Evaluation of the antibacterial potential of crude extracts and essential oils of three *Baccharis species*

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ABSTRACT

The purpose of this work was to analyze the antimicrobial potential of *Baccharis trimera*, *B. gaudichaudiana* and *B. dracunculifolia*, which were used to perform antibiogram tests, with *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and a wild microorganism isolated from soil, which was submitted to molecular identification characterized as *Bacillus cereus*. The sensitivity or resistance of each microorganism subjected to hydro-alcoholic extracts and essential oils of the three plants was analyzed, and the inhibition was quantitatively evaluated according to the halo formed around the disks of paper. Data were subjected to statistical evaluation using a completely randomized design, in a three-factor split-plot scheme and four replications. The results of the microorganism susceptibility tests allow us to infer that *B. trimera* (Less.) DC. has a greater inhibitory action in the essential oils. The two microorganisms with Gram-positive characteristic cell walls *(B. cereus* and *S. aureus*) suffered growth inhibition by the three species of *Baccharis*, both in essential oils and in extracts. In the case of Gram-negative (*E. coli* and *P. aeruginosa*) there was no growth inhibition at the concentrations of extracts and essential oils used. The microorganism *B. cereus* suffered a greater inhibitory influence from the species *B. trimera* and *B. gaudichaudiana*. Chemical analysis showed similarity of five compounds in the tested species, namely caryophyllene; bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-; y-gurjunene; D-amorphene, and spathulenol. Some of these are already known in the literature due to their antibacterial capacity. The sum, in percentage, of these compounds for *B. dracunculifolia*, *B. gaudichaudiana* and *B. trimera* was 39.08, 31.45 and 37.15%, respectively.

Keywords: Antibacterial potential, Genetic identification, Medicinal plants, methodologies of extraction.

INTRODUCTION

The increase in drug-resistant microorganisms and the scarcity in the discovery of new antimicrobials has been causing concern worldwide (Jindal et al. 2015; Ramírez et al. 2021; Jadeja and Worrich, 2022). According to Pena da Costa (2017), due to the exaggerated and discrepant use of available drugs, an accelerated natural process of bacterial resistance against synthetic antibiotics has been observed. Allied to this, another factor responsible for the rapid growth in the number of multiresistant bacterial species is the ability of bacteria to transfer resistance genes to other bacteria (Caumo et al. 2010).

According to a study by British economist Jim O'Neill (2016), by 2050, ten million deaths per year will be attributed to antimicrobial resistance and the impact on the global economy will be around one hundred trillion dollars, and according to the World Health Organization the bacteria *Escherichia coli* and *Staphylococcus aureus* are notorious examples of bacteria which are showing resistance to antimicrobials (World Health Organization et al. 2018).

In addition to the aforementioned microorganisms, *Bacillus cereus* has the ability to form biofilms and adhere to different surfaces. This makes both the elimination and the reduction of this microorganism difficult in industrial and food environments. This microorganism is more resistant to antimicrobials of the β-lactam class (penicillin, oxacillin and cefepime) due to its ability to produce β-lactamase (Costa 2019).

Another pathogenic microorganism

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is *Pseudomonas aeruginosa*, which acts in an opportunistic manner, mainly affecting immunocompromised patients, patients with cystic fibrosis, pacients with burns and neutropenics. Data presented by SENTRY (Global Antimicrobial Surveillance Program) report that *P. aeruginosa* was responsible for 31.2% of pneumonias and 13.8% of skin and soft tissue infections (Gales et al. 2001; Sader et al. 2014).

In this sense, there is a need to search for new antimicrobial agents, since the bacteria have developed resistance to most antibiotics used (Al-Bari 2006). Plant research has proven its antimicrobial potential in the treatment of various infectious diseases, in addition to showing a reduction in side effects when compared to synthetic antimicrobials (de Lima 2006; Bella 2010). Furthermore, Amor (2009) emphasizes that traditional medicinal plants should receive significant attention as a source of new chemical properties, because their phytochemicals have potential in the discovery of new drugs.

Furthermore, plant extracts contain a variety of phytoconstituents that can act synergistically to inhibit bacterial growth and prevent the development of drug resistance in bacteria (da Silva et al. 2020). An example of this is the genus *Baccharis* which is the largest genus of the Asteraceae family, with more than 500 species distributed throughout the North and South American continent (Abad and Bermejo 2007).

The biological activity of *B. trimera* is justified by the presence of secondary compounds produced by the plant, such as phenols, saponins, flavonoids, tannins, coumarins, among others (Rabelo and Costa, 2018; Garcia et al. 2018). Among the species of *Baccharis*, *B. dracunculifolia* DC., which is a native plant of Brazil, known as “Alecrim-do-Campo”, stands out in this context (Budel et al. 2018) and, in addition, several pharmacological activities have been attributed to this plant, including antibacterial activity (Cazella 2019). An example of this is cited by da Silva et al. (2018) who analyzed the antimicrobial capacity of the tincture from the leaf of *B. trimera* and concluded that the tincture has relevant antimicrobial activity against methicillin-resistant *S. aureus* (clinical isolate).

In this sense, and aiming to broaden the discussion on this topic, which is of extreme public health relevance, the objective of this work was to analyze the antimicrobial potential of three crude extracts and three essential oils from plants of the genus *Baccharis*, belonging to the Asteraceae family, being the species *B. trimera* (carqueja-amargosa), *B. dracunculifolia* (alecrim-do-campo) and *B. gaudichaudiana* DC. (carqueja-doce) against standard bacterial strains, which are *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and a wild microorganism initially called MIC 01, collected from soil, in an experimental area at the UFSM campus in the municipality of Frederico Westphalen-RS-Brazil.

MATERIALS AND METHODS

The research activities were carried out at the Laboratory of the Environmental Management and Water Resource Management Research Group (GAMRH), at the Frederico Westphalen campus of the Federal University of Santa Maria.

Plant species were collected from September to December 2019, in the early hours of the day, without rainfall in a ruderal area, located on the campus of the Federal University of Santa Maria, in the municipality of Frederico Westphalen, Rio Grande do Sul/Brazil, at the coordinates latitude: 270 25’ 43’’S and longitude: 530 43’ 25”W. The species were identified as: *B. trimera* (SMDB21593), *B. gaudichaudiana* (SMDB21594), and *B. dracunculifolia* (SMDB21592), and the project A891533 was registered in the SISGEN database.

The identification was carried out using the method of comparison with other existing species in the herbarium of the Department of Forestry at the Frederico Westphalen campus of the Federal University of Santa Maria. After collecting the plants, their aerial parts were fragmented and placed for drying in an oven at 45 ºC until the weight remained constant (Brazil 2019).

Obtaining the raw extracts

To obtain the extract, 200 g of previously dehydrated samples were crushed in a knife mill and subsequently homogenized. Subsequently, the samples were suspended in 1 l of hydroalcoholic solution (70:30, ethanol: distilled water), being in extraction for a period of 10 days at room temperature and in the absence of light (Brazil 2019).

After this period, the extracts were vacuum filtered and the residues re-extracted. Finally, the filtrates were evaporated under pressure and constant temperature until resulting in about 10% of the volume. Subsequently, the extracts were taken to the kiln to obtain the crude extract and, subsequently, stored in a refrigerator at a temperature of 5 ºC (Brazil 2019).

Obtaining the essential oils

To obtain the essential oils, crushed fresh leaves (500 g) were submitted to the extraction process by hydrodistillation, in a modified Clevenger apparatus and adapted to a round-bottom flask with a capacity of 4 l, for a period of 4 h, according to the methodology described by Guimarães et al. (2008).

The material obtained was centrifuged (5000rpm) for 5 min and, later, the volatile oils were placed in an amber glass bottle and stored, in the dark, in a refrigerator at 15 °C.

Microbiological tests

The microorganisms used in the antimicrobial activity assay were: *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), being these standard strains, purchased from the company NEW Prov. In addition, a bacterium from the soil was isolated from the experimental area on the UFSM campus in the municipality of Frederico Westphalen-RS and identified. Initially, this was called MIC 01.

For initial identification of MIC 01, a fraction of the original sample was inoculated into Mueller Hinton Agar Kasvi® (MHA), for 24 h at a temperature of 37.0 ºC (Chierrito et al. 2019), with subsequent microscopic analysis, resulting in an arrangement of Gram-positive streptobacilli. Furthermore, amylolytic activity was analyzed, forming halos when grown in medium enriched with 1% starch in MHA. Afterwards, with the uniform colonies grown in the medium, an aliquot followed for molecular characterization and identification. The species MIC 01 identity confirmation occurred by molecular method (PCR).

Antibiotic Sensitivity Test (AST)

Before the antimicrobial activity tests with the plant extracts, the culture medium MHA, was prepared. The method of direct suspension of colonies in saline was used, in order to obtain a density equivalent to the turbidity standard 0.5 of the McFarland, by spectrophotometry (absorbance 0,08 – 0,13 at 625 nm) scale which corresponds approximately to 1-2 x10^8 CFU. The antimicrobial activity of the extract was verified by the paper disk diffusion technique according to NCCLS (2003) and BrCAST-EUCAST (2021).

Finally, sterilized blank discs (without the presence of extracts/ essential oils) were added to the extracts, which contained 500 mg/ml resulting in a concentration of 0.0025 mg/mm^2, and directly to the essential oils without dilution. The disks were then applied to the plates and, subsequently, they were incubated at 35.0 +/- 1 ºC for 18 +/- 2 h in an inverted position. After the incubation period, visual readings were taken in order to observe halos of inhibition of bacterial growth and quantify them in millimeters with the aid of a digital caliper.

Qualitative and quantitative evaluation of the extracts

For analysis, 20 g of each plant were crushed and diluted in 100 ml of methanol. The mixture in maceration and after a period of 10 days was filtered in two stages, the first with a paper filter to remove coarse residues and the second in syringe filters of Mixed Cellulose Esters - MCE, with a porosity of 0.45 µm. The content was stored in a vial and then taken to the equipment.

The chromatograph in this experiment used helium as carrier gas, a Varian® capillary column, FactorFourTM model, VF-5 ms, 30 m, 0.25 mm – 0.25 µm, and 1 µl of sample was inserted with a split/ splitless injector (250 ºC). The heating program was (50 ºC – 1 min; 120 ºC – 5 °C/ min, 3 min; 280 ºC – 20 °C/min, 24 min) and the detector temperature was set at 290ºC.

The qualitative evaluation of the crude extract was performed by gas chromatography coupled with mass spectrometry (GC/MS - Shimadzu, model QP 5050A). The identification of the constituents was performed by comparison of the retention indices, calculated using the equation of Van den Dool and Kratz (1963) in relation to the homologous series of linear alkanes (nC8-nC18), extrapolation to C19 and C20 and also by comparison of the spectral data with the data available in the literature (Adams 2007) and also with the use of the NIST107 and NIST21 libraries. In the quantitative evaluation, a Shimadzu CG-17A gas chromatograph equipped with a flame ionization detector (DIC) was used, and the quantification of each constituent was obtained by area normalization (%) (Flach et al. 2021).

Statistical design

The experiment was carried out in a completely randomized design, in a three-factor scheme with split plots and four replications. The main plots were composed of Petri dishes in which the combinations of the extracts factor (hydroalcoholic extract and oils) with the microorganisms factor (MIC 01, *E. coli*, *P. aeruginosa* and *S. aureus*) were allocated. The Petri dishes were divided into quadrants (subplots), and in three of these quadrants the plants soaked in the disks (*B. trimera*, *B. dracunculifolia* or *B. gaudichaudiana*) were allocated.

The diameter of the halo was measured in each subplot and the analysis of variance was performed according to a three-factor mathematical model with a subdivided plot in a completely randomized design given by: Yijkl = µ + ai + dj + (ad)ij + εPP + ck + (ac)ik + (dc)jk + (adc)ijk + εSP. In this model, Yijkl is the mean observed value of the response variable in the ijkl portion; µ is the overall mean; ai is the fixed effect of level i (i = 1 and 2) of the extracts factor (composed of: 1 = hydroalcoholic extract and 2 = oils); dj is the fixed effect of level j (j = 1, 2, 3, and 4) of the microorganism factor (1=MIC 01; 2=*E. coli*; 3=*P. aeruginosa* and 4=*S. aureus*); (ad) ij is the effect of the interaction of the level i of the Extracts factor with the level j of the microorganism

factor; εPP is the effect of the experimental error in the main plot; ck is the fixed effect of the level k (k = 1, 2, and 3) of the plant factor (1= *B. trimera*; 2= *B. dracunculifolia* and 3= *B. gaudichaudiana*); (ac)ik is the effect of the interaction of the extracts factor level i with the plant factor level k; (dc)jk is the effect of the interaction of the microorganism factor level j with the plant factor level k; (adc)ijk is the effect of the triple interaction of the extracts factor level i with the microorganism factor level j and the plant factor level k. The effect of the experimental error in the subplot is εSP, assumed to be normal and independently distributed with zero mean and common variance σ2 (Storck et al. 2016).

Next, the experimental precision was calculated and the means were grouped using the Scott-Knott test, both for main effects and for the unfolding of interactions. In all analyses, 5% probability of error was established, and the analyzes were performed using the Microsoft Office Excel application and the GENES software (Cruz 2013).

RESULTS AND DISCUSSION

Species confirmation by PCR

After the analysis, through the Technical Report Identification of Microorganisms (OS 210591), the microorganism previously identified as MIC 01 was defined by the following taxonomic classification: *B. cereus* Frankland and Frankland, 1887, cellular organisms; Bacteria; Terrabacteria group; Firmicutes; Bacilli; Bacillales; Bacillaceae; *Bacillus*; *B. cereus* group.

Phytochemical analysis

Altogether, in the three species of *Baccharis* evaluated, 28 different chemical compounds were found. According to table 1, it is possible to observe the difference in composition between the three species.

Of these, 19 compounds are present in *B. dracunculifolia,* 12 compounds in *B. gaudichaudiana* and 12 in *B. trimera.* Five compounds are common to the three *Baccharis,* namely*:* Caryophyllene;

Table 1. Chemical composition of methanolic extracts of each species studied.

CAS	Chemical compound name	R.T.	B.D. %	B. G. %	B. T. %
127-91-3	β-Pinene	7.609	7.92	13.775	-
503-93-5	Eucarvone	14.581	-	-	4.182
103-25-3	Methyl β-phenylpropionate	16.122	3.808	-	-
471-01-2	beta-Isophorone	16.766	-	-	24.63
3856-25-5	α-Copaene	19.264	-	-	2.016
515-13-9	Elemene	19.527	-	-	10.063
87-44-5	Caryophyllene	20.058	7.822	9.04	3.144
6753-98-6	α-Humulene	20.585	1.016	4.522	-
150320-52-8	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	20.903	2.366	4.072	10.521
22567-17-5	γ-Gurjunene	21.075	3.495	5.208	5.734
483-76-1	D-Amorphene	21.292	1.415	1.91	4.837
NI	Elemol	21.613	-	-	2.014
1139-30-6	Caryophyllene oxide	21.688	5.216	-	-
77171-55-2	Spathulenol	21.920	23.978	11.221	12.912
1460-73-7	Agarospirol	22.084	4.365	-	8.288
NI	Isoaromadendrene epoxide	22.321	4.154	-	-
51317-08-9	Isocadinene	22.578	0.771	-	3.747
NI	Murolan-3,9(11)-diene-10-peroxy	22.784	2.519	-	-
NI	cis-Z-.alpha.-Bisabolene epoxide	22.896	2.457	-	-
NI	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydrona	23.128	6.009	3.708	-
NI	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	23.592	1.031	-	-
34624-81-2	2,6-Bis(t-butyl)-4-(dimethylbenzyl)phenol	24.619	7.072	-	-
NI	(6-Isopropyl-3,4-bis(methylamino)-2,4,6- cycloheptatrienylidene) malononitrile	25.974	2.213	-	-
NI	3-Oxatricyclo [20.8.0.0(7,16)] triaconta - 1(22),7(16),9,13,23,29-hexaene	27.616	1.578	2.641	-
85760-81-2	Alloaromadendrene oxide	28.808	4.987	24.999	-
111-02-4	Squalene	29.022	5.807	-	7.912

RT – Retention Time; BD - *B. dracunculifolia*; BG- *B. gaudichaudiana* and BT- *B. trimera*.

Bicyclo [4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-; γ-Gurjunene ; D-Amorphene, and Spathulenol (Flach et al. 2021).

Among the compounds observed stands out the spathulenol for introducing biological importance with moderate antibacterial and cytotoxic activity (Limberger et al. 2004). Although the concentrations of these compounds were different in the studied plants, there was a similar result with regard to the size of the halo formed. This may have occurred due to a diversity of effects among the large amount of compounds observed in the sample that may be synergistic, antagonistic or not significantly interfere with the activity of the others.

Monoterpenes, such as β-pinene, interfere with the integrity and functioning of the cell membrane, through the change in membrane potential, loss of cytoplasmic material and inhibition of the respiratory chain. Exposure to terpenes can interfere with the expression of genes encoding virulence factors, as when considering enterotoxin-producing *S. aureus* strains (Li et al. 2011). In the present study β-pinene was found only in species *B. dracunculifolia* and *B. gaudichaudiana,* on the other hand, caryophyllene and azulene derivative, for example, appeared in the three species of *Baccharis*.

Statistical analysis

It was found that the main effects of the three factors and the double and triple interactions were significant by the F test of the analysis of variance (p<0.05 Table 2). These results indicate that the different extraction processes, microorganisms and plants used affect the diameter of the halos. Furthermore, the significant interactions indicate that there are combinations between extraction processes, microorganisms and plants that change the diameter of the halos, indicating synergism in some combinations and antagonism in others. The experimental precision was medium in the main plots and high in the subplots. Greater precision in the subplots is expected due to the greater number of repetitions at this level and the error degrees of freedom, as described by Storck et al. (2016).

As the triple interaction was significant, all the double interactions were unfolded (Tables 3, 4 and 5) and the main effects will be discussed in the background. When comparing the diameters of the halos between extraction processes obtained from different plants, it was found that for the hydroalcoholic extract, there were no differences between the tested plants (Table 3). When the essential oil of *B. trimera* (plant 1) was used, a larger diameter of halos was formed, that is, the tested microorganisms were inhibited with this extract. There were no statistical differences between halo diameters when using hydroalcoholic extract or *B. trimera* oil. On the other hand, for *B. dracunculifolia* (plant 2), larger halo diameter occurs using hydroalcoholic extract. In *B. gaudichaudiana* (plant 3) there is no significant difference in the diameter of halos with the use of hydroalcoholic extract or oil.

Regarding the diameters of halos, no differences were found between hydroalcoholic extract and oil in the microorganisms *B. cereus*, *E. coli* and *P. aeruginosa* (Table 4). The microorganism *B. cereus*, in general, was more influenced by extracts and essential oils, resulting in a larger diameter of halos, differing statistically in relation to the diameter of the formed halos compared to *S. aureus*. In the case of the microorganisms *E. coli* and *P. aeruginosa*, there was no halo growth.

Table 2. Analysis of variance in a three-factor scheme with sources of variation (SV) and their respective degrees of freedom (DF), sums of squares (SS), mean squares (MS), F calculated statistic values (Fcalc) and p value (P). Experimental precision in the main plot (CV pp) and in the subplot (CV sp).

SV	DF	SS	MS	Fcalc	P
Extraction processes	1	0.027	0.027	7.529*	0.011
Microorganisms	3	31.564	10.521	2970.706	0.000
Extraction processes × Microorganisms	3	0.034	0.011	3.216*	0.041
Error A	24	0.085	0.004	-	-
Plants	2	0.133	0.066	20.548	0.000
Extraction processes × Plants	2	0.092	0.046	14.161	0.000
Microorganisms × Plants	6	0.173	0.029	8.936	0.000
Extraction processes × Microorganisms × Plants	6	0.108	0.018	5.559	0.000
Error B	48	0.155	0.003	-	-
Total	95	32.370	0.341	-	-
Average: 0.5729 cm		CVpp: 10.388%		CVsp: 9.919%	

*Extraction processes = Extracts or Essential Oils

Table 3. Comparisons of general averages and between combinations of plants and extracts for the diameter of the halo (cm).

	Baccharis species*			Grand Total
Extracts	**Plant 1**	**Plant 2**	**Plant 3**	
Hydroalcoholic extract	0.6125Aa**	0.6125Aa	0.5438Aa	0.5896
Oil	0.6375Aa	0.4938Bb	0.5375Ba	0.5563
Grand Total	0.6250	0.5531	0.5406	0.5729

* Plant 1- *B. trimera*, Plant 2- *B. dracunculifolia* and Plant 3- *B. gaudichaudiana*
**Averages followed by the same uppercase letters horizontally and lowercase letters vertically constitute a statistically homogeneous group by the Scott-Knott test at 5% probability of error.

Table 4. Comparisons of general averages and between combinations of extracts and microorganisms for the diameter of the halo (cm).

	Microorganisms *				
Hydroalcoholic Extract / Oil	**1**	**2**	**3**	**4**	**Grand total**
Hydroalcoholic extract	1.2000Aa**	0.0000Ba	0.0000Ba	1.1583Aa	0.5896
Oil	1.1583Aa	0.0000Ca	0.0000Ca	1.0667Bb	0.5563
Grand total	1.1792	0.0000	0.0000	1.1125	0.5729

* 1- *B. cereus*, 2- *E. coli*, 3- *P. aeruginosa* and 4- *S. aureus*.
**Averages followed by the same uppercase letters horizontally and lowercase letters vertically constitute a statistically homogeneous group by the Scott-Knott test at 5% probability of error.

This may occur due to the intrinsic resistance of Gram-negative bacteria, which have cell wall specializations that prevent or hinder the action of antibacterial compounds. This group of bacteria can promote the activation of efflux pumps, change the drug binding site and membrane permeability, use degradation enzymes and conformational change of the drug culminating in its inactivation (Oliveira and Reygaert 2019).

According to Bernardes (2014), extracts, fractions and substances isolated from *B. dracunculifolia* have antibacterial activity, mainly against Gram-positive bacteria. Aleixo et al. (2013) obtained similar results for tests with the species *B. trimera*, which, at the concentration used, inhibited 100% of the Gram-positive strains tested, not happening with the Gram-negative ones.

Despite the lack of literature on antimicrobial effects of *B. gaudichaudiana* extracts, the present study brought very similar resulting data between the three species evaluated.

In evaluating the interaction between plants and microorganisms, it was found that for *B. trimera* (plant 1), the most sensitive microorganism was *B. cereus*, followed by *S. aureus* (Table 5). In *B. dracunculifolia* (plant 2), the microorganisms *B. cereus* and *S. aureus* did not differ in the size of halo formation. In *B. gaudichaudiana* (plant 3), again the microorganisms *B. cereus* was the most sensitive, followed by *S. aureus*. In the microorganisms *E. coli* and *P. aeruginosa* there was no halo formation, regardless of the extraction process (Table 4) and the plant used (Table 5). Among the plants, a larger diameter of halos was found when *B. trimera* was used, both in the microorganism *B. cereus* and in *S. aureus*. Thus, in general, *B. trimera* for the microorganism *B. cereus* presented the largest halo diameter, indicating that this microorganism is sensitive to the phytochemicals present in this species.

Considering these results, it is intended to refine the research, improve and further deepen the evaluation methods, fractioning the extractions into groups or isolating compounds to analyze the isolate and the individual behavior of escapes. From this, it will be possible to confirm the hypothesis that was strengthened in this study, these extracts serve as promising antimicrobials.

Table 5. Comparisons of general averages and between combinations of plants and microorganisms for the diameter of the halo (cm).

	Microorganisms**				Grand total
Plant*	1	2	3	4	
1	1.3125Aa***	0.0000Ca	0.0000Ca	1.1875Ba	0.6250
2	1.1000Ab	0.0000Ba	0.0000Ba	1.1125Ab	0.5531
3	1.1250Ab	0.0000Ca	0.0000Ca	1.0375Bb	0.5406
Grand total	1.1792	0.0000	0.0000	1.1125	0.5729

*1- *B. trimera*, 2- *B. dracunculifolia* and 3- *B. gaudichaudiana*

** 1- *B. cereus*, 2- *E. coli*, 3- *P. aeruginosa* and 4- *S. aureus*.

***Averages followed by the same uppercase letters horizontally and lowercase letters vertically constitute a statistically homogeneous group by the Scott-Knott test at 5% probability of error.

FINAL CONSIDERATIONS

The microorganism isolated in soil, initially called MIC 01, was identified as *B. cereus* an important pathogen responsible for food poisoning, with the ability to form endospores and biofilms.

The species *B. trimera* has the greatest inhibitory action among the tested crude extracts and essential oils. The two microorganisms with characteristic Gram-positive cell walls (*B. cereus* and *S. aureus*) suffered growth inhibition by the three species of *Baccharis*. In the case of Gram-negative (*E. coli* and *P. aeruginosa*) there was no growth inhibition in the concentrations of extracts and essential oils used.

The microorganism *B. cereus* suffered the greatest inhibitory influence from the species *Baccharis trimera* and *B. gaudichaudiana*.

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AUTHORS' CONTRIBUTION

All authors contributed to the study. Conceptualization, G.V. and G.R.; methodology, validation, investigation, formal analysis, G.V., K.F., G.R., M.T., U.B. and J.J; software, M.T.; resources and funding acquisition, G.R., J.J.; data curation, G.R., M.T.; writing original draft preparation, G.V., K.F., G.R., M.T., U.B.; writing review and editing, G.R., K.F. and U.B.; visualization, K.F., G.R., U.B.; supervision and project administration, G.R. and J.J. All authors read and approved the final manuscript.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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