

EXTRACTION AND ANTIMICROBIAL EVALUATION OF CAPSAICINÓIDES OBTAINED OF PEPPER CAPSICUM SP.

VIDIANY APARECIDA QUEIROZ SANTOS*
KARIN MARTINS NUNES**
CRISPIN HUMBERTO GARCIA CRUZ***

Capsicum sp. peppers besides conferring distinctive flavor in foods, have antimicrobial potential, which is interesting from the hygienic-sanitary aspect. Thus, the aim of this study was to extract and evaluate the antimicrobial potential of three peppers extracts in front of different microorganisms. Three different types of peppers, were crushed and added to pure ethanol (crude extract). This extract was fractionated with three organic solvents of different polarity and the antimicrobial activity was determined in front of eight microorganism in crude extracts and fractions of hexane, chloroform and ethyl acetate. The size zone inhibition was used as a parameter for indicating the susceptibility of these microorganisms to the extracts and compared to those produced with antibiotics. The antibiograms results were compared with the Scoville scale values. Gram-negative bacteria and fungi were the most sensitive microorganisms to antibiotics and plant extracts. Gram-positive bacteria were resistant to all extracts tested. In general, among all the extracts, the hexane was less effective against all microorganisms, chloroform and ethyl acetate extracts and the most efficient. Among the three tested peppers, chilli pepper was the one that caused more inhibitions, followed by cumari pepper and dedo-de-moça.

KEY-WORDS: PEPPER; ANTIMICROBIAL ACTIVITY; CAPSAICINOIDS.

* Bióloga, Pesquisadora de Pós-doutorado pelo programa de Pós-Graduação em Processos Químicos e Bioquímicos (UTFPR), Pato Branco, PR, Brasil (E-mail: vidiandyqueiroz@yahoo.com.br).

**Engenheira de Alimentos, R&D Packaging Analyst na PepsiCo do Brasil, Itu, SP, Brasil (E-mail: karin.m.nunes@hotmail.com).

***Doutor em Ciência de Alimentos, Professor Titular Livre Docente na Universidade Estadual Paulista – IBILCE (UNESP), São José do Rio Preto, SP, Brasil (E-mail: crispin@ibilce.unesp.br).

1 INTRODUCTION

The natural preservatives use as spaces in substitution of synthetics has ever more occupied space, since, the latter may cause different harms including toxic and carcinogenic potential (KAPPEL, 2007, COSTA, 2007 e ANTONIOUS *et al.*, 2006). *Capsicum* sp. includes peppers which confer distinctive food flavor and help in digestion, also have preservative power due to antimicrobial potential conferred by capsaicinoids (SCHERER, 2010, SURH e LEE, 1995).

Peppers are sources of different vitamins (C, A, E, B complex, β -carotene and β -cryptoxanthin), besides the capsaicinoids which are alkaloids responsible for spicy peppers flavor (ARAÚJO, 2009, CARVALHO e BIACHETTI, 2007, SANTOS *et al.*, 2008, SUNG, 2005).

Brazil is a major center of genetic diversity of *Capsicum* sp. The most marketed species are the dedo-de-moça pepper (*Capsicum baccatum*); malagueta pepper (*Capsicum frutescens*) and Cumari pepper (*Capsicum baccatum* L. var. *Praetermissum*) (ZHUANG *et al.*, 2012). Among these peppers, the malagueta has a high content of A vitamin, between 10.500 and 11.000 IU, near to carrots level (13.000 IU), therefore is considered an interesting source of A vitamin (FRAIFE, 2010).

There are different forms of pepper commercialization, including the fresh pepper to *in natura* consume and homemade preserves, to the export of processed and industrialized (CARVALHO e BIACHETTI, 2004, FURTADO, DUTRA e DELIZA, 2006).

Brazilian pepper production occupies nearly five thousand ha with 75 thousand tons of production per year, mainly in Minas Gerais, São Paulo, Ceará, Rio Grande do Sul, Bahia and Sergipe states. This production largely is directed to domestic consumption as sauces and preserves and is exported as dried pepper, paprika or paste (MOURA *et al.*, 2013).

In front of the necessity to food preservation using natural methods as well as the sensory quality maintaining of them, the aim of this study was to extract capsaicinoids of the three *Capsicum* sp. varieties and evaluate the antimicrobial activity *in vitro* against different microorganisms.

2 MATERIAL AND METHODS

2.1 RAW MATERIAL

As raw material were used fresh fruits of three peppers: *Capsicum frutescens* (malagueta pepper), *Capsicum baccatum* (dedo-de-moça pepper) and *Capsicum baccatum* L. var. *praetermissum* (Cumari pepper) obtained in the Municipal Market of São José do Rio Preto-SP, Brazil.

2.2. EXTRACTION AND FRACTIONATION OF CAPSAICINOIDES WITH ORGANIC SOLVENTS

Whole raw fruits of three pepper species were used, which were previously triturated in a food processor, and added in ethanol solution (96 ° GL) in mass-volume ratio 1:1 and macerated during 48 h. Then, the hydro-alcoholic solution it was concentrated in a rotatory evaporator at 40 °C under reduced pressure to remove the alcohol excess and in the end was obtained the raw extract (RE). The raw extract was fractionated with three different organic solvents: hexane, chloroform and ethyl acetate, in increasing polarity order, resulting in the hexane fraction (FH), the chloroform fraction (CF) and the fraction of ethyl acetate (FA). These fractions and raw extracts were used in tests for antimicrobial activity evaluation.

2.3 MICROORGANISM AND CULTURE CONDITION

The microorganisms used was *Salmonella* sp.; *Escherichia coli*; *Bacillus cereus*;

Staphylococcus aureus; *Rhodotorula rubra*; *Saccharomyces cerevisiae*; *Aspergillus ochraceus* and *Penicillium expansum*. All microorganisms were obtained from culture collection of Food Microbiology Laboratory of the Department of Food Engineering and Technology - São Paulo State University - UNESP, São José do Rio Preto, SP, Brazil.

Microorganisms were previously cultured in nutritive broth at 30°C during 24 h. After were transferred to Petri dishes, using sterilized *swabs*, contained plate count agar (PCA) to bacteria and potato dextrose agar with tartaric acid (10%) to yeasts and filamentous fungi.

2.4. EVALUATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of capsaicin extracts was found using paper disc diffusion method. The paper discs (65 mm diameter) were previously immersed for 24 h in amber vials containing the pepper extracts: raw (RE) and fractionation in hexane (FH), chloroform (CF) and ethyl acetate (FA). After this discs were added in each Petri dish containing the microorganisms. Furthermore, the antibiogram test was performed using the Gram-negative and Gram-positive bacteria with sensitivity discs series (SENSIBIODISC - CECON), described in Table 1, and fluconazole for yeast and filamentous fungi. The incubation time was 72 h at 30°C. The experiments were performed in triplicate.

3 RESULTS AND DISCUSSION

The results obtained to *Salmonella* sp., after antibiogram and antimicrobial effect of pepper extracts are shown in the tables 2 and 3. *Salmonella* sp. was sensitive to most antibiotics analyzed, the antibiotics CIP 5 and TT30 were more efficient with inhibition zone of 2.84 and 2.8 cm⁻¹, respectively. This inhibitory effect of tetracycline against the *Salmonella* sp., was also observed by Spricigo *et al.* (2008), but these authors found resistance to tetracycline in 41% of samples. Such behavior was also observed by Duarte *et al.* (2009) who found tetracycline resistance in 31% of samples evaluated.

TABLE 1. ANTIBIOTICS DISCS FROM GRAM-NEGATIVE AND GRAM-POSITIVE SERIES BOUGHT FOR SUSCEPTIBILITY TEST (SENSIBIODISC - CECON).

Gram-negative	Gram-positive
Ampiclin 10 mcg ⁻¹	Norfloxacin 10 mcg ⁻¹
Tetracilin 30 mcg ⁻¹	Eritromicin 15 mcg ⁻¹
Gentamicin 10 mcg ⁻¹	Oxacilin 1 mcg ⁻¹
Cefoxitin 30 mcg ⁻¹	Sulfazotrim 25 mcg ⁻¹
Kanamycin 30 mcg ⁻¹	Gentamicin 10 mcg ⁻¹
Cefazolin 30 mcg ⁻¹	Amicacin 30 mcg ⁻¹
Sulfazotrim 25 mcg ⁻¹	Ampicilin 10 mcg ⁻¹
Ciprofloxacin 5 mcg ⁻¹	Clindamicin 2 mcg ⁻¹
Cefotaxim 30 mcg ⁻¹	Penicilin G 10 mcg ⁻¹
Cefalotin 30 mcg ⁻¹	Tetracilin 30 mcg ⁻¹
Cloranfenicol 30 mcg ⁻¹	Cloranfenicol 30 mcg ⁻¹
Amicacin 30 mcg ⁻¹	Cefalotin 30 mcg ⁻¹

TABLE 2. RESULTS TO GRAM NEGATIVE SERIES ANTIBIOGRAM TO SALMONELLA SP. AND ESCHERICHIA COLI.

Antibiotic	<i>Salmonella sp.</i>	<i>Escherichia coli</i>
	Diameter of inhibition zone cm ⁻¹	Diameter of inhibition zone cm ⁻¹
GN 10	1.18±0.02	1.19±0.02
AP 10	2.43±0.62	x
CZ 30	1.98±0.48	0.66±1.14
TT 30	2.80±0.06	2.42±0.16
KN 30	1.68±0.07	1.55±0.02
CFO 30	1.54±1.13	x
CTX 30	2.20±1.14	3.92±0.07
CIP 5	2.84±0.10	2.89±0.05
CF 30	0.76±1.31	0.51±0.89
SFT	1.28±1.21	0.55±0.52
AM 30	1.57±0.03	1.50±0.04
CO 30	1.94±0.55	2.52±0.17

GN10= Gentamicin 10 mcg⁻¹; AP10= Ampicilin 10 mcg⁻¹; CZ30= Cefazolin 30 mcg⁻¹; TT30= Tetraciclín 30 mcg⁻¹; KN30= Kanamicin 30 mcg⁻¹; CFO30= Cefoxitin 30 mcg⁻¹; CTX30= Cefotaxim 30 mcg⁻¹; CIP5= Ciprofloxacín 5 mcg⁻¹; CF30= Cefalotin 30 mcg⁻¹; SFT= Sulfazotrim 25 mcg⁻¹; AM30= Amicacin 30 mcg⁻¹; CO30= Cloranfenicol 30 mcg⁻¹; RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

Among pepper extracts malagueta tested the raw extract showed the highest inhibition (0.74 cm⁻¹). In the other hand, the ethyl acetate extract showed the lowest inhibition zone with 0.15 cm⁻¹. It was also observed that *Salmonella sp.* was resistant to hexane extract, as shown in Table 3. Among the cumari pepper extracts, raw extract also showed greater inhibition zone 0.28 cm⁻¹. The remaining extracts showed low or no action to *Salmonella sp.*, as shown in the table 4. Among pepper extracts dedo-de-moça was observed which none had inhibitory effect to microorganism tested (Table 5).

The results to antibiogram and antimicrobial effect of extracts of three peppers to *Escherichia coli* bacteria are shown in tables 2, 3, 4 and 5. In relation to antibiogram, the CTX 30 antibiotic was more effective with inhibition zone of 3.92 cm⁻¹ as showed in table 2. In relation to ampicillin and cefoxitin antibiotics the microorganism showed total resistance (table 2). Moreover, Fields *et al.* (2006) observed an inhibitory effect ampicillin and cefoxitin against different *E. coli* strains.

In relation to malagueta pepper extracts against *E. coli* the ethyl acetate extract showed the largest inhibition zone (0.61 cm⁻¹) compared to the other extracts. Similarly to *Salmonella sp.*, the hexane extract caused no inhibition, as shown in Table 3.

Carvalho *et al.* (2005) reported same behavior when evaluated the malagueta pepper alcoholic extract effect in front of *E. coli* bacteria. In malagueta effect opposition, cumari extracts did not shown inhibitory effect against *E. coli* bacteria, with exception of raw extract that showed inhibition zone of 0.87 cm⁻¹. On the other hand, it was found that inhibition zone formed by cumari pepper raw extract was higher than the malagueta pepper, as showed in table 4.

For dedo-de-moça pepper the chloroform and ethyl acetate extracts, were the ones who showed inhibition against *E. coli*. In this case, among all substances tested, the ethyl acetate extract was that caused a smaller inhibition zone (0.16 cm⁻¹) as shown in table 5.

TABLE 3. RESULTS TO GRAM POSITIVE SERIES ANTIBIOGRAM TO B. CEREBUS AND S. AUREUS.

Antibiotic	<i>B. cereus</i>	<i>S. aureus</i>
	Diameter of inhibition zone cm ⁻¹	Diameter of inhibition zone cm ⁻¹
CO 30	1.47±0.75	2.44±0.15
CF 30	x	1.22±2.12
TT 30	1.75±0.55	2.28±1.11
CI 2	1.41±0.30	2.35±0.11
EI 15	0.78±0.02	2.40±0.15
AP 10	x	1.73±1.43
NOR 10	0.71±0.02	2.31±0.05
PN 10	x	1.24±2.14
SFT	x	0.95±1.64
AM 30	1.65±0.02	1.71±0.31
GN 10	1.25±0.04	1.27±0.03
OX 1	x	0.28±0.48

GN10= Gentamicin 10 mcg⁻¹; AP10= Ampicilin 10 mcg⁻¹; CZ30= Cefazolin 30 mcg⁻¹; TT30= Tetraciclín 30 mcg⁻¹; KN30= Kanamicin 30 mcg⁻¹; CFO30= Cefoxitin 30 mcg⁻¹; CTX30= Cefotaxim 30 mcg⁻¹; CIP5= Ciprofloxacín 5 mcg⁻¹; CF30= Cefalotin 30 mcg⁻¹; SFT= Sulfazotrim 25 mcg⁻¹; AM30= Amicacín 30 mcg⁻¹; CO30= Cloranfenicol 30 mcg⁻¹; RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

Table 6 shows the results for effect of pepper extracts and antibiotic susceptibility to *Bacillus cereus*. In general, the most antibiotics caused inhibition of *B. cereus* and the largest inhibition zone observed was to TT30 antibiotic with 1.75 cm⁻¹. Such behavior observed to tetraciclín was also observed by Luna *et al.* (2007) where 100% of strains were susceptible to this antibiotic. On the other hand, these authors found the opposite result to ampicillin, which was effective in 40% strains tested, whereas for this study *B. cereus* was resistant to antimicrobial. The extracts of three peppers evaluated were not able to inhibit the growth of *B. cereus* regardless of the solvent used.

The results to *Staphylococcus aureus* was shown in the tables 3, 4, 5 and 6. Among the antibiotics tested, the EI15 and CO30 showed the greatest inhibition zones with 2.40 and 2.44 cm⁻¹, respectively (table 6). These results indicate that this bacterium was more sensitive than *B. cereus*. In contrast, Cruvinel *et al.* (2011) evaluated the resistance of *S. aureus* strains against different antibiotics, found resistance to erythromycin in 30% of samples.

TABLE 4. RESULTS OF ANTIMICROBIAL EFFECT OF MALAGUETA PEPPER EXTRACTS IN FRONT OF DIFFERENT MICROORGANISMS.

microorganism	Average of diameter (cm ⁻¹)			
	RE	HE	CE	AE
<i>Salmonella</i> sp.	0.74±0.32	x	0.19±0.33	0.15±0.25
<i>E. coli</i>	0.37±0.03	x	0.56±0.02	0.61±0.22
<i>B. cereus</i>	x	x	x	x
<i>S. aureus</i>	0.10±0.17	x	x	0.10±0.17
<i>R. rubra</i>	0.73±0.02	x	0.89±0.04	0.27±0.0
<i>S. cerevisiae</i>	0.26±0.05	x	0.43±0.17	0.38±0.03
<i>P. expansum</i>	0.95±0.09	0.09±0.16	0.10±0.17	x
<i>A. ochraceus</i>	0.15±0.21	0.15±0.21	0.20±0.28	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

TABLE 5. RESULTS OF ANTIMICROBIAL EFFECT OF CUMARI PEPPER EXTRACTS IN FRONT OF DIFFERENT MICROORGANISMS

microorganism	Average of diameter (cm ⁻¹)			
	RE	HE	CE	AE
<i>Salmonella</i> sp.	0.28±0.48	0.11±0.20	x	0.14±0.23
<i>E. coli</i>	0.87±0.23	x	x	x
<i>B. cereus</i>	x	x	x	x
<i>S. aureus</i>	0.18±0.16	x	x	x
<i>R. rubra</i>	0.88±0.13	x	0.40±0.14	x
<i>S. cerevisiae</i>	0.76±0.14	x	0.69±0.02	0.20±0.35
<i>P. expansum</i>	0.16±0.14	x	x	x
<i>A. ochraceus</i>	x	x	x	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

TABLE 6. RESULTS OF ANTIMICROBIAL EFFECT OF DEDO-DE-MOÇA PEPPER EXTRACTS IN FRONT OF DIFFERENT MICROORGANISMS

microorganism	Average of diameter (cm ⁻¹)			
	RE	HE	CE	AE
<i>Salmonella</i> sp.	x	x	x	x
<i>E. coli</i>	x	x	0.20±0.35	0.16±0.02
<i>B. cereus</i>	x	x	X	x
<i>S. aureus</i>	x	x	0.49±0.05	x
<i>R. rubra</i>	x	x	X	x
<i>S. cerevisiae</i>	x	x	x	x
<i>P. expansum</i>	x	x	x	x
<i>A. ochraceus</i>	x	x	x	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

In relation to malagueta extracts, the raw extract and ethyl acetate were the only ones that inhibited microorganism with inhibition zone of 0.10 cm⁻¹ for both (Table 3). For cumari pepper it was found that the raw extract had only efficient in inhibiting *S. aureus* (0.18 cm⁻¹) among all the extracts evaluated. Regarding the dedo-de-moça pepper, the chloroform extract was the one who showed inhibitory effect on *S. aureus*, forming a inhibition zone of 0.49 cm⁻¹ as shown in Table 5.

Carvalho *et al.* (2010) obtained the opposite result for *Staphylococcus aureus* which was resistant to the extracts of malagueta pepper. In the same way, Molina *et al.* (1999) observed *Escherichia coli* inhibition using capsaicin solution (300 mg mL⁻¹) and against *Bacillus* sp. of 25 µg mL⁻¹.

Cichewicz and Thorpe (1996) evaluated the influence of capsaicinoids extracted from Chilean pepper and also observed antimicrobial effect against the *Bacillus cereus*, which was not observed in this work for the extracts of malagueta, cumari and dedo-de-moça peppers extracts. Similarly as observed in this work, Dima *et al.* (2013) evaluating the antimicrobial activity of pure capsaicin and emulsion observed inhibitory effect of both forms against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp.

In contrast to that observed in this work Dorantes *et al.* (2000) evaluated the effect of three types of peppers (habanero, serrano and dulce pepper) and observed that *Salmonella* sp. was more resistant. The *S. aureus* and *Bacillus cereus* were the most sensitive against all extracts evaluated.

Nascimento *et al.* (2013) used alcoholic extract of malagueta peppers against different microorganisms and observed antimicrobial effect on *Escherichia coli* and *Staphylococcus aureus* in minimum inhibitory concentration of 5 and 10 mg mL⁻¹, respectively.

The results obtained for *Rhodotorula rubra* using fluconazole and extracts of three pepper types of are shown in table 7. Among all the extracts evaluated, the hexane extract no showed efficiency in front of yeast studied for the three peppers. This behavior was also observed for

fluconazole antibiotic, which had no effect on the yeast. The extracts obtained with cumari and malagueta pepper were those with the largest inhibition zone of the microorganism with 0.89 and 0.88 cm⁻¹ for raw extract and chloroform, respectively. The dedo-de-moça pepper had no effect on the microorganism in any of extracts as showed in table 7.

TABLE 7. RESULTS OF ANTIBIOGRAM TESTS TO FLUCONAZOL AND PEPPER EXTRACTS IN FRONT OF *RHODOTORULA RUBRA*.

Antibiótic	<i>Rhodotorula rubra</i>		
	Malagueta Pepper	Cumari Pepper	Dedo-de-moça Pepper
	Diameter of inhibition zone cm ⁻¹		
Fluconazol	x	x	x
RE	0.88±0.13	0.73±0.02	x
HE	x	x	x
CE	0.40±0.14	0.89±0.04	x
AE	x	0.27±0.0	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

The results obtained for the yeast *Saccharomyces cerevisiae* using fluconazole and extracts of three peppers are showed in Table 8. Regarding the fluconazole it was found that it showed inhibitory zone of 0.52 cm⁻¹. Pepper extracts of cumari and malagueta again showed the highest inhibition zones except for the hexane extract, as shown in Table 8.

Zeyrek and Oguz (2005) not observed inhibitory effect of *Saccharomyces cerevisiae* even at capsaicin high concentration. They found even a proportional increase in the growth rate of microorganism in the highest concentration compound Table 8.

TABELA 8. RESULTS OF ANTIBIOGRAM TESTS TO FLUCONAZOL AND PEPPER EXTRACTS IN FRONT OF *SACCHAROMYCES CEREVISIAE*.

Antibiotic	<i>Saccharomyces cerevisiae</i>		
	Malagueta Pepper	Cumari Pepper	Dedo-de-moça Pepper
	Diameter of inhibition zone cm ⁻¹		
Fluconazol	0.52±0.91	0.52±0.91	0.52±0.91
RE	0.26±0.05	0.76±0.14	x
HE	X	X	x
CE	0.43±0.17	0.69±0.02	x
AE	0.38±0.03	0.20±0.35	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

In Tables 9 and 10 are presented the result obtained for antibiotic susceptibility using the fluconazole and peppers extracts of three types against *Penicillium expansum* and filamentous fungi *Aspergillus ochraceus*, respectively.

The extracts that showed the highest inhibitory effect against both fungi were those obtained from malagueta pepper, with the largest inhibitory zone to the raw extract with 0.95 cm⁻¹ for *Penicillium expansum* and 0.20 cm⁻¹ for *Aspergillus ochraceus* (Tables 9 and 10). It was also found that the fluconazole had no effect on either filamentous fungi studied. The extracts obtained from cumari pepper and dedo-de-moça pepper had low effect or no effect, as shown in Tables 9 and 10.

TABELA 9. RESULTS OF ANTIBIOGRAM TESTS TO FLUCONAZOL AND PEPPER EXTRACTS IN FRONT OF *PENICILLIUM EXPANSUM*.

Antibiotic	Malagueta Pepper	Cumari Pepper	Dedo-de-moça Pepper
	Diameter of inhibition zone cm ⁻¹		
Fluconazol	x	x	x
RE	0.95±0.09	0.16±0.14	x
HE	0.09±0.16	x	x
CE	0.10±0.17	x	x
AE	x	x	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

TABELA 10. RESULTS OF ANTIBIOGRAM TESTS TO FLUCONAZOL AND PEPPER EXTRACTS IN FRONT OF *ASPERGILLUS OCHRACEUS*.

Antibiotic	Malagueta Pepper	Cumari Pepper	Dedo-de-moça Pepper
	Diameter of inhibition zone cm ⁻¹		
Fluconazol	x	x	x
RE	0.15±0.21	x	x
HE	0.15±0.21	x	x
CE	0.20±0.28	x	x
AE	x	x	x

RE=raw extract; HE= hexane extract; CE= chloroform extract; AE= ethyl acetate extract; x = no inhibition zone; Média±Desvio padrão.

It is also important to emphasize that the antimicrobial activity of capsaicin is conferred by its lipophilic potential, which allows you the ability to interact with the lipids of the cell membrane inducing their fluidization and consequently cell death (TSUCHIY, 2001).

Besides the antimicrobial activity was also evaluated the ability of pungency of the three pepper varieties, using the Scoville scale as a parameter. These results were showed in Table 11. Overall, among the three tested peppers, malagueta pepper that was caused larger number of

inhibitions, cumari and dedo-de-moça pepper was less efficient. These results can be explained by the concentration of capsaicinoids in each pepper. The Scoville Scale emphasizes that malagueta pepper has the highest concentration of capsaicinoids ranging from 60 to 100,000 Scoville units (SHU), followed by cumari, which ranges from 30 to 50,000 SHU and lastly, pepper dedo-de-moça who does not exceed 5 mil SHU (Table 11).

TABELA 11. SCOVILLE SCALE TO DEDO-DE-MOÇA, CUMARI AND MALAGUETA PEPPER.

Pepper	Scoville Scale (SHU)
Dedo-de-moça	5 a 15 thousand
Cumari verdadeira	30 a 50 thousand
Malagueta	60 a 100 thousand

SHU = a unit of scale Scoville corresponds to the proportion 1:1000, ie 1 cup pepper equals 1000 cups water (BONTEMPO, 2007).

Similarly at the observed in this study were observed for Bara and Vanetti (1998) using the Scoville scale to evaluate the antimicrobial activity of some flavorings and spices and those observed with low pungency had no inhibitory effect as in the case Green pepper (*C. annuum*), which by the Scoville Scale has zero SHU.

Based on the above results, it can be conclude that among all the extracts, the hexane was the least efficient in the inhibition of all microorganisms and the chloroform and ethyl acetate were of better efficiency. The malagueta pepper was the that caused the largest number of inhibitions followed by cumari and dedo-de-moça pepper was less efficient. This fact is related to the concentration of capsaicinoids in each pepper, since, malagueta has the highest concentration, followed by cumari and dedo-de-moça according to the Scoville Scale.

EXTRAÇÃO E AVALIAÇÃO DO POTENCIAL ANTIMICROBIANO DE CAPSAICINÓIDES OBTIDOS DE PIMENTAS DO GÊNERO CAPSICUM SP.

As pimentas do gênero *Capsicum* sp. além de conferir sabor diferenciado aos alimentos, apresentam potencial antimicrobiano, o que é interessante do ponto de higiênico-sanitário. Diante disso, o objetivo deste trabalho foi extrair e avaliar o potencial antimicrobiano de três extratos de pimentas frente a diferentes microrganismos. Foram utilizados frutos de três tipos de pimentas, as quais foram trituradas e adicionadas em etanol puro (extrato bruto). Este extrato foi fracionado com três solventes orgânicos de polaridade diferente e foi determinada a atividade antimicrobiana do extrato bruto e das frações de hexano, clorofórmio e acetato de etila das três espécies de pimentas, sobre oito tipos de microrganismos. O tamanho do halo de inibição foi utilizado como parâmetro para indicar a suscetibilidade destes microrganismos aos extratos e comparados àqueles produzidos com antibióticos. Por sua vez, os resultados obtidos dos antibiogramas foram comparados com os valores da Escala de Scoville. As bactérias Gram-negativas e os fungos foram os microrganismos mais sensíveis aos antibióticos e aos extratos testados. As bactérias Gram-positivas se mostraram resistentes a todas as substâncias testadas. No geral, dentre todos os extratos obtidos, o extrato de hexano foi o menos eficiente contra todos os microrganismos, e os extratos de clorofórmio e acetato de etila os mais eficientes. Dentre as três pimentas testadas, a pimenta malagueta foi a que causou maior número de inibições, seguida da pimenta cumari e a dedo-de-moça.

PALAVRAS-CHAVE: PIMENTA; ATIVIDADE ANTIMICROBIANA; CAPSAICINÓIDES

REFERENCES

- 1 ANTONIOUS, G. F.; KOCHHAR, T. S.; JARRET, R. L.; SNYDER, J. C. Antioxidants in pepper: Variation among accessions. **Journal of Environmental Science and Health**, v.41, p.1237-1243, 2006.
- 2 ARAÚJO, E. R.; SILVA, P. K.; RÉGO, E. R.; BAIRRAL, M. A. A.; SANTOS, R. M. C.; SAPUCAY, M. J. L. C.; FARIAS, G. A.; RÉGO, M. M. Análise sensorial e de aceitação comercial de geléia de pimenta com acerola. **Horticultura Brasileira**, v. 27, n. 2, p. 06, 2009.
- 3 BARA, M. T. F.; VANETTI, M. C. D. Estudo da atividade antibacteriana de plantas medicinais, aromáticas e corantes naturais. **Revista brasileira farmacognosia**. [online], v.7-8, n.1, p. 22-34, 1998.
- 4 BONTEMPO, M. **Pimenta e seus benefícios à saúde**, Alaúde Editorial - São Paulo, 2007. 18p.
- 5 CAMPOS, M. R. H.; KIPNIS, A.; ANDRÉ, M. C. D. P. B.; VIEIRA, C. A. S.; JAYME, L. B.; SANTOS, P. P.; SERAFINI, Á. B. Caracterização fenotípica pelo antibiograma de cepas de *Escherichia coli* isoladas de manipuladores, de leite cru e de queijo "Minas Frescal" em um laticínio de Goiás, Brasil. **Ciência Rural**, v.36, n.4, p.1221-1227, 2006.
- 6 CARVALHO, S. I. C.; BIANCHETTI, L. B. **Sistema de produção de pimentas (*Capsicum* sp.): Botânica**. EMBRAPA, 2007.
- 7 CARVALHO, H. H. C.; CRUZ, F. T.; WIEST, J. M. Atividade antibacteriana em plantas com indicativo etnográfico condimentar em Porto Alegre, RS/Brasil. **Revista Brasileira de Plantas Mediciniais**, v.7, n.3, p.25-32, 2005.
- 8 CARVALHO, H. H.; WIEST, J. M.; CRUZ, F. T. Atividade antibacteriana *in vitro* de pimentas e pimentões (*Capsicum* sp.) sobre quatro bactérias toxinfecivas alimentares. **Revista brasileira de plantas medicinais**. [online], v.12, n.1, p. 8-12., 2010.
- 9 CICHEWICZ, R. H.; THORPE, P. A. The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in Mayan medicine. **Journal of Ethnopharmacology**. v. 52, n. 2, p. 61-70, 1996.
- 10 COSTA, L. M. **Avaliação da atividade antioxidante e antimicrobiana do gênero *Capsicum***. 2007. 91 f. Dissertação (Mestrado em Ciências Ambientais) - Universidade Comunitária Regional de Chapecó, Chapecó, Brasil, 2007.
- 11 CRUVINEL, A. R.; SILVEIRA, A. R.; SOARES, J. S. Perfil antimicrobiano de *Staphylococcus aureus* isolado de pacientes hospitalizados em uti no distrito federal antimicrobial profile of *Staphylococcus aureus* isolated from hospitalized patients in uti in the distrito federal. **Cenarium farmacêutico**, v.4, n. 4, 2011.
- 12 DIMA, C.; COMAN, G.; COTÂRLET, M.; ALEXE, P.; DIMA, S. ANTIOXIDANT AND Antibacterial Properties of Capsaicine Microemulsions†. **Food Technology**, v. 37, n.1, p. 39-49, 2013.
- 13 DORANTES, L.; COLMENERO, R.; HERNANDEZ, H.; MOTA, L.; JARAMILLO, M.E.; FERNANDEZ, E.; SOLANO, C. Inhibition of growth of some foodborne pathogenic bacteria by *Capsicum annum* extracts. **International Journal of Food Microbiology**. v. 57, n. 1-2, p. 125-128, 2000.
- 14 DUARTE, D. A. M.; RIBEIRO, A. R.; VASCONCELOS, A. M. M.; SANTOS, S. B.; SILVA, J. V. D.; ANDRADE, P. L. A.; FALCÃO, L. S. P. C. A. Occurrence of *Salmonella* spp. in broiler chicken carcasses and their susceptibility to antimicrobial agents. **Brazilian Journal of Microbiology**, v. 40, p. 569-573, 2009.
- 15 FRAIFE, G. A. F. **Cultura da pimenta**: CEPLAC/CEPEC, 2010. Disponível em: <http://www.ceplac.gov.br/radar/pimenta.htm>. Acesso em: 16/02/2010.
- 16 FURTADO, A. A. L.; DUTRA, A.S.; DELIZA, R. **Processamento de "Pimenta Dedo-de-Moça" (*Capsicum baccatum* var. *pendulum*) em conserva**. 2006. 4 f. Comunicado técnico – Ministério da Agricultura, Pecuária e Abastecimento, Rio de Janeiro, RJ, Brasil, 2006.
- 17 KAPPEL, V. D. **Avaliação das propriedades antioxidante e antimicrobiana de extratos de *Capsicum baccatum* L. var. *pendulum***. Dissertação (Pós-Graduação em Ciências Biológicas: Bioquímica) Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande da Sul, Porto Alegre, 2007.
- 18 LUNA, V. A.; KING, D. S.; GULLEDGE, J.; CANNONS, A. C.; AMUSO, P. T.; CATTANI J. Susceptibility of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus Pseudomycoides* and *Bacillus thuringiensis* to 24 antimicrobials using sensititre automated microbroth dilution and Etest agar gradient diffusion methods. **Journal of Antimicrobial Chemotherapy**, v. 60, p. 555-567, 2007.
- 19 MOLINA, T. J.; GARCÍA, C. A.; RAMÍREZ, C. E. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. **Journal of Ethnopharmacology**, v. 64, p. 241-248, 1999.

- 20 MOURA, A. P. M. M. F.; GUIMARÃES, A.; AMARO, G. B.; LIZ, R. S. **Manejo integrado de pragas de pimentas do gênero *Capsicum* sp.** Ministério da Agricultura, Pecuária e Abastecimento: EMBRAPA, 2013.
- 21 NASCIMENTO, P. L. A.; NASCIMENTO, T. C. E. S.; RAMOS, N. S. M.; SILVA, G. R.; CAMARA, C. A.; SILVA, T. M. S.; MOREIRA, K. A.; PORTO, A. L. F. Antimicrobial and antioxidant activities of *pimenta malagueta* (*capsicum frutescens*), **African Journal of Microbiology Research**, v. 7, n. 27; p. 3526-3533, 2013.
- 22 SANTOS, J. A. B.; SILVA, G. F.; OLIVEIRA, L. C. Avaliação dos capsaicinóides em pimentas malagueta. **Revista Faculdade José Augusto Vieira: FJAV** - Ano I - nº 02. 2008.
- 23 SCHERER, B. S. **Avaliação dos efeitos antifibróticos do suco total e frações da pimenta *capsicum baccatum* na linhagem celular grx.** 2010. 56 f. Dissertação: Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul, Rio Grande do Sul, 2010.
- 24 SPRICIGO, D. A.; MATSUMOTO, S. R.; ESPÍNDOLA, M. L.; FERRAZ, S. M. Prevalência, quantificação e resistência a antimicrobianos de sorovares de *Salmonella* sp. isolados de lingüiça frescal suína. **Ciência e Tecnologia de Alimentos**, Campinas, v. 28, n. 4, p. 779-785, 2008.
- 25 SUNG, Y., CHANG, Y.; TING, N. Capsaicin biosynthesis in waterstressed hot pepper fruits. **Botanical Bulletin of Academia Sinica**. v. 46, p. 35-42, 2005.
- 26 SURH, Y. J. LEE, S. S. Capsaicin, a double-edged sword: Toxicity, metabolism, and chemopreventive potential. **Life Sciences**, v. 56, n. 22, p. 1845-1855, 1995.
- 27 TSUCHIYA, H. Biphasic membrane effects of capsaicin, an active component in *Capsicum* species. **Journal of Ethnopharmacology**, v. 75, p. 295 - 299, 2001.
- 28 ZHUANG Y., CHEN L., SUN L., CAO J. Bioactive characteristics and antioxidant activities of nine peppers. **Journal of Functional Foods**. v. 4, p. 331-338, 2012.
- 29 ZEYREK, F. Y.; OGUZ, E. In vitro activity of capsaicin against *Helicobacter pylori*. **Annals of Microbiology**, v. 55, n. 2, p. 125-127, 2005.

ACKNOWLEDGMENT

The Research Foundation of the State of São Paulo - FAPESP for financial support.