dilution	
		L. monocytogenes CECT 935	3	>3	Tube Dilution	
		S. enteritidis CECT 4155	1.5	>3	Tube Dilution	
		E. coli O157:H7 CECT 4267	>3	>3	Tube Dilution	
		P. aeruginosa CECT 110	>3	>3	Tube Dilution	
	<b>Mentha</b>	S. aureus ATCC 29213	0.25	0.25	Micro-dilution	Chraibi et al (2017)
		B. cereus	0.25	0.25	Micro-dilution	
E. coli ATCC 25922		0.5	1	Micro-dilution		
S. typhi		0.5	1	Micro-dilution		
P. aeruginosa ATCC 27853		8	>8	Micro-dilution		
<b>Garlic</b>	L. monocytogenes CECT 4032	0.01	nd	Tube Dilution	Somrani et al., 2020	
	L. monocytogenes	0.4	>0.8	Micro-dilution	S. Pedrós-Garrido et al., 2020	
	S. enterica ATCC 25957	0.2	nd	Broth Dilution	H. Al-Talib et al., 2015	
	E. coli ATCC 43889	0.1	nd			
	S. aureus	0.8	nd	Agar Dilution	S. Sethi et al., 2013	
<b>Lavandula mairei</b>	L. monocytogenes CECT 4032	0.008	0.01	Macro-dilution	A. El Hamdaoui et al., 2018	
	Salmonella	nd	nd			
	E. coli	nd	nd			
	S. aureus CECT 976	0.012	0.012			

**ArtiSaneFood – D4.1 MIC and MBC of natural antimicrobials**

Partner	Essential oil or active substance	Strain Identification	MIC (% v/v) <sup>‡</sup>	MBC (% v/v) <sup>‡</sup>	Method Applied	Reference
ISBST/UMA	Lemon peel ( <i>Citrus lemon</i> )	<i>Staphylococcus aureus</i>	2.5	nd	Micro-dilution	Otang and Afolaya, 2016
		<i>Escherichia coli</i>	2.5	nd		
		<i>Bacillus cereus</i>	0.02	nd		
ISBST/UMA	<i>Date (Phoenix dactylifera L.)</i>	<i>Bacillus cereus ATCC 11778</i>	1.56	3.13	Broth Dilution	El Arem et al., 2013
		<i>Staphylococcus aureus ATCC 25923</i>	0.78	1.56		
		<i>Listeria monocytogenes ATCC 19115</i>	0.09	0.19		
		<i>Escherichia coli ATCC 35218</i>	12.50	25		
ISBST/UMA	<i>Sesame seeds (Sesamum indicum)</i>	<i>Bacillus subtilis</i>	0.092	nd	Streak method	Sandeep et al., 2014
		<i>Escherichia coli</i>	0.095	nd		
ISBST/UMA	Oleuropein (olive leaves)	<i>Pseudomonas aeruginosa</i>	0,10	nd	Micro-dilution	Djenane et al., 2012
		<i>Staphylococcus aureus</i>	0.05	nd		
		<i>Salmonella enterica</i>	0.1	nd		
ISBST/UMA	Hesperidin (orange byproduct)	<i>Aeromonas hydrophila ATCC 7966</i>	3125	12500	Broth micro-dilution	Abuelsaad et al., 2013
		<i>Bacillus subtilis</i>	45	nd	Agar diffusion	Abass et al., 2014
		<i>Escherichia coli</i>	75	nd		
		<i>Salmonella typhi</i>	175	nd		
		<i>Staphylococcus aureus</i>	175	nd		
ISBST/UMA	Garlic	<i>Staphylococcus aureus</i>	0.00016	0.00064	Tube-dilution	Elsom et al., 2000
		<i>Escherichia coli</i>	0.0032	0.0032	Broth Dilution	Belguith et al., 2010
		<i>Salmonella</i>	1 - 1.25	1.3 - 1.5		
ISBST/UMA	Mint	<i>Listeria monocytogenes</i>	0.5	0.5	Micro-dilution	Bouyahya et al., 2017
	Fennel seeds ( <i>Foeniculum vulgare</i> )	<i>Escherichia coli</i>	0.13	0.13	Broth Dilution	Sayed Ahmad et al., 2017
		<i>Staphylococcus Aureus</i>	0.13	0.13		
	Mint ( <i>Mentha spicata</i> )	<i>Listeria monocytogenes</i>	15.3		Micro-dilution	Teixeira et al. 2012
ISBST/UMA	Corriander (EO)	<i>Listeria monocytogenes</i>	<0.01	nd	Broth Dilution	Delaquis et al., 2004
		<i>Escherichia coli 25922</i>	0.2	0.2		Casetti et al., 2012
		<i>Staphylococcus Aureus ATCC 25923</i>	0.2	1.6		Silva et al., 2011
		<i>Salmonella ATCC 13311</i>	0.4	0.8		Silva et al., 2012
ISBST/UMA	Red pepper	<i>Listeria monocytogenes</i>	0.40	-	Micro-dilution	Omolo et al., 2014
		<i>Escherichia coli</i>	0.06	-		