

Biological Activity of Hexane Extracts of the Northern Amazon Species *Capsicum* spp.

Karla S. Morais^{*a}, Antonio A. de Melo Filho^b, Lucianne B. O. Vilarinho^c, Bruna S. Morais^a, Pollyana C. Chagas^b, Ricardo C. dos Santos^d, Ana C. G. Reis Melo^d, Jacqueline A. Takahashi^e

^a Department of Health, Federal Institute of Roraima, IFRR, Boa Vista Campus, CEP 69.303-340, Boa Vista-RR-Brazil.

^b Post-Graduate in Biotechnology and Biodiversity Program, Departament of Chemistry and Post-Graduate Program in Chemistry, PPGQ, Center for Research and Graduate Studies in Science and Technology, NPPGCT, Federal University of Roraima, UFRR, Paricarana Campus, CEP 69304-000, Boa Vista-RR-Brazil.

^c Post Graduate in Agronomy, POSAGRO, UFRR, Campus Paricarana, CEP 69304-000, Boa Vista-RR-Brazil

^d National Postdoctoral Program of CAPES, PNPD/CAPES, associated to the Postgraduate Program in Agronomy of UFRR (POSAGRO/UFRR), Cauamé Campus, BR 174, s/n, Km 12, District Monte Cristo, CEP 69.301-970, Boa Vista-RR-Brazil.

^e Department of Chemistry, Institute of Exact Sciences, Federal University of Minas Gerais, UFMG, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil.

karlasantana.m@gmail.com

The objective of this study was to evaluate the presence of inhibitory substances from the fruits of *Capsicum* spp. var. Canaimé against micro-organisms indicators. Mature fruits (1.0 kg) were purchased at an agricultural fair, dried in an oven, and then was continuously extracted on a Soxhlet apparatus for 6 h, using 500 mL of hexane. Gram-positive *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 15313) and *Bacillus cereus* (ATCC 49456) were used to perform the antibacterial activity, as well as three Gram-negative strains: *Salmonella typhimurium* (ATCC 14028), *Citrobacter freundii* (ATCC 8090) and *Pseudomonas aeruginosa* (ATCC 25922). The antibacterial activity of hexane extract of *Capsicum* spp. fruits in some concentrations, was quite high when compared with the antibiotic. The level of inhibition was better towards Gram (-) bacteria compared to Gram (+) bacteria. The excellent bioactivity of the extract against *S. typhimurium* may be related to its selectivity. The present study demonstrated the efficacy of *Capsicum* spp. extract against pathogens that affect the health of human beings and presence of chemical assets that aid in the prevention of diseases. However, more in-depth studies are still needed to consolidate its use and application in pharmaceutical area.

1. Introduction

Since ancient times, human societies have accumulated information and experiences about the environment around them, to interact with it and to provide for their survival needs. Among the many practices disseminated by popular culture, plants have always had fundamental importance for many reasons, and their therapeutic potential is stressed throughout the generations (Rangel and Bragança, 2009).

Brazilian flora receives special attention of researchers and specialists for having vegetal material composed of substances with chemical potential which contributes to the scientific and technological advances (Braz Filho, 2010). Condiment plants, such as chilies and chilies of the genus *Capsicum*, have always been used by Indians and ancient civilizations to make food more palatable and to treat diseases (Reifschneider, 2000). The species of peppers of *Capsicum* genus originated in the Americas and were first reported in the 15th century by Chanca, a physicist who accompanied Columbus on his second expedition to the West Indies (De Souza and Rossi, 2014). Peppers of *Capsicum* genus belong to the Solanaceae family and present fruits with great genetic diversity in terms of color, size, shape, chemical composition and degree of pungency or spiciness (Cisneros-Pineda et al., 2007; Chuah et al., 2008). The total number of species of *Capsicum* spp. known to date is 35, distributed according to the degree of domestication: 5 domesticated, 10 semi-domesticated and

20 wild (Porto and Silva, 2013). The diversity of varieties of these Solanaceae species is explained by their reproductive system that provides interspecific and intraspecific interbreeding (Simões, 2014). Evidently this diversification reflects on their phytochemicals production. Domesticated species of the genus *Capsicum* are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*. *C. chinense* is probably the most important species cultivated in the Amazon region. It is believed that it was domesticated by the natives who first inhabited this region (Reifschneider, 2000). The first report of presence of this species in the region date back to the period of the exploratory expeditions from the 16th century, in search of drugs from the north Brazilian countryside (Nascimento Filho et al., 2007).

Nascimento Filho et al. (2007), in a study on the use of peppers in Roraima, reported that some old quotations pointed to the great importance of peppers in feeding the Amazon populations. According to them, in the present day, the cultivation of peppers occurs practically in all the regions of the state of Roraima. They are marketed in different forms by the population, as in the form of in natural fruits, pulp, jiquitaia, sauces, preserves and ornamental (Nascimento Filho et al., 2007).

In Roraima, one of the first studies with the chemical composition of fruits of this genus was conducted by Marangon et al. (2014), where they made the characterization of minerals in the fruits of two species. In the following year, Borges et al. (2015) conducted another research with six varieties of this genus, being identified some morpho-anatomical and physico-chemical properties of the fruits and among them, the fatty acids contents.

Due to the abundance of the various types of peppers in the state of Roraima and to a few research studies directed to their analysis, the objective of this work was to perform an *in vitro* biological assay on a variety of *Capsicum spp.* genus. In this context, the antibacterial activity of hexane extracts of *Capsicum spp.* was tested against the standard strains (bacterial) of the American Type Culture Collection (ATCC) in a variety of pepper widely produced and consumed in the state of Roraima (Brazil).

2. Material and Methods

The experiment was carried out at the Federal University of Roraima, in a pepper species of the genus *Capsicum spp.* which is widely consumed by residents in the state, and often marketed in the free trade fairs of the state of Roraima. This variety was selected and the fruits were bought at the fair of the rural producer of Boa Vista city.



Figure 1: Variety CA: Pepper purchased at the fair of the rural producer.

In order to prepare the hexane extracts, the collected material was previously sanitized and weighed to obtain the initial mass, after which it was taken to a forced ventilation oven at 65 °C for 72 h until constant biomass to stabilize the compounds and remove the moisture of the material according to the methodology described by Silva and Queiroz (2002). The extracts were then prepared from the dried fruits, in which 33 g were obtained in a dry mass of the fruits, after greenhouse. Then they were submitted to a continuous extraction in Soxhlet apparatus at the Laboratory of Environmental Chemistry of the Federal University of Roraima-UFRR. In this stage 500 mL of hexane solvent was used for a continuous extraction of 6 hours. Each extraction was carried out in triplicate of 11 g each and after the conclusion of the extractions, were submitted to rotary evaporation (Silva and Queiroz, 2002). The extracts already concentrated by rotaevaporation were transferred to a glass bottle and weighed in an analytical balance whose final extract weight of the triplicates was 5.83 g.

2.1 Microorganisms

The microorganisms tested were standard strains ATCC (*American Type Culture Collection*) and the assays were carried out at the Biotechnology and Bioassay Laboratory of the Department of Chemistry of Federal University of Minas Gerais. The following bacterial strains were used: *Listeria monocytogenes* (ATCC 15313), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311) and *Citrobacter freundii* (ATCC 8090).

2.2 Antibacterial activity (*in vitro* bioassay)

The antimicrobial activity evaluation analyzes were performed at the Biotechnology Laboratory of the Federal University of Minas Gerais, following the Minimal Inhibitory Concentration (MIC) method described below. The tested concentrations of extracts of the pepper variety studied were 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90625 $\mu\text{g mL}^{-1}$ respectively. The antibiotic Ampicillin was used as a control at a concentration of 12.5 $\text{mg}\cdot\text{mL}^{-1}$. The procedures established (Zacchino and Gupta, 2007) were used to perform the bioassay. For the preparation of BHI (Brain Heart Infusion) culture medium 3.4 g of BHI was weighed and dissolved in 200 mL of distilled water. The solution was homogenized and then autoclaved for 15 min at 121 °C. As for inoculum preparation, one pre-inoculum was prepared in test tubes by adding 3.0 mL of BHI solution and 100 μL of bacteria. The test tubes containing the bacteria were incubated in an oven at 37 °C for 24 hours. Then, with the aid of a micropipette, 500 μL of bacterial pre-inoculum was added to an erlenmeyer containing approximately 40 mL of sterile distilled water. The inoculum was standardized in a spectrophotometer at 600 nm, in the transmittance range of 74-75% indicated for bacteria. For the preparation of the samples, 10 mg of extract was next weighed and then solubilized in 200 μL of DMSO, resulting in a solution with a concentration of 50 $\text{mg}\cdot\text{mL}^{-1}$. After, the solution was homogenized in a vortex mixer. Then a volume of 20 μL of the solution was added in eppendorf containing 1980 μL of BHI solution, thus obtaining the solution with a final concentration of 250 $\mu\text{L}\cdot\text{mL}^{-1}$. Then we performed the antibacterial activity test, in which the assays were performed in a 96-well ELISA plate in duplicate. Dilutions were initiated by adding 100 μL BHI medium and 100 μL working solution into the first wells. The solution is then homogenized and 100 μL are transferred to the next well and so on. The remaining 100 μL were neglected. Then at the end of the microdilution, 100 μL of inoculum was added to each of the 96 wells. To control the quality of the assay, we substitute the antibiotic-working solution, 100 μL of Ampicillin solution (12.5 $\text{mg}\cdot\text{mL}^{-1}$) plus 100 μL of inoculum. For the blank, the inoculum was not added, 100 μL solution of the working solution (250 $\mu\text{L}\cdot\text{mL}^{-1}$) plus 100 μL of sterile distilled water. For control of bacterial growth (cell viability checked) was added 100 μL of BHI medium plus 100 μL of inoculum. As for the sterility control of the culture medium, 100 μL of BHI medium plus 100 μL of sterile distilled water was added. The microplates were homogenized for 5 min and incubated in an oven at 37 °C for 24 hours. The reading was performed in a Elisa plate spectrophotometer at 492 nm. The results were calculated as percent inhibition using the formula:

$$\% \text{ Inhibition} = 100 - \text{AC1} - \text{AC2} \times 100 \text{AH} - \text{AM}$$

Where AC1 = absorbance of the sample; AC2 = absorbance of control sample; AH = absorbance in the control of the microorganism and AM = absorbance of the control of the culture medium.

3. Results and Discussion

Gram (+) and Gram (-) bacteria (Tables 1 and 2) bacterial strains were inhibited in the assay showing activity for the hexane extract of *Capsicum* spp. The extracts concentrations ranged from 3.90625 $\mu\text{g mL}^{-1}$ to 250 $\mu\text{g mL}^{-1}$, and potentially some very satisfactory results in this bioassay. Ampicillin was used as a control, of standard clinical use of antibiotics, was very efficient in the concentrations shown.

The activity test against *S. typhimurium*, *P. aureginosa*, *L. monocytogenes* showed remarkable inhibition at almost all extracts concentrations. However, *S. aureus*, *C. freundii* and *B. cereus* showed little inhibited or none at most of the concentrations tested with the extracts. The antibacterial activity against *S. typhimurium* was higher. Using 250 mg L^{-1} extract, the extent of inhibition reached 97% and at the lowest concentration tested (3.90625 mg L^{-1}) the inhibition level was quite high (84%). *S. typhimurium* is a Gram (-) bacterium that can cause diarrhea, severe fever and even death. It is mainly transmitted by fecal-oral contamination, very common in countries without basic sanitation, as well as the inadequate handling of food (Moura et al., 2012; Butler, 2011). The activity of the extract against *S. aureus* was not satisfactory, since less than 40% of inhibition was observed in almost all the concentrations used. Likewise, inhibition of *C. freundii* and *B. cereus* was less than 50% at most of the concentrations tested. The low inhibition of *Capsicum* spp. in relation to some bacterial strains and high inhibition against others is indicative of a mechanism of selective action, which deserves further investigation. *S. aureus* and *C. freundii* are Gram (+) bacteria that also cause serious health

problems for humans (Evans et al., 2014; Sung et al., 2008). The World Health Organization (WHO, 2014) pointed out that there is a global concern about the indiscriminate use of antibiotics because this causes bacteria to develop resistance to current medications. Thus, it is necessary to look for new medicines that meet this need.

Table 1: Inhibition of Gram (+) bacteria by the hexane extract of *Capsicum* spp.

Concentration ($\mu\text{g mL}^{-1}$)	<i>S. aureus</i> (%)	Ampicillin (%)	<i>L. monocytogenes</i> (%)	Ampicillin (%)	<i>B. cereus</i> (%)	Ampicillin (%)
250	13.71	84.89	92.00	95.00	57.00	93.00
125	43.06	83.92	100.0	96.00	17.70	90.00
62.5	10.02	84.95	100.0	96.00	11.03	94.00
31.25	8.50	91.44	54.00	97.00	6.98	93.00
15.625	-	94.24	40.00	96.00	-	93.70
7.8125	-	95.87	-	63.00	-	83.90
3.90625	-	82.00	-	-	-	97.00

-: No inhibition

Table 2: Inhibition of Gram (-) bacteria by the hexane extract of *Capsicum* spp.

Concentration ($\mu\text{g mL}^{-1}$)	<i>C. freundii</i> (%)	Ampicillin (%)	<i>S. typhimurium</i> (%)	Ampicillin (%)	<i>P. aureginosa</i> (%)	Ampicillin (%)
250	83.00	94.00	97.80	100.0	100.0	95.00
125	49.00	95.00	90.03	97.00	90.00	96.00
62.5	42.00	96.00	91.18	95.00	100.0	97.00
31.85	60.00	95.00	53.45	79.00	47.00	97.00
15.625	-	80.00	55.30	30.00	43.00	95.00
7.8125	-	-	29.70	20.00	39.87	58.00
3.90625	-	-	84.70	30.00	57.42	-

-: No inhibition

The antimicrobial effect of *Capsicum* fruits has been verified in other studies as demonstrated in the review by Fontoura et al. (2017) when describing 74 Brazilian plant species. The results are presented for *C. annum*, *C. baccatum* and *C. frutescens* when analyzing the effects of hydroalcoholic extracts by suspension, whose efficacy was verified for the bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

The chilli pepper, *C. frutescens*, presents the largest number of studies in the literature regarding microbiological action, probably because it is one of the most widespread and appreciated in the consumer market. In extracts of *C. frutescens*, Barbosa (2012) observed the inhibition of *S. aureus*, *E. coli* and *S. typhimurium*. Tests with the genus *Capsicum* species extracts of *C. frutescens*, *C. annum* and *C. baccatum* and bacterial strains of *S. enteritidis*, *E. coli*, *S. aureus* and *Enterococcus faecalis* show that bacterial *C. frutescens* have better efficacy (Carvalho et al., 2010).

When classifying extracts of different species according to their bacteriostatic or bactericidal effect, it was found that cayenne showed bactericidal activity, as well as garlic *nirá*, leek, sage and "manjerona preta", for inactivating the bacteria *S. enteritidis*. Calabrian and finger peppers as well as tarragon did not promote the inhibitory effect, showing bacteriostatic action. *E. coli* was also inhibited by *C. frutescens*, as well as extracts of garlic *nirá*, garlic, and sage. *E. faecalis* and *S. aureus* were not inhibited by *C. frutescens*. Inhibition was greater than the inactivation of these bacteria (Carvalho et al., 2005).

Three species of the Solanaceae family were cited by Fontoura et al. (*Solanum mauritianum*) and *S. sisymbriifolium* (Mata-Cavalo) and root extracts of *S. paniculatum* (Jurubeba), and showed that the first two

Solanum species showed no effect antibacterial activity in *S. aureus* strains. This review showed that for most plants, the alcoholic extract presented better action against the bacteria. Mota et al. (2011) and Passos et al. (2009) justify that this occurrence is due to the loss of volatile essential oils in the extraction by decoction by the high temperatures as opposed to the hydroalcoholic extraction that happens with low temperature.

Santos (2010) in his experiments observed that extracts of *C. annuum* L significantly inhibited the fungus *Candida albicans*, but did not cause inhibition in *E. coli*. Pereira (2015) when investigating antimicrobial peptides in ethanol extracts of *C. annuum* L. inhibited the growth of phytopathogenic fungi *C. lindemuthianum*, *C. gloeosporioides* that cause the anthracnose and the bacterium *Xanthomonas euvesicatoria* that causes bacterial spotting in plants.

According to Tenório et al. (2016) differences in minimum inhibitory concentration may be related to the solvent used for the extraction of the metabolites and also to the type of strain selected for the antibacterial assays. However, it is necessary to purify, isolate and identify the bioactive components of this plant for further testing, which act to inhibit bacterial growth and bactericidal activity.

4. Conclusions

The hexane extracts of fruits *Capsicum* spp. in some concentrations, the antibacterial activity was quite high when compared with the antibiotic. The level of inhibition was better in Gram (-) bacteria compared to Gram (+) bacteria. Inhibition of Gram-positive bacteria, *S. aureus* was unsatisfactory. The excellent bioactivity of the extract against *S. typhimurium* may be related to the selectivity. In this context and due to the excellent results the use as an antibiotic is suggested, but it needs new tests.

Acknowledgments

To CAPES for a fellowship, to CCT (Sao Paulo) and CML (UFLA, MG) for donation of microorganisms, to FAPEMIG for a grant and to the Research Oleoquímicos Group UFRR.

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