Escola Superior da Saúde - Instituto Politécnico do Porto

Study of the bioactivity of medicinal plants' extracts: *Diplotaxis muralis and Cochlospermum angolensis*

July 2018

Márcio Paulo Cardinal Ferreira

Study of the bioactivity of medicinal plants' extracts: *Diplotaxis muralis and Cochlospermum angolensis*

Project submitted to the Porto High School of Health, ESS, Polytechnic of Porto to exercise the requirements for obtaining a master's degree in Health Biochemistry, Biotechnology Branch, conducted under the guidance of Teacher Mónica Vieira, Adjunct Professor and Teacher Cristina Prudêncio, Coordinating Professor, with Agregation.

J u l y 2 0 1 8

Abstract

The leaves of the *Diplotaxis muralis* (Arugula) are the most metabolic part of the plant. Here, photosynthesis plays an essential role. Arugula is a fast-growing perennial plant that grows in the winter and produces new shoots in the spring. It has a spicy taste and pleasant odor and it is originated in Europe and West Asia.

Cochlospermum angolensis (Borututo) is a plant with medicinal properties used for the treatment in prophylaxis against malaria and hepatic pathology. However, further studies are needed to prove its chemical composition and biological activity.

As the use of *D. muralis* is recurrent within the Portuguese population and the use of *C. angolensis* is frequent within the Angolan population, the objectives of this study were to describe the state of art for their uses, through popular knowledge and existing bibliography, and to evaluate their anti-bacterial activity, through aqueous and organic extracts.

Organic and aqueous extracts were obtained by the classical extraction technique, using as solvents distilled water, ethanol and dimethylsufoxide.

Measurement of 3-nitrotyrosine (3-NT) in biological samples can be used as a biomarker of nitrosative stress, since it is very stable and suitable for analysis.

The results indicate that Arugula does not present antibacterial properties for the studied strains. However, Borututo presents chemical compounds that confer antibacterial properties on the organic extract. With these results, it possible to concluded that Borututo may present properties of interest to the pharmaceutical and food industries.

Key words: *Diplotaxis muralis*, *Cochlospermum angolensis*, anti-bacterial activity, organic and aqueous extracts, oxidative stress

INDEX

Index of abbreviations

Abbreviation	In full
Abs	Absorbance
ATCC	American Type Culture Collection
CFU	Colony forming units
DMSO	Dimethylsulfoxide
HPLC	High Perfomance Liquid Chromatography
MIC	Minimum inhibitory concentration
MRSA	*Staphylococcus aureus* resistant to methicillin
OD	Optical Density
ROS	Reactive Oxygen Species
RPM	rotations per minute
T0	OD reading zero test time
T24	OD reading after incubation for 24h
TSA	Tryptic Soy Agar
TSB	Tryptic soy broth

Index of figures

Index of tables

INTRODUCTION

1. Use of medicinal plants

Traditional medicine is an old practice of societies. About 80% of the population in underdeveloped countries depends on traditional medicine as primary health care. Medicinal plants are a rich source of diverse types of compounds with potential to be used as therapeutic agents or as a basis for the development of new drugs. There are many reasons that lead people to the use of traditional medicine, especially for socio-economic circumstances and the fact that traditional medicine is more affordable and within reach of populations compared to licensed medicines (Manuel et al. 2009) (Islam et al. 2014).

The widespread belief in medicinal plants, being a factor of variability transmitted from generation to generation, traditional uses of medicinal plants may result in toxicity and adverse effects on human consumption without prior knowledge, some populations are not aware of this.

2. *Diplotaxis muralis*

Diplotaxis muralis (Brassicaceae) is known by the local population as "Arugula" Figure 1. The plant parts used are leaves. In the leaves, photosynthesis plays an essential role, because it is the most metabolically active part of the plant. In addition, the collection and drug method of preparation of leaves are much easier than other parts of the plant and makes it the first choice for use. In some cases, the processing involves simply drying the vegetal material (Islam et al. 2014).

Figure 1- Leaves of arugula (*https://www.cantinhodasaromaticas.pt/etiqueta-produto/diplotaxis-muralis/*, consulted on 10/07/2018)

Arugula is a perennial plant being grown in the winter and producing new shoots in the spring. Arugula plant is characterized by average height of 80 cm, a deep root, oblong, lobed leaves with pointed apices (Tripodi *et al.* 2017). Arugula has a spicy taste and pleasant odor, is a fast-growing plant, originating in Europe and West Asia. Its nutritional and phytotherapeutic properties are varied. Its chemical composition includes vitamins, minerals and fibers, besides the presence of calcium, sulfur compounds, sulfur, iron, fibers, phosphorus and potassium (Basu *et al*. 2001).

The arugula helps in the treatment of lung diseases, lack of appetite, intestinal gas, anemias, aid in the process of detoxification of the organism, also helps the treatment of triglycerides, due to the presence of omega 3, fatty acid that has the ability to unclog the arteries, which provides better blood circulation (Filgueira 2000). There are still few studies that involve the chemical characterization of arugula.

3. *Cochlospermum angolensis*

Cochlospermum angolensis, Borututo Figure 2, has been used by the Angolan population for many years. The population uses the root rich in quinones, catechins, polyphenols, highly detoxifying and purifying bioflavonoids. Due to its richness in substances with physiological activity, it acts in great part in our body according to the needs of the cells, being still recognized in the treatment of diseases of the stomach, liver, gallbladder, spleen and all urinary tract. As a purifier allows blood to flow by fighting cholesterol and normalizing blood pressure. It has also been used against *Plasmodium berghei*, a common malaria parasite. (Abourashed, E. & Fu, 2017) (Carla Pereira et al. 2013) (Ferreres, et al., 2013).

Figure 2 – Tree of Borututo (*http://borututuroot.com/*, consulted on 10/07/2018)

4. Bioactivity study models of natural extracts

In laboratory experiments, in recent years have achieved great results in animal experiments, in particular microorganisms because many animals are needed to ensure statistically significant results, the microorganisms are easier to handle and have rapid growth (Soares, D. G., *et al.* 2005).

There are several study models to evaluate the bioactivity of natural extracts, from bacterial models: *Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli,* MRSA, producers of *β-lactamases*). Some protozoa such as *Plasmodium berghei*, *P. falciparum* and yeasts (C. *et al*. Pereira 2015).

5. Evaluation of oxidative / nitrosative stress

3-Nitrotyrosine (3-NT) is a biomarker of nitrosative stress. When increased levels of 3-NT in biological samples are associated with pathological and physiological changes, that is, an increase in oxidative/nitrosative stress is represented by cellular antioxidant defenses that are not able to eliminate reactive oxygen species (ROS) and nitrogen reactive species (RNS). One of the effects is the damage of the bases of nucleic acid, proteins, lipids and carbohydrates. This effect may impair cell health and viability (Teixeira, et al., 2016). The nitration of tyrosine residues in proteins is associated with nitrosative stress, resulting in the formation of 3-nitrotyrosine (3-NT) (Teixeira, et al., 2017).

AIMS

The main objective of this work is to study the bioactivity of the medicinal plants *Diplotaxis muralis* and *Cochlospermum angolensis*.

The specific objectives are:

- To study the state of the art for the use of *Diplotaxis muralis* and *Cochlospermum angolensis*;
- To prepare aqueous and organic extracts of *Diplotaxis muralis* and *Cochlospermum angolensis*;
- To evaluate the bioactivity of the extracts obtained against Gram-positive *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 6538P, *S. aureus* MRSA and Gram-negative isolates (S3R9, S3R22) and *Escherichia coli* ATCC 25922.

MATERIALS AND METHODS

Materials:

Samples

The *D. muralis* leaves were collected in Canidelo parish, Vila Nova de Gaia municipality, Porto district (Portugal), on January 2, 2018. The harvest method used was the traditional method. It consists of cutting the fresh leaves of the arugula by the stem, washed to keep it clean for better conservation in the laboratory of the School of Health of Porto, Polytechnic of Porto.

The *C. angolensis* root, from Angola, was stored in the freezer of the Porto High School of Health, Politécnico do Porto. I knew by my coordinator that a student of this school José Herculado left the root Borututo for future academic work. This student was in Angola, however he had the opportunity to bring the Borututo root, washed, cleaned of earth and cut into small pieces for better conservation in the laboratory.

Reagents / Equipment

The equipment used was heating blanket (J.P.selecta, s.a, Barcelona); balance (Kern ABJj); tub for grinding; distiller (GFL Gesellsehoft Labotechnik); autoclave (Uniclave 88-40x60, A. J. Costa); greenhouse (Selecta); vortex (Labnet international, inc); Thermo Spectronic (American APPraiSAL); Bunsen burner (CAMPINGAZ); Thermo Scientific MultiskanFc (Vantoa Filand); Thermo Scientific MultiskanFc (Vantoa Filand) and incubator Orbital Skaka. 96-well plates (Frilabo).

The consumables used were 0.45µm and 0.22µm Filters (VWR – International); Test tubes 12x75mm (Kimble); loops of 1µm-10µm; Petri Dishes; swipe; 96-well plates (Frilabo) and micropipettes 10/100µL 100/1000µL.

The culture media used were *Tryptic Soy Agar* (TSA) (Merck) and *Tryptic Soy Broth* (TSB) (Merck) for bacteria. Nutritious agar for bacteria.

The reagents were etanol (≥96%) (José Manuel Gomes dos Santos, LDA); and dimethylsulfoxide (DMSO) (Biochemica Applichen Panreac ITW Companies).

The strains used were *S. aureus* ATCC 6538, *S. aureus* ATCC 6538P, *S. aureus* MRSA, *E. coli* ATCC 25922 and environmental isolates from the working group, identified by S3R9 and S3R22 (Gram-negative rods).

Methods:

1. Extraction (aqueous and organic)

The fresh sample of *D. muralis* was cold and protected from light.

The *C. angolensis* sample was dried at room temperature and protected from light.

The extracts were prepared. Therefore:

(i) For aqueous extraction, the extraction was carried out using distilled water in the proportions 2:10 (2 g of leaf, *D. muralis* or 2 g of root, *C. angolensis* to 10 mL of distilled water). The mixture was boiled for 60 minutes at 60ºC and then filtered;

(ii) For organic extraction, ethanol was added after maceration of the leaves (*D. muralis*) and root (*C. angolensis*) in the ratio of 2:10 (2g of sheet to 10 mL of ethanol) for extraction for 30 minutes 30ºC and then filtered.

Both extracts were stored at 4 ° C and protected from light until use.

2. Preparation of the inoculates

Cultures of microorganisms were maintained at 4 °C in nutrient agar and were recovered, when necessary, in TSA for the bacteria under study, and incubated 24h at 37 °C.

Suspensions of the culture in saline solution (0.85% NaCl) and TSB according to the point of inoculation, 125 minutes, exponential phase, for *S. aureus* ATCC 6538P and *S. aureus* ATCC 6538 according to the work developed by Herculano Hossi José, 2017, were prepared using the 0.5 MacFarland scale to approximately 1.5×10^8 CFU/mL for bacteria, this scale corresponding to an optical density (OD) of 0.08 to 0.10 at 620 nm, as measured by visible spectrophotometry (Almeida et al. 2014).

3. Preliminary tests

The microorganism was then inoculated by the technique of distension on the surface of a Petri dish. Then 20µL of each extract and control by drop technique were placed in the Petri dishes with TSA medium in duplicate. Bacteria were incubated 24 h at 37 °C. After incubation, the halos of inhibition of microbial growth were measured in millimeters, using a millimeter ruler.

The extract with antimicrobial activity was then concentrated by rotary evaporation. Then the extract was dissolved in DMSO and the tests were performed.

For the controls were used according to the drop method, since no growth inhibition was observed on the Petri dish, it indicates to us that the inhibition verified by the extracts is of origin of the extract.

4. Determination of sensitivity of the strains to the extract

In this assay the solvent with the highest antimicrobial activity used in the preparation of the antimicrobial extract of *C. angolensis* (ethanol) was used. Dilutions of one culture of each strain (*S. aureus* ATCC 6538P and *S. aureus* ATCC 6538) were performed in the solvent in 96 well plates and the OD were checked at 620nm (T0). After incubation in an orbital incubator with shaking (120rpm) for 24h at 37 ° C for bacteria with positive results, OD (T24) was read.

In this assay the crude ethanolic extract of the Borututu root obtained from the extraction process was used. The volume of culture medium TSB was distributed in each well in triplicate, then the volume of extract and finally the volume of suspension of strain under study was added. The OD was checked at 620nm (T0). After incubation the OD (T24) was read.

Both controls, negative and positive, and the tests were performed in triplicate in the proportions shown, in figure 3 and figure 4, for a total volume of 200 µL.

The sensitivity of the strains to the extracts can be understood as the smaller volume of extract in µL, capable of inhibiting the microbial growth, that is, the minimum inhibitory concentration (MIC).

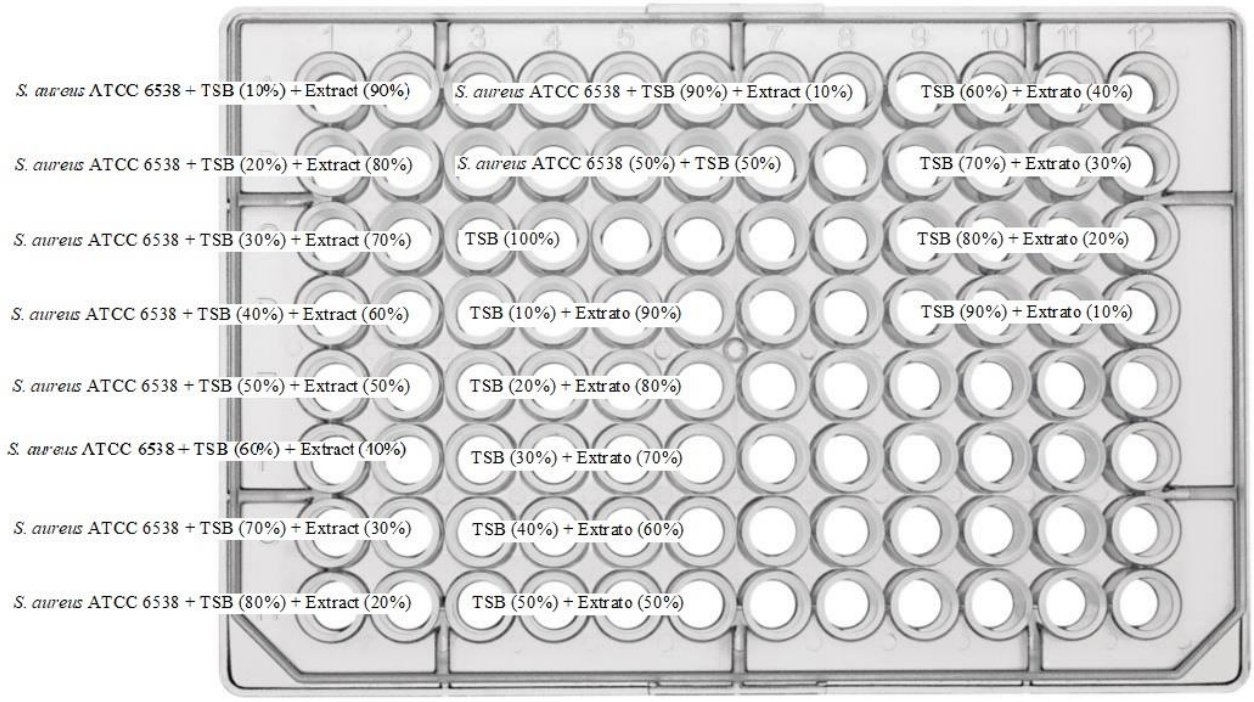

Figure 3 - Quantities of bacterial suspension *S. aureus* ATCC 6538, TSB and ethanolic extract of Borututo in 96-well plate. When in the well shown there is an amount of bacterial suspension and culture medium together, the above percentage is divided equally.

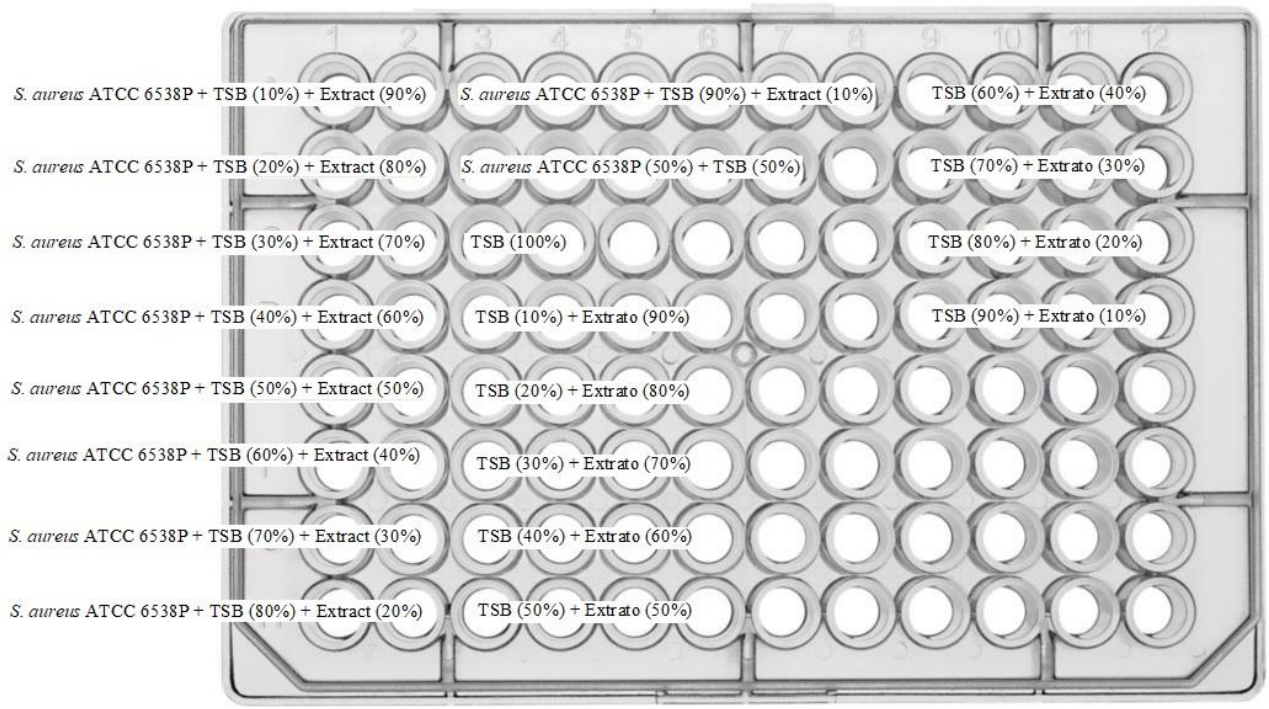

Figure 4 - Quantities of bacterial suspension *S. aureus* ATCC 6538P, TSB and ethanolic extract of Borututo in 96-well plate. When in the well shown there is an amount of bacterial suspension and culture medium together, the above percentage is divided equally.

5. Evaluation of oxidative / nitrosative stress

The 3-nitrotyrosine was evaluated in *S. aureus* ATCC 6538 and *S. aureus* ATCC 6538P where the MIC corresponding to the crude ethanolic extract of Borututo was obtained. The method used for the detection and quantification of 3-nitrotyrosine was performed at 356 nm, according to Teixeira et al (2017). Initially, in the preparation of the sample, 2 cycles were performed at -80 ° C and room temperature (10 minutes in each cycle). After that, the ultrasound was used for 15 minutes. For 1 mL of sample, 1 mL of trifluroacetic acid was used. Spin at 14500 rpm for 10 minutes. The sample was filtered with a 0.45 μm filter. Thus, the sample was doped with a final concentration of 0.05 mg/mL.

6. Data processing

The results were expressed as mean and standard deviation calculated in the Microsoft Excel program. All tests were performed in triplicate (n = 3).

RESULTS

1. Extracts

Arugula

Inhibition of bacterial growth in the aqueous and ethanolic extract of arugula in the strains described below is shown in Tables 1, and 2.

In Table 1, the aqueous extract of the arugula did not obtain antibacterial activity in the studied strains, 0 mm. Distilled water as is innocuous there was no inhibition of bacterial growth, 0 mm.

Table 1 - Aqueous extract of arugula in Petri dish.

	Petri dish 1 (mm) - Aqueous extract of arugula	Petri dish 2 (mm) - Distilled water
S. aureus ATCC 6538	0	0
S. aureus ATCC 6538P	0	0
S. aureus MRSA	0	0
E. coli ATCC 25922	0	0
S3R9	0	0
S3R22	0	0

Arugula

In Table 2, the ethanolic extract of arugula did not obtain antibacterial activity in the studied strains. The ethanol 96% did not show any inhibition of bacterial growth, 0 mm.

Table 2 - Ethanolic extract of arugula in Petri dish.

	Halo (mm) - Ethanolic extract of arugula		Halo (mm) - Ethanol 96%	
	Petri dish 1	Petri dish 2	Petri dish 1	Petri dish 2
S. aureus ATCC 6538	0	0	0	0
S. aureus ATCC 6538P	0	0	0	0
S. aureus MRSA	0	0	0	0
E. coli ATCC 25922	0	0	0	0
S3R9	0	0	0	0
S3R22	0	0	0	0

Borututo

The inhibition of bacterial growth in the Borututo aqueous extract, in the strains described below, is presented in Table 3.

In Table 3, the aqueous extract of Borututo did not obtain antibacterial activity in the studied strains, 0 mm.

Table 3 - Aqueous extract of Borututo in Petri dish.

	Halo (mm) - Aqueous extract of Borututo	
	Petri dish 1	Petri dish 2
S. aureus ATCC 6538	0	0
S. aureus ATCC 6538P	0	0
S. aureus MRSA	0	0
E. coli ATCC 25922	0	0
S3R9	0	0
S3R22	0	0

Borututo

In Tables 4, and 5, the ethanolic extract of Borututu presented greater inhibition against used strains of *S. aureus* ATCC 6538 and *S. aureus* 6538P than in the evaporated extract (DMSO).

In Table 4, ethanolic extract of Borututo obtained antibacterial activity in strains S. aureus ATCC 6538, 23 mm and S. aureus 6538P, 23 mm. However, 96% ethanol had no inhibition of bacterial growth, 0 mm.

Table 4 - Ethanolic extract of Borututo in Petri dish.

	Halo (mm) - Ethanolic extract of Borututo		Halo (mm) - Ethanol 96%	
	Petri dish 1	Petri dish 2	Petri dish 1	Petri dish 2
S. aureus ATCC 6538	**23**	**23**	0	0
S. aureus ATCC 6538P	**23**	**23**	0	0
S. aureus MRSA	0	0	0	0
E. coli ATCC 25922	0	0	0	0
S3R9	0	0	0	0
S3R22	0	0	0	0

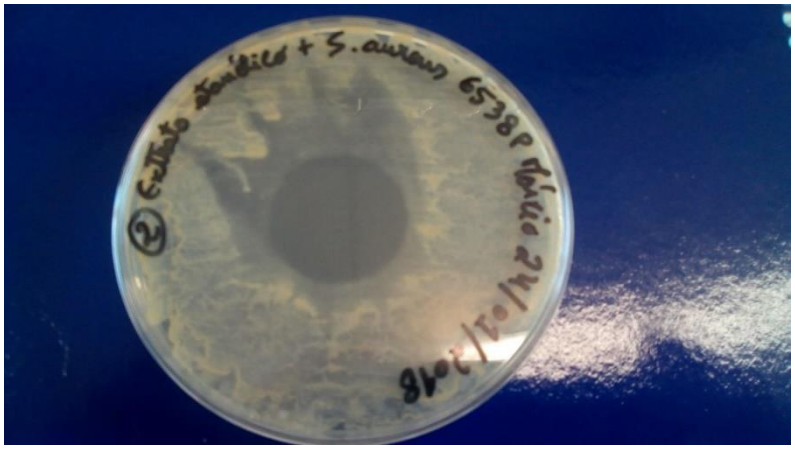

Figure 5 - Growth inhibition by ethanolic Borututo extract of S. aureus ATCC 6538P.

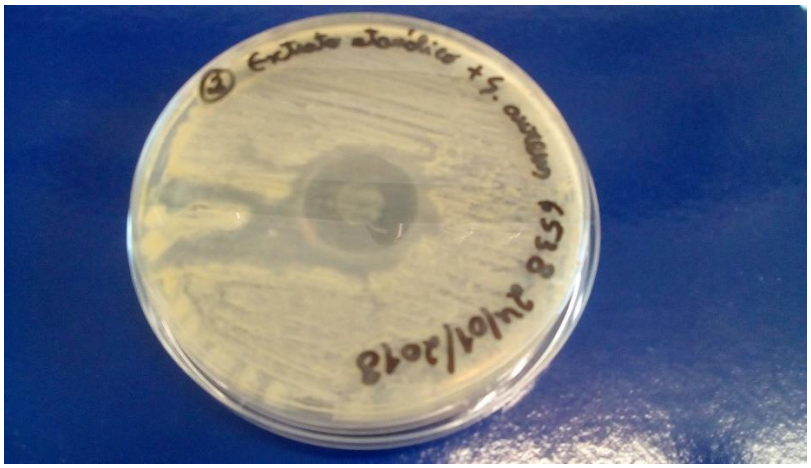

Figure 6 - Growth inhibition by ethanolic Borututo extract of S. aureus ATCC 6538.

In Table 5, evaporated extract (DMSO) of Borututo obtained antibacterial activity in strains *S. aureus* ATCC 6538, 14 mm and *S. aureus* 6538P, 15 mm. However, DMSO had no inhibition of bacterial growth, 0 mm.

Tabela 5 - Evaporated extract (DMSO) of borututu in Petri dish.

	Halo (mm)		Halo (mm) - DMSO	
	Petri dish 1	Petri dish 2	Petri dish 1	Petri dish 2
S. aureus ATCC 6538	**14**	**14**	0	0
S. aureus ATCC 6538P	**15**	**15**	0	0
S. aureus MRSA	0	0	0	0
E. coli ATCC 25922	0	0	0	0
S3R9	0	0	0	0
S3R22	0	0	0	0

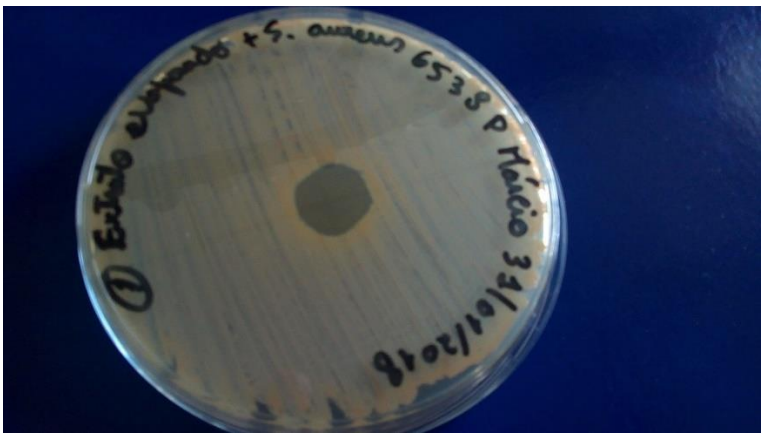

Figure 7 - Growth inhibition of ethanolic Borututo extract evaporated and diluted in DMSO of *S. aureus* ATCC 6538P.

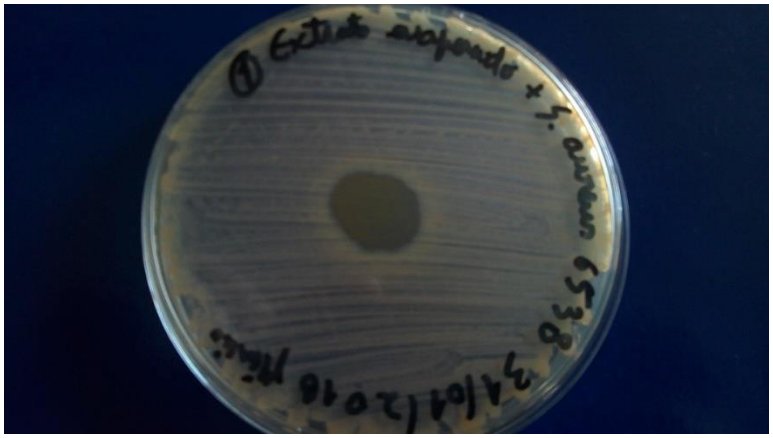

Figure 8 - Growth inhibition of ethanolic Borututo extract evaporated and diluted in DMSO of *S. aureus* ATCC 6538.

Considering that the sensitivity results of the ethanolic extract of Borututo were more expressive than the results of the ethanolic extract of the Borututo evaporated and diluted in DMSO followed the remaining tests with the first formulation.

2. Test of sensitivity to different concentrations of Borututo

Dilutions of a culture of *S. aureus* ATCC 6538P and *S. aureus* ATCC 6538 in the solvent were performed in 96-well plates and the OD was checked at 620 nm (T0). After incubation, in an orbital incubator with shaking (120 rpm) for 24 h at 37 °C for the bacteria under study, OD (T24) was read.

In Table 6 there was a greater difference of T24-T0 growth in 20% and 10% of extract compared to the other percentages of Borututo extract, that is, there was bacterial growth in these two percentages.

Table 6 – *S. aureus* ATCC 6538P T0 and T24 to the control.

	T0 %	**T24 %**	**T24-T0 %**
S. aureus ATCC 6538P (5%) + TSB (5%) + Extrato (90%)	138,6	138,7	0,1
S. aureus ATCC 6538P (10%) + TSB (10%) + Extrato (80%)	114,9	114,9	0,0
S. aureus ATCC 6538P (15%) + TSB (15%) + Extrato (70%)	100,5	98,9	-1,6
S. aureus ATCC 6538P (20%) + TSB (20%) + Extrato (60%)	94,4	92,1	-2,2
S. aureus ATCC 6538P (25%) + TSB (25%) + Extrato (50%)	74,2	103,6	29,4
S. aureus ATCC 6538P (30%) + TSB (30%) + Extrato (40%)	91,1	107,0	15,9
S. aureus ATCC 6538P (35%) + TSB (35%) + Extrato (30%)	127,2	155,7	28,5
S. aureus ATCC 6538P (40%) + TSB (40%) + Extrato (20%)	141,4	374,1	232,7
S. aureus ATCC 6538P (45%) + TSB (45%) + Extrato (10%)	142,0	415,1	273,2

Table 7 shows a larger T24-T0 growth difference in 10% of extract compared to the other percentages of Borututo extract, that is, there was bacterial growth in that percentage.

Table 7 - *S. aureus* ATCC 6538 T0 and T24 to the control.

	T0 %	**T24 %**	**T24-T0 %**
S. aureus ATCC 6538 (5%) + TSB (5%) + Extrato (90%)	65,0	65,3	0,3
S. aureus ATCC 6538 (10%) + TSB (10%) + Extrato (80%)	77,7	75,9	-1,8
S. aureus ATCC 6538 (15%) + TSB (15%) + Extrato (70%)	63,9	62,5	-1,5
S. aureus ATCC 6538 (20%) + TSB (20%) + Extrato (60%)	78,8	78,3	-0,5
S. aureus ATCC 6538 (25%) + TSB (25%) + Extrato (50%)	94,9	94,6	-0,3
S. aureus ATCC 6538 (30%) + TSB (30%) + Extrato (40%)	131,1	130,7	-0,4
S. aureus ATCC 6538 (35%) + TSB (35%) + Extrato (30%)	166,5	162,0	-4,5
S. aureus ATCC 6538 (40%) + TSB (40%) + Extrato (20%)	146,6	165,4	18,9
S. aureus ATCC 6538 (45%) + TSB (45%) + Extrato (10%)	64,4	132,4	68,0

In figure 9, minimum inhibitory concentration of at least 20% of *S. aureus* ATCC 6538 with ethanolic extract of Borututo.

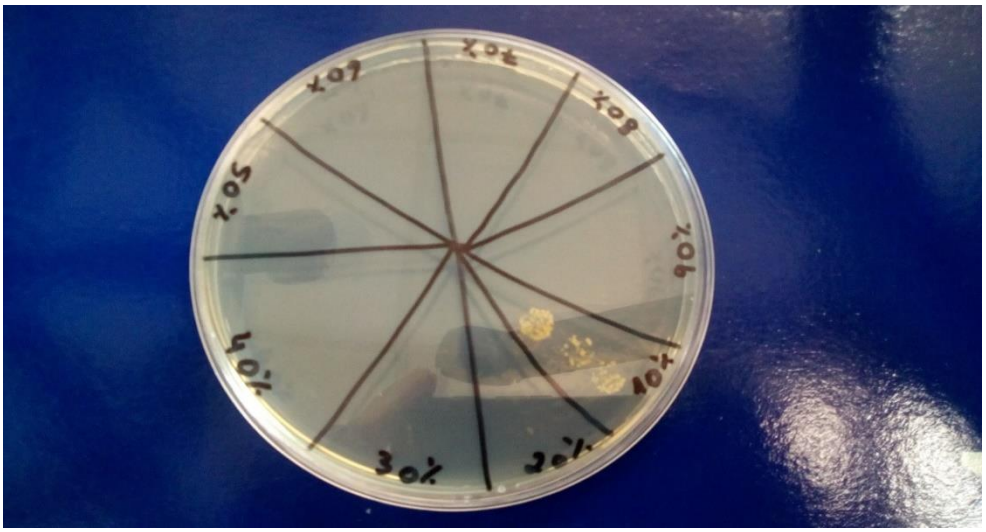

Figure 9 - Minimum inhibitory concentration of at least 20% of *S. aureus* ATCC 6538 with ethanolic extract of Borututo.

In figure 10, minimum inhibitory concentration of at least 30% of *S. aureus* ATCC 6538P with ethanolic extract of Borututo.

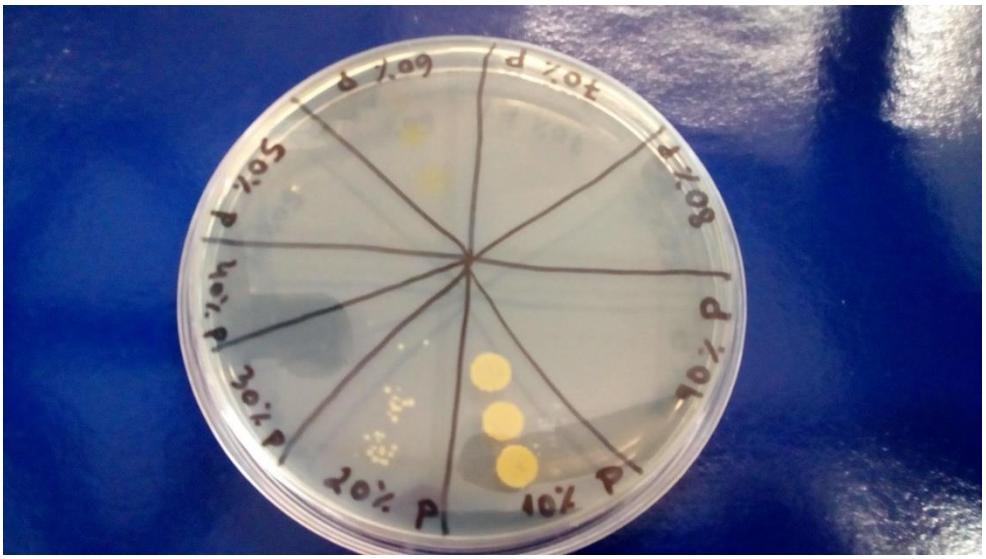

Figure 10 - Minimum inhibitory concentration of at least 30% of *S. aureus* ATCC 6538P with ethanolic extract of Borututo.

3. Evaluation of oxidative/nitrosative stress

After chromatographic analysis, it was verified that there are no differences in the concentration of 3-NT between the different samples.

DISCUSSION AND CONCLUSION

There are some studies on the discovery of new antimicrobial agents from plant extracts and other natural products (Abourashed, E. & Fu, H. 2017) (Brusotti G, *et al* 2014). In this work the aim was to verify the antimicrobial activity of organic and aqueous extract of the leaf of the arugula and the root of the Borututo so that it is possible to use both for medicinal purposes.

According to the results obtained in this study, it can be concluded that the extracts of the arugula plant do not have antimicrobial properties for the studied strains. While the extracts of the Borututo plant contain components with inhibitory properties for the studied strains, namely *S. aureus* ATCC 6538, and *S. aureus* ATCC 6538P.

For the sensitivity tests on studied strains, the Borututo organic extract showed inhibition in the abovementioned strains, comparing with the control that did not inhibit, and it can be concluded that the organic extract of Borututo may have inhibitory capacity.

Minimum inhibitory concentration of at least 20% of *S. aureus* ATCC 6538 with ethanolic extract of Borututo while minimum inhibitory concentration of at least 30% of *S. aureus* ATCC 6538P with ethanolic extract of Borututo..

After chromatographic analysis, the were no differences in the 3-NT concentration, a possible reason is that it is outside the detection limit of 3-NT.

Furthermore, there were several limitations encountered, both in the fact that there is little literature available and during the thesis the occurrence of solvent evaporation in the 96-well plate assays. The 96-well plate method has some drawbacks, such as the cells of some microorganism's adhesion to the base of the well, or remaining in suspension, some compounds present in some extracts may precipitate. However, it is a cheap method, it is sensitive, it has reproducibility, it can be used for many samples, it requires a small amount of sample and leaves a permanent record.

The Borututo requires more evaluation studies of the components and put them to the

service of the pharmaceutical industry.

References

Abourashed, E. & Fu, H. 2017. "Hydroxybenzoic Acids Are Significant Contributors to the Antioxidant Effect of Borututu Bark, Cochlospermum Angolensis Welw. Ex Oliv. Antioxidants 6, 9 (2017)." 9: 2017.

Almeida, Eliana, Mira De Bona, Fabiana Gisele, Thomas Kehrwald Fruet, Tereza Cristina, Marinho Jorge, and Alexandre Carvalho De Moura. 2014. "Comparação de Métodos Para Avaliação Da Atividade Antimicrobiana E Determinação Da Concentração Inibitória Mínima (CIM) de Extratos Vegetais Aquosos E Etanólicos." *Arq. Inst. Biol. (Sao. Paulo)*, no. 81: 218–25. doi:10.1590/1808-1657001192012.

Andrade, L. S., Santos, D. B., Castro, D. B., Guillo, L. a & Chen-Chen, L. 2008. "Absence of Antimutagenicity of Cochlospermum Regium (Mart. and Schr.) Pilger 1924 by Micronucleus Test in Mice." *Braz. J. Biol.* 68: 155–59.

Basu, Hemendra N, Anthony J Del Vecchio, Frank Flider, and Frank T Orthoefer. 2001. "Nutritional and Potential Disease Prevention Properties of Carotenoids." *Northeast Consultant Resources* 78 (7): 665–75.

Brusotti G, Cesari I, Dentamaro A, Caccialanza G, Massolini G. 2014. "Isolation and Characterization of Bioactive Compounds from Plant Resources: The Role of Analysis in the Ethnopharmacological Approach." *J Pharm Biomed Anal*, 87:218-28.

Ferreres, Federico, Clara Grosso, Angel Gil-Izquierdo, Patrícia Valentão, and Paula B. Andrade. 2013. "Ellagic Acid and Derivatives from Cochlospermum Angolensis Welw." *Phytochemical Analysis* 24 (6): 534–40. doi:10.1002/pca.2429.

Filgueira, F. A. R. 2000. "Novo Manual de Olericultura: Agrotecnologia Moderna Na Produção E Comercialização de Hortaliças." *Viçosa: UFV*, 284295.

Islam, Khirul, Sanjib Saha, Imran Mahmud, Khalit Mohamad, Khalijah Awang, Shaikh Jamal, and Jamil A Shilpi. 2014. "An Ethnobotanical Study of Medicinal Plants Used by Tribal and Native People of Madhupur Forest Area, Bangladesh." *Journal of Ethnopharmacology* 151: 921–30. doi:10.1016/j.jep.2013.11.056.

José, Herculano Hossi. 2017. "Estudo Da Bioatividade de Compostos Fitoquímicos de Plantas Medicinais Africanas: Cochlospermum Angolensis," 37.

Manuel, José, Carla Matos, Carla Moutinho, Glória Queiroz, and Lígia Rebelo. 2009. "Ethnopharmacological Notes about Ancient Uses of Medicinal Plants in Trás-Os-Montes (Northern of Portugal)." *J Ethnopharmacol.* 124: 270–83.

doi:10.1016/j.jep.2009.04.041.

Pereira, C. et al. 2015. "Phenolic Profile and Antimicrobial Activity of Different Dietary Supplements Based on Cochlospermum Angolensis." *Welw. Ind. Crops Prod.* 74: 412 – 416.

Pereira, Carla, Ricardo C. Calhelha, Lillian Barros, and Isabel C F R Ferreira. 2013. "Antioxidant Properties, Anti-Hepatocellular Carcinoma Activity and Hepatotoxicity of Artichoke, Milk Thistle and Borututu." *Industrial Crops and Products* 49. Elsevier B.V.: 61–65. doi:10.1016/j.indcrop.2013.04.032.

Soares, D. G., Andreazza, A. C. & Salvador, M. 2005. "Avaliação de Compostos Com Atividade Antioxidante Em Células Da Levedura Saccharomyces Cerevisiae." *Rev. Bras. Ciências Farm.* 41: 95 – 100.

Teixeira, Dulce, Rúben Fernandes, and Cristina Prudêncio. 2016. "Biochimie 3-Nitrotyrosine Quantification Methods : Current Concepts and Future Challenges." *Biochimie* 125: 1–11. doi:10.1016/j.biochi.2016.02.011.

Teixeira, Dulce, Cristina Prudêncio, and Mónica Vieira. 2017. "Development of a New HPLC-Based Method for 3-Nitrotyrosine Quantification in Different Biological Matrices." *Journal of Chromatography B* 1046. Elsevier B.V.: 48–57. doi:10.1016/j.jchromb.2017.01.035.

Tripodi, P., G. Francese, and G. Mennella. 2017. "Rocket Salad: Crop Description, Bioactive Compounds and Breeding Perspectives Rocket Salad: Crop Description, Bioactive Compounds and Breeding Perspectives." *Adv. Hort. Sci.* 31 (2): 107–13. doi:10.13128/ahs-21087.

Vinha, Ana F, Anabela Costa, Marta Soares, and Pedro Pires. 2012. "Atividade Antioxidante de Extratos Aquosos de Cochlospermum Angolense (Borututu)." In , 2–5. Portugal.