Antibacterial Activity of Habanero Chili Sauces against Selected Pathogenic Bacteria

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Abstract

Aim of this study was researched antibacterial activity of habanero chili sauces against selected pathogenic bacteria, as *Escherichia coli*, *Bacillus thuringiensis*, *Yersinia enterocolitica* and *Salmonella enterica* subsp. Typhimurium. Antibacterial activity of chili sauces was determined by two-fold broth microdilution methods in 96 well plates. Determination of microbial density before and after experiment was measured by ELISA reader and measured values were counted out. Absorbance differences which were greater than 0.05, it were considered as bacterial growth. The best antibacterial activity of chili sauce had against *Salmonella enterica* subsp. Typhimurium (33 %) and *Yersinia enterocolitica*(16.65 %). Other concentration has weaker inhibition effect to growth of bacteria. Also chili sauces have weaker antibacterial effect against *Escherichia coli* and *Bacillus thuringiensis*. This experiment confirms that habanero chili sauces have antibacterial activity and it showed the next possibilities to study of antimicrobial activity of habanero chili sauces.

Keywords: Antibacterial activity, habanero chili sauces, pathogenic bacteria

1. Introduction

In the process of developing new pharmacologically active compounds, natural resources of vegetable origin represent an important source of drugs [1]. However, it is worth emphasizing the importance of defining criteria in the selection of the material for scientific research. Concerning those criteria, it has been found that ethnopharmacological approaches have provided important subsidies in establishing criteria for inclusion and/or exclusion of species for development of validation studies, allowing to evaluate efficacy and safety of plant resources employed therapeutically by the population [2,3]. Chili (*Capsicum annuum* L.) is one of the five domesticated and the most cultivated species from an economic and nutritional viewpoint worldwide [4]. Chili fruit contains a broad variety of antioxidant vitamins especially vitamin A and C, capsaicin, which determine the great variability of the fruit's smell, flavour, taste and consequently consumer preference [5]. Capsaicin is an alkaloid (capsaicinoid) found in chili and it is present in large amounts in the placental tissues that hold the seeds in chilies. The pungency of chili is caused by at least two and possibly all related capsaicinoids. According to previous studies, capsaicin (8-methylN-vanillyl-6-nonenamide) and dihydrocapsaicin contribute almost 90% of the pungency [6,7]. Other capsaicinoids such as

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nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, and nonivamide are present in a small quantity [8]. Capsaicin and its synthetic derivatives have been extensively investigated in the fields of pharmacy [9], neuroscience [10], and antimicrobial drugs [11]. The plain and heated extracts were found to exhibit varying degrees of inhibition against microbes. Recently researchers have shown that capsaicin can inhibit the growth of some foodborne pathogenic bacteria [12].

In this study we researched antibacterial activity of red and yellow habanero chili suaces by microdillution methods.

2. Materials and methods

Sample preparation

For this experiment was used sauces of chili „Habanero“ in two type varietes. The first was yellow variete (SHU 350 000 – 450 000) and second red variete (SHU 400 000 – 500 000). Sauces were prepared by milling of chili and salt was added only in 16.4 % amount. Chili was harvested in Spain at September 2014. Chili sauces were stored in room temperature for 6 months.

Agar plate dilution method

For determination of CFU counts, agar plate dilution method was used. A 100 µl of pure chili sauces were put on the agars and spreader by sterile L-rod in three replicates. Crude chili sauces in 10^0 dilution was used in this method only. We cultivated three groups of microorganisms, TPC – total plate counts on TGY agar (Biolife, Itally), coliforms on MacConkey agar (Biolife, Italy) and anaerobes on Anaerobe agar (Oxoid, UK). Total plate count and coliform bacteria were cultivated under aerobic conditions and anaerobes under anaerobic condition in anaero-jars. Coliforms and anaerobes were cultivated at 35±2 °C at 24-48 hours (coliforms) and 48-72 hours (anaerobes). Total plate counts at 30 °C for 48-72 hours. After cultivation numbers of colonies were counted.

Antibacterial assay

For antibacterial activity *Escherichia coli* CCM 2024, *Bacillus thuringiensis* CCM 19^T, *Yersinia enterocolitica* CCM 5671 and *Salmonella enterica* subsp. TyphimuriumCCM 3807 were used in this experiment. These bacterial strains were collected from Czech Collection of Microorganisms. Antibacterial activity of habanero chili sauces were tested by microdilution methods in 96 well plates (Dispolab, Slovak republic) with some following modification. For detection of absorbance and following bacterial growth Elisa reader (Biotek EL-808, US) was used before and after the experiment. Measured absorbance values were counted out and absorbance differences which were greater than 0.05, it were considered as bacterial growth. We prepared three components for microtitrate dillution method. The first was prepared pure habanero chili saucespreviously. Second component was pure sterile Mueller-Hinton broth and third component was solutions of different type of microorganisms equaled to 1.5 McF° in physiological solution. These three components were added to the first column of wellsin proportion 1:1:1 in 100 µl amount. To each other columns were added 50 µl of sterile pure Mueller-Hinton broth expect the last column. Dilution of tested antibacterial component (Habanero chili sauces) was started in 33,3 % and finished in 0.02 %. Two-fold dilution method was used in this experiment. The pure Mueller-Hinton broth as control of purity and each bacterial solution with Mueller-Hinton broth as growth control were used.

Statistical methods

For averages determination of CFUs and evaluation of antibacterial activity in percetange from the absorbance values, Microsoft Office – Excel was used. One hundred percent into the graph is representing full growth of microorganisms without chili sauces.

3. Results and discussion

After incubation of microorganisms 2.59 log $cfu.ml^{-1}$ in red habanero chili sauce and 1 log $cfu.ml^{-1}$ in yellow habanero chili sauce of TPC were determined. We detected coliforms too 3.06 log $cfu.ml^{-1}$ in red habanero chili sauce and 3.24 log $cfu.ml^{-1}$ in yellow habanero chili sauce. Authors Estrada-Garcia et al., [13] tested chili sauces from street-vended industry and they detected coliforms in their samples, also enterotoxinogenic *E. coli* was determined in their study. They detected 40% faecaly contaminated

samples of chili sauces by coliforms. Also, authors Meldrum et al., [14] tested chili sauces and they detected coliform and *Bacillus* species. They identified *Salmonella* species too in chili sauces. Equally we determined anaerobes and we detected 2.78 log cfu.ml^{-1} in red habanero chili sauce and 1.3 log cfu.ml^{-1} in yellow habanero chili sauce. We didn't found any articles about microbiological determination of total plate count and anaerobes in chili sauces. Every result about log CFU.ml^{-1} is described into the Table 1.

Table 1. The averages numbers of different type of microorganisms isolated from Habanero chili sauces in log cfu.ml^{-1}

Microorganisms	Red Habanero chili sauce	Yellow Habanero chili sauce
TPC	2.59	1
Coliform	3.06	3.24
Anaerobes	2.78	1.3

Legend: TPC – total plate count

Habanero chili sauces were tested for antibacterial activity against some selected bacterial strains collected from Czech Collection of Microorganisms. We measured absorbance of solutions in 96 well plates by Elisa reader before and after the experiment and we determined growth of microorganisms with habanero sauces. The highest percentage level of red habanero sauces tested in this experiment was 33.3%, which has strong antibacterial effect against *Y. enterocolitica* and *S. enterica* subsp. Typhimurium. In compare with growth control we detected weaker inhibition in the first two concentrations of red habanero sauce. Then 16.65 % of red habanero sauce had strong antibacterial effect against *S. enterica* subsp. Typhimuriumonly. The last small antibacterial effect showed samples with 8.33% red habanero sauce. Very interesting is rapid growth of bacteria with 4.16 and 2.08% of red habanero sauce. After this concentration of red habanero sauce bacterial strains showed settled growth which was slightly below normal growth condition without red habanero sauce. The more described results are showed into the Figure 1.

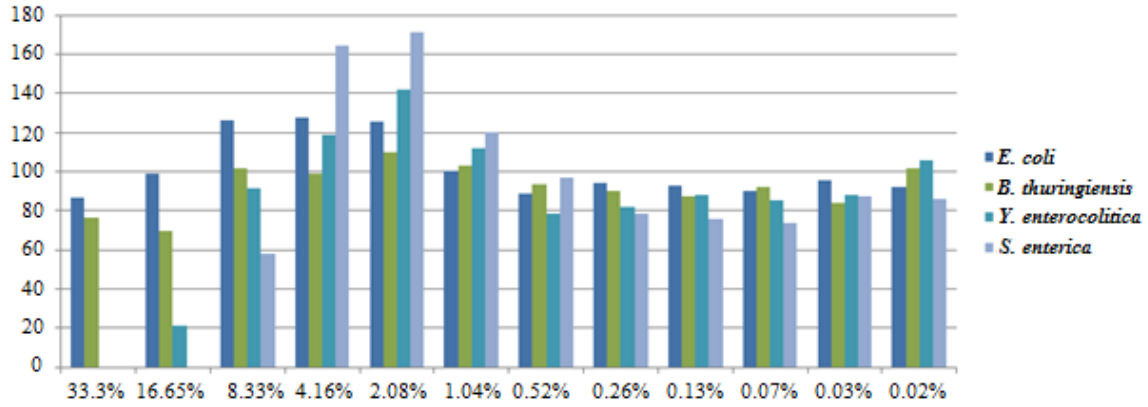

Figure 1. Antibacterial activity of red habanero chili sauce in percentage

Very similar results were showed in the case of yellow habanero sauce. Equally the most antibacterial effect in the comparing with normal growth was in the case of 33.3 and 16.65% of mentioned sauce. Some differences we determined in the case of 8.33% of yellow habanero sauce, where we detected stronger growth of bacterial strains, as well as 4.16 and 2.08% of habanero sauce concentration. In the following concentration of habanero sauce was growth of microbes settled slightly below normal growth without habanero sauce. More results are showed into the Figure 2. Authors Soetarno et al., [15] studied ethanolic extracts of three species of *Capsicum* and they determined antibacterial activity against Gram-positive and Gram-negative bacterial strains. These authors demonstrated that capsaicin is the main compounds with antimicrobial effect and Cowan et al., [16] reported that capsaicin kills bacteria by disruption of microbes membranes. Molina-Torres *et al.* [17] reported that high concentrations of capsaicin retarded the growth of *Escherichia coli* and

Pseudomonas solanacearum, whereas growth of *Bacillus subtilis* was strongly inhibited. This suggests that capsaicin has a variable effect on different microorganisms. Zeyrek and Oguz [18] observed bactericidal effect of capsaicin against *Helicobacter pylori*, the major cause of gastroduodenal disease, gastric cancer, peptic and gastric ulcer. Therefore, it promises to be an added benefit for patients with ulcer. Jones *et al.*[19] also noticed that capsaicin specifically inhibited growth of *Helicobacter pylori* in a dose-dependent manner at concentrations greater than 10 µg.mL^{-1}.

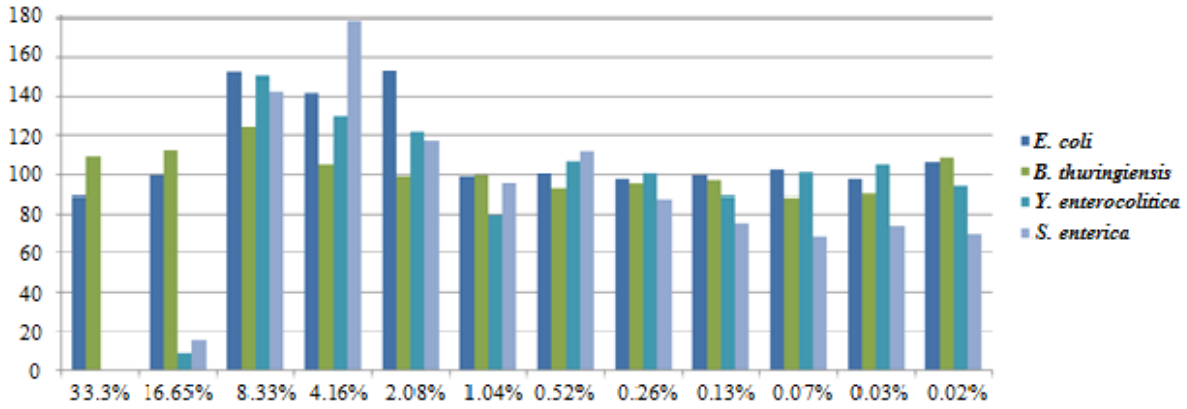

Figure 2. Antibacterial activity of yellow habanero chili sauce in percentage

Also many researchers studied antimicrobial activity of the base compounds of chili, capsaicins, and they determined very good antimicrobial activity against many species of yeasts, fungi and bacteria [20-22].

Conclusions

This study showed that Habanero chili sauce products contain some counts of microorganisms, but it has very good antibacterial activity against pathogenic bacteria too. It is very needed to know differences between bacteria which can and not resist of capsaicin disruption activity to microbes membranes. So and this work showed the next possibilities to study of antimicrobial activity of habanero chili sauces.

Acknowledgements

This work was funded by European Community under project no 26220220180: Building Research Centre „AgroBioTech" and by grant of VEGA 1/0611/14.

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