ANTIBACTERIAL ACTIVITY OF LEMON, CARAWAY AND BASIL EXTRACTS ON LISTERIA SPP.

Gordana R. Dimić*, Sunčica D. Kocić-Tanackov, Olivera O. Jovanov, Dragoljub D. Cvetković, Siniša L. Markov and Aleksandra S. Veličanski

*a University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
b Jugoinspekt, Dunavska 23, 21000 Novi Sad, Serbia

Commercial extracts of three spices (lemon, caraway and basil) against Listeria monocytogenes, L. innocua and L. welshimeri were investigated using disc diffusion method. Lemon and basil extracts inhibited all the organisms at the level of ≥ 5 µl (lemon) and 20 µl (basil). These extracts produced inhibitory zones of 9-19 mm (lemon) and 8-11.5 mm (basil). The extract of caraway showed activity only against L. innocua at the highest level (20 µl), producing an inhibitory zone of 14.7 mm. Generally, the lemon extract was the most effective. This extract exhibited greater inhibitory activity against L. monocytogenes, while the basil extract had the strongest effect on L. welshimeri.

KEY WORDS: Antibacterial activity, essential oils of spices, Listeria spp., food contamination

INTRODUCTION

Food contamination by microorganisms and their development and, hence, its decontamination represent a serious problem. Chemical agents to prevent microbial growth and various additives that are used in food industries, are considered to be potentially harmful to human health.

In seeking of possible alternatives antimicrobial activities of compounds of natural origin that can be found in many plants are currently in progress worldwide. Spices are aromatic plants that are widely used in the food industry and culinary food preparation for flavouring. However, their essential oils and extracts can have another role, and that is to control the growth of harmful microorganisms. It is necessary the spice to be effective enough to ensure that the product is safe and also have acceptable both odour and taste.

Numerous studies document the inhibitory effects of some essential oils and extracts of spices, plants, or their major active constituents on the bacteria Escherichia coli, Aeromonas spp., Enterococcus faecalis, Salmonella enterica Typhimurium, Staphylococcus aureus, Shigella spp., Bacillus spp., Listeria monocytogenes, Micrococcus spp., Yersinia enterocolitica, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus spp., Lacto-
bacillus spp., Enterobacter spp. (1-11). Gram-positive bacteria are generally more sensitive than Gram-negative bacteria (12, 13).

Species of the genus *Listeria* (gram-positive rod-shaped bacteria) are frequent in the human environment and their growth at low temperatures and high salt concentrations, in vacuum packed food and that packaged under modified atmospheres (MAP) could be a problem, especially if the contamination with pathogenic *L. monocytogenes* is present. Also, ready-to-eat (RTE) products are popular and can be consumed without further cooking. Salads and sandwiches are at risk of contamination because they require much manual manipulation during their preparation. Even the products that are held at refrigerator temperature during their manufacture, the storage and distribution provide the opportunity for growth of psychrotolerant pathogens and spoilage bacteria. Hence, the consumption of the food contaminated with *L. monocytogenes* can constitute a serious health risk (listeriosis) to humans. *Listeria* spp. was isolated from homemade white cheeses from various public bazaars (*L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. grayi*, *L. ivanovii* and *L. welshimeri*) (14), from fresh salad vegetables (*L. monocytogenes*, *L. welshimeri* and *L. murray*) (15), liquid whole eggs (*L. innocua*) (16). *L. innocua* was the dominant species in fresh chicken meat and chicken products that are examined by Kosek-Paszkowska et al. (17) and the highest prevalence in food products from supermarkets in Bangkok (18). The results of this study indicate the highest number of cases of *Listeria* spp. of contamination in meat, meat products and raw vegetables. Previously, we have reported that *Listeria* spp. were frequent in raw chicken, pork, and beef meat (19).

In this work we studied the antibacterial activity of some commercial spice extracts against pathogenic and nonpathogenic of *Listeria* species that contaminate food.

### EXPERIMENTAL

#### Spice extracts

Three different commercial extracts, of lemon, caraway and basil, were provided by ETOL, Celje, Slovenia. The botanical name and main components as typical contents of the spice samples are listed in Table 1. These extracts are intended for use in the food industries. Test concentrations for antibacterial examination were 0, 5, 10 and 20 µl.

#### Table 1. List of spice extracts tested

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Plant family</th>
<th>Main component</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td><em>Citrus limon</em></td>
<td>Rutaceae</td>
<td>Limonene</td>
<td>65.0</td>
</tr>
<tr>
<td>Caraway</td>
<td><em>Carum carvi</em></td>
<td>Apiaceae</td>
<td>Carvone</td>
<td>70.0</td>
</tr>
<tr>
<td>Basil</td>
<td><em>Ocimum basilicum</em></td>
<td>Lamiaceae</td>
<td>Estrapole</td>
<td>86.7</td>
</tr>
</tbody>
</table>

#### Test bacteria

The foodborne and spoilage bacteria used as test organisms were *L. monocytogenes*, *L. innocua* and *L. welshimeri*, all isolated from fresh meat. Until the experiment cultures of the bacteria were kept on tryptone soy yeast extract (TSYE) slants agar at 4°C.
Preparation of the inoculum

Bacterial inoculums were prepared from overnight culture on the TSYEA slant. Cultures were directly suspended in saline and the optical density of the suspension was measured by MacFarland standards and then the suspensions were diluted to the appropriate bacterial concentrations (10^6 cells/ml) needed in the experiments, using the same solution.

Antibacterial assays

In these studies, the disc diffusion method was used. TSYEA was poured in the Petri plates (9 mm diam) in a thickness of 4 mm. Each of test cultures was inoculated with a sterile swab by rotating the plate three times for 1/3 rounds between each smear and left to dry for 10 min at ambient temperature. Sterile discs (diameter of 6 mm), were placed at the center of each plate, after which the spice extracts were added in the above amounts. The plates were incubated at 37°C for 48 h. If the organism was more or less sensitive to the extract, around the disc appeared a clear zone of the growth inhibition. The diameter of the inhibitory zone was measured in mm. All tests were done in triplicate. The values are presented as means ± SD of three measurements.

RESULTS AND DISCUSSION

The results of the antibacterial disc diffusion assays are summarized in Table 2.

Table 2. Antibacterial activity of lemon, caraway and basil extracts on the growth of Listeria spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Quantity of spice extract (μl/disc)</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone diameter (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>9.5 ± 0.26</td>
<td>12.5 ± 0.35</td>
<td>19 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>L. innocua</td>
<td>9 ± 0.21</td>
<td>16.9 ± 0.44</td>
<td>17.5 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>9.5 ± 0.27</td>
<td>15.5 ± 0.35</td>
<td>18 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Caraway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>L. innocua</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>14.7 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Basil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>10 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>L. innocua</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>8 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>0 ± 0.00</td>
<td>10 ± 0.22</td>
<td>11.5 ± 0.26</td>
<td></td>
</tr>
</tbody>
</table>
The extracts of lemon, caraway and basil showed inhibitory potential against *Listeria* spp., the lemon extract being the most efficient. The inhibitory effect of this extract at the lowest volume for all three *Listeria* species was not practically different. The data indicate that *L. innocua* was equally sensitive to the increase in the level of oil at 10 and 20 µl, with the inhibition zones of 16.9 and 17.5 mm, respectively. At the level of 20 µl, *L. monocytogenes* was the most sensitive, with a slightly larger inhibitory zone (19 mm). The volumes of the caraway extract less than 20 µl did not affect the growth of the examined species. With 20 µl, it was effective only against *L. innocua*. The basil extract appeared to be a weaker inhibitor of *L. innocua*, but it was effective against *L. welshimeri* and *L. monocytogenes*.

The study showed that of the different spices tested for their antibacterial effect only lemon extract inhibited the growth of all bacteria, with *L. monocytogenes* being the most and *L. innocua* the least susceptible, while *L. monocytogenes*, *L. innocua* and *L. welshimeri* showed moderate sensitivity to basil extract. On the other hand, no inhibition zones of caraway were observed on *L. monocytogenes* and *L. welshimeri*. However, the basil extract was less potent than the caraway extract against *L. innocua*.

Smith-Palmer et al. (12) examined the inhibitory activity of essential oils of 23 species plant and two essences against some Gram-negative and Gram-positive bacteria, including also *L. monocytogenes*, and they found low activity of pure lemon oil and basil against *L. monocytogenes* when they were used in quantities of 25 µl in agar well technique. Fernandez-Lopez et al. (20) showed that the lemon extract (from an industrial by-product) having a high water content was not active against *L. monocytogenes* and *L. innocua*, as well as against lactic acid bacteria (except for one strain of *Lactobacillus lactis*, with an inhibitory zone of 8.5 mm). Friedly et al. (21) studied the sensitivity of *L. monocytogenes* and *L. innocua* to commercial citrus essential oils and the results of the disc diffusion assay showed inhibition zone diameters of 8.4 and 8.8 mm, for 10 µl of lemon essential oil. Espina et al. (22) evaluated the antimicrobial effects of three essential oils of lemon, orange, and mandarin against six microorganisms: *L. monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecium*, *Salmonella Enteritidis*, *Escherichia coli* O157 : H7 and *Pseudomonas aeruginosa*. Lemon and orange oils (15 µl/disc) showed weak/no inhibition zone (< 12 mm) against these microorganisms. The results that were obtained using broth dilution method showed minimum inhibitory concentration (MIC) of lemon oil of 1.0 µl/ml for *L. monocytogenes* and minimum bactericidal (MBC) > 30 µl/ml. Shan et al. (23) tested the antimicrobial properties of 46 extracts from spices and herbs and reported that caraway extract had no inhibitory activity against five food-borne bacteria, *L. monocytogenes*, *B. cereus*, *S. aureus*, *E. coli* and *S. anatum*. *L. monocytogenes* was the most resistant of the tested Gram-positive organisms in this investigation. Pure oil from caraway and suspensions in ethanol in the ratio of 2:1 and 1:1 showed microbistatic effect on *L. monocytogenes* ATCC 19115 (more sensitive) and *L. monocytogenes* ATCC 19112, with an inhibitory zone of ≤ 13 mm (24). In several report, basil essential oil was less effective in inhibiting *L. monocytogenes* and *Pseudomonas aeruginosa* compared to the other Gram-positive and Gram-negative bacteria (25). However, Hossain et al. (26) reported that essential oils and methanol extracts of basil displayed a great antibacterial activity against *L. monocytogenes* and other tested bacteria. Nguefack et al. (27) investigated the inhibitory effects of essential oils from five aromatic plants on
the strains of \textit{L. monocytogenes}, \textit{L. innocua} and \textit{S. aureus}. \textit{L. innocua} and three strains \textit{L. monocytogenes} were more sensitive to oil isolated from basil \textit{Ocimum gratissimum} than \textit{Ocimum basilicum}, while one strain of \textit{L. monocytogenes} was resistant to \textit{O. basilicum}.

Lemon, caraway and basil essential oils and extracts contain many biologically active compounds that give rise to their antimicrobial actions. The most important active ingredients of lemon are monoterpenes such as limonene, $\gamma$-terpinene, $\beta$-pinene and the aldehydes geranial and neral (22, 28). Iacobellis et al. (29) determined carvone, limonene, germacrene D and $\text{trans}$-dihydrocarvone as the main components of caraway. Linalool was found as a main component in basil oil investigated by Hanif et al. (30). Kocić-Tanackov et al. (31) identified estragol as the major component present in basil extract.

Numerous publications have documented different data on antibacterial activity of the spice essential oils and extracts. The data are based on different test methods, different methods of obtaining and composition of oils and extracts and the characteristics of the test microorganisms. Although it is not always easy to compare different studies, there are indications that the application of these natural antimicrobial agents can slow or prevent the growth of microorganisms (32, 33), and the mechanisms of the action are being intensively examined. The flow cytometry of \textit{L. innocua} stained with carboxy fluorescein diacetate showed that essential oil permeabilized the cytoplasmic membrane (27).

**CONCLUSION**

The results of this study indicate that lemon, caraway and basil extracts have the potential as a natural antimicrobial agents to be used for the future practical application in preservation of the food from microbiological spoilage and prevention of foodborne diseases.

**Acknowledgement**

These results are part of the Project No. TR - 31017 financially supported by the Ministry of Science and Technological Development of the Republic of Serbia.

**REFERENCES**


АНТИБАКТЕРИЈСКА АКТИВНОСТ ЕКСТРАКАТА ЛИМУНА, КИМА И БОСИЉКА НА LISTERIA SPP.

Гордана Р. Димића, Сунчица Д. Коџић-Танацков, Оливера О. Јованов, Драгољуб Д. Цветковића, Синиша Л. Марков и Александра С. Велићански

Универзитет у Новом Саду, Технолошки факултет, Булевар цара Лазара 1, 21000 Нови Сад, Србија

Југоинспект, Дунавска 23, 21000 Нови Сад, Србија

Три комерцијална екстракта зачина (лимун, ким и босилак) против Listeria monocytogenes, L. innocua и L. welshimeri су били испитивани коришћењем диск дифузионе методе. Екстракти лимуна и босилака су инхибирали све организме на нивоима од ≥ 5 µl (лимун) и 20 µl (босилак). Ови екстракти произвели су зоне инхибиције од 9-19 mm (лимун) и 8-11,5 mm (босилак). Екстракт кима је показао активност само против L. innocua на највишем нивоу (20 µl), производећи зону инхибиције од 14,7 mm. Генерално, екстракт лимуна је био најефективнији. Овај екстракт је показивао већу инхибиторну активност против L. monocytogenes, док је екстракт босилака најјаче деловао на L. welshimeri.

Кључне речи: антибактеријска активност, етарска уља заchina, екстракти, Listeria spp., контаминација хране

Received: 02 July 2012
Accepted: 13 September 2012