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# *In Vitro* Antimicrobial effects of Extracts from Leaves of Medicinal Herbs and Native Brazilian Plants

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**Abstract**

The objective of this study was to evaluate the antimicrobial effects of crude and lyophilized extracts of leaves from sweet passion fruit (*Passiflora alata*), araçá (*Psidium cattleianum*), rosemary (*Rosamrinus officinalis*) and oregano (*Origanum vulgare*) on planktonic cells of *Staphylococcus aureus* and *Aspergillus parasiticus*. Sweet passion fruit showed no inhibitory effect against the micro-organisms tested. However, crude and lyophilized extracts from Araçá had the highest ($P < 0.05$) antimicrobial activity against S. *aureus*, with minimum inhibitory concentrations (MIC) of 0.39 and 0.35 mg/ml, respectively. MIC values against S. *aureus* for lyophilized extracts from rosemary and crude extracts from oregano were 0.57 and 0.65 mg/ml, respectively. None of the extracts demonstrated effective results against A. *parasiticus*, although araçá and oregano extracts had the lowest ($P < 0.05$) MIC values when compared with the other extracts. This preliminary screening study indicated that araçá, rosemary and oregano are interesting alternatives as antimicrobial agents in food substrates, although further studies are needed to develop commercial formulations based on field trials.

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**Keywords**:

A. *Parasiticus*;
Antimicrobial Activity;
Plant extracts;
S. *aureus*

## Introduction

Microbial pathogens like certain bacteria and fungi species has been a major threat to human and animal health. The introduction of antimicrobial agents began in the mid-1930s, with increasing applications in the following years[1]. However, the

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excessive use of these compounds rapidly ended up selecting resistant strains that developed mechanisms for survival, making their combat using antimicrobials or biocides a complicated task.[2] In the food industry, antimicrobial agents are generally not permitted as additives in raw or processed products.[2] Thus, preventive measures to control the microbial contamination of foods are of particular importance to avoid foodborne infections. *Staphylococus aureus* and *Aspergillus parasiticus* are important causative agents of foodborne diseases and mycotoxin-producing in cereals,[3,4] respectively.

The possibility of using natural antimicrobial agents is an attractive alternative to control or reduce the bacterial and fungal load in food products, provided that they are biodegradable, environmental friendly, and biologically safe.[5-7] Previous studies indicated that essential oils and plant extracts containing secondary metabolites have antimicrobial properties similar to common antimicrobials.[6,8] However, information regarding the specific antimicrobial activity of major compounds found in plant extract is still scarce.[9,10] There are several categories of secondary metabolites with possible antimicrobial action in plant extracts, including terpenes, phenols and nitrogen compounds.[11] Additionally, the possibility of synergism between these molecules may increase the antimicrobial efficiency of several types of plants.[12]

The main types of plants that have been studied regarding their antimicrobial activity are those related to cooking, such as seasoning.[13,14] Among them, rosemary and oregano are the most investigated mainly because they are seasoning used in several culinary preparations worldwide, including in Brazil. The Brazilian native flora is very rich in diversity of species due to the presence of different regions and biomass in the country. A large number of Brazilian plants are popularly used as medicinal herbs, although the pharmacological bases of action for some plants are not completely understood. An example is the sweet passion fruit (*Passiflora alata*), which is used in several remedy preparations,[15] but it is little explored regarding its antimicrobial activity.[16] Araçá (*Psidium cattleianum*) is also empirically used for treatments of diarrhea, hemorrhages and cramps.[17] In this study, crude and lyophilized extracts of leaves from two Brazilian native plants with little information regarding their antimicrobial action, sweet passion fruit and araçá, and two seasonings already known in the literature, rosemary and oregano,[18,19] were evaluated *in vitro* regarding their antimicrobial effects on planktonic cells of S. *aureus* and A. *parasiticus*.

## Materials and Methods

### Plant Materials

Leaves were collected from three different types of family plants (Passifloraceae, Myrtaceae and Lamiaceae) during the summer and fall period of 2017 in the Southern region of Brazil. The species studied were *Passiflora alata* (sweet passion fruit), *Psidium cattleianum* (araçá), Rosmarinus *officinalis* (rosemary) and *Origanum vulgare* (oregano). Exsicate samples of each plant were sent to the São Paulo Institute of Botany for identification, and two of them were added to the Herbarium SP (P. *alata* SP499802 and P. *cattleianum* SP499801).

### Preparation of Extracts

Extracts were prepared according to recommendations of the Brazilian Pharmacopeia,[20] with some modifications. All leaves were dried, grinded and mixed (4 g) with 100 mL of ethyl alcohol (96 °GL). The mixture was incubated at 37 °C for 15 days, and manually shaken three times per day. The organic solvent was evaporated by fractional distillation under reduced pressure in an evaporator (Heidolph, Schwabach, Germany). Next, the aqueous extract from each plant was divided into 2 aliquots, one (crude extract) reserved for analysis of bioactive compounds by gas chromatography coupled to mass spectrometry (GC-MS) and evaluation of antimicrobial activity, and the other submitted to freeze-drying (lyophilized extract) before running the antimicrobial evaluation. The concentrations of dry matter in crude extracts of sweet passion fruit, araçá, rosemary and oregano were 28.3, 60.0, 32.2 and 6.5 mg/mL, respectively. The freeze-drying process started with freezing of extracts at -18 oC for 24 h. After this period, the sediment was placed into the lyophilizer (LC 1500 - Terroni Equipment Ltda., São Carlos, Brazil) under vacuum (DV-142N vacuum pump - JB Platinum, Aurora, IL, USA) for 24 h, with initial and final temperatures of -5.0 oC and 19.5 oC, respectively. For subsequent evaluations of antimicrobial activity, all the lyophilized extracts were re-dissolved in sterile distilled water at 40 mg/mL.

**Analysis of Bioactive Compounds**

Samples were analyzed in a GC-MS system (Shimadzu, Japan), equipped with a ZB-5HT column (30 m x 0.25 mm x 0.25 µm). GC-MS detection was conducted with ionization energy of 70 eV. Helium gas was used as the carrier gas at a constant flow rate of 1.8 mL/min. Injector, transfer line and ionization source temperatures were adjusted to 280 °C. The initial column temperature was kept at 60 °C for 1 min, then gradually increased to 280 °C at a rate of 10 °C/min and held for 35 min at 280 °C. Samples were injected manually into the GC-MS system in the split mode 2:1. Components were identified by matching their recorded mass spectra with those of standards from the National Institute of Standards and Technology libraries (NIST 14). The relative percentage of each component was calculated by peak areas, as percentages of total components.

**Evaluation of Antibacterial Activity**

The antibacterial activities of crude and lyophilized plant extracts were evaluated using a strain of S. *aureus* (ATCC 29213), previously cultured in Brain Heart Infusion broth (Oxoid, Basingstoke, UK) at 37 ± 0.5 °C for 24 h until reaching the turbidity of 0.5 on the McFarland scale. The minimum inhibitory concentration (MIC) of plant extracts was tested using the broth microdilution reference technique.[21] Fifty µL of each plant extract were mixed with 50 µl of saline solution (0.85%) in triplicate wells in 96-well polystyrene microplates, and successively diluted (1:2) until the 1:250 dilutions. Considering the initial concentrations of crude extracts, the concentration ranges for sweet passion fruit, araçá, rosemary and oregano were 14.16 to 0.11 mg/ml, 30 to 0.23 mg/ml, 16.12 to 0.13 mg/ml and 3.23 to 0.025 mg/ml, respectively. All lyophilized extracts had concentrations in the range of 20 to 0.16 mg/ml.

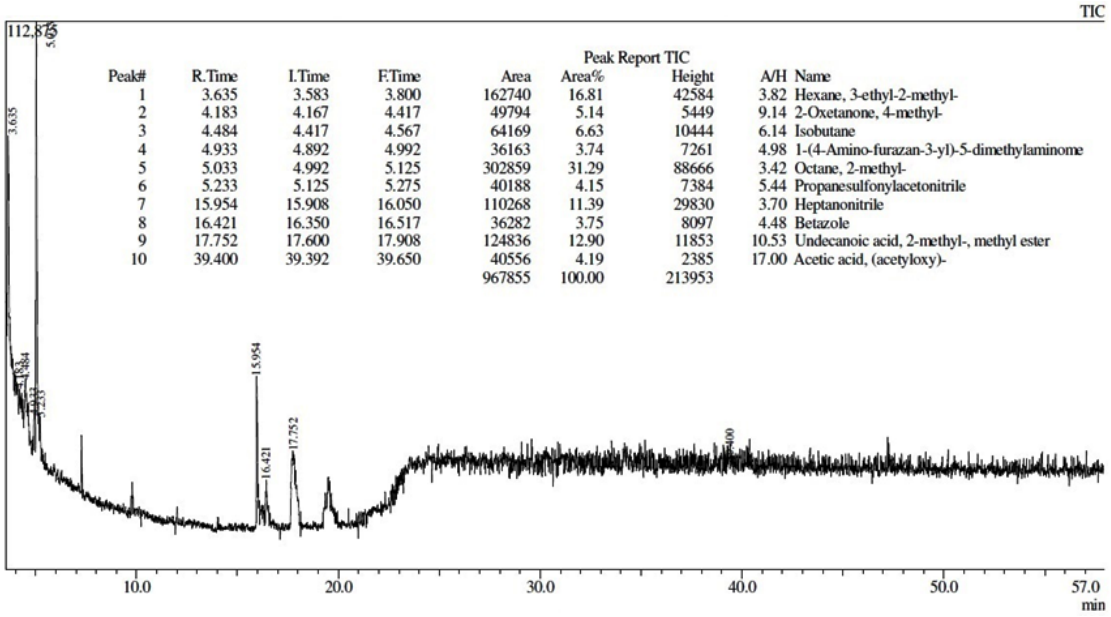
After the dilution procedure, 50 µl of Mueller-Hinton broth (Kasvi, São José dos Pinhais, Brazil) and 5 µl of the bacterial suspension were added in each well. Triplicate positive controls (only bacterium), negative controls (only extracts) and two antibiotic references (cephalexin and ciprofloxacin) were also prepared as previously described. The microplates were incubated statically at 35 °C for 24 hr. After this period, the turbidity in each well was visually inspected.[22] Ten µl of resazurin aqueous solution (0.01%) were added in each well, followed by a new incubation period at 37 °C for 2 hr to assess the cell viability and improve the visual inspection of wells.[23] The solutions that remained blue were considered as containing non-viable bacterial cells (no growth), and those which color changed to purple or pink were considered as displaying viable cells (bacterial growth).[24,25] All experiments were performed in triplicate. The minimal bactericidal concentration (MBC) of plat extracts was also determined by placing 5 µl aliquots from each well onto Tryptone Soy Agar plates (Oxoid, Basingstoke, UK). The lowest concentration showing no bacterial growth on agar medium was considered the MBC.

**Evaluation of Antifungal Activity**

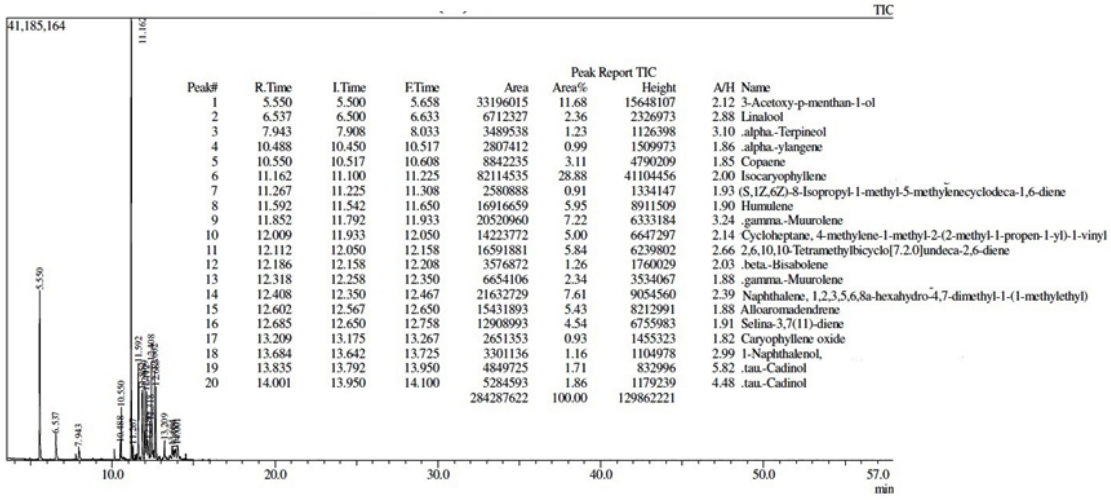
The MIC values of crude and lyophilized plant extracts were evaluated using a strain of A. *parasiticus* (NRRL 2999), according to the National Committee for Clinical Laboratory Standards.[26] Fungal inoculums were prepared on potato-dextrose agar and incubated at 35 °C for 7 days. A colony loop was suspended in 1 mL of saline solution (0.85%) containing 0.01 ml of Tween 20 (Sigma-Aldrich, St. Louis, MO), and the suspension was adjusted spectrophotometrically to 0.5 on the McFarland scale. Fifty µL of each plant extract were placed in triplicate wells in 96-well polystyrene microplates, mixed with 50 µl of RPMI-1640 medium (Inlab, São Paulo, Brazil) supplemented with MOPS [3-(N-morpholino propanesulfonic acid)] at final concentration of 0.165 mol/l[26], and successively diluted (1:2) until the 1:250 dilution. The dilutions and respective concentrations of crude and lyophilized plant extracts were the same as previously described for evaluation of antibacterial activity. Triplicate positive controls (only fungi), negative controls (only extracts) and one antifungal reference (Fluconazol) were also prepared.[27] The microplates were incubated statically at 35 °C for 50 hr. After this period, the turbidity in each well was visually inspected. Ten µL of resazurin aqueous solution (0.01%) were added in each well, and then the microplate was incubated for at 37 °C for 2 hr to assess the cell viability by visual inspection. All experiments were performed in triplicate. Additionally, the minimum fungicidal concentration (MFC) was assessed by adding 10 µl from each well onto Sabouraud -dextrose agar (Kasvi, São José dos Pinhais, Brazil). The lowest concentration showing no fungal growth on agar medium was considered the MFC.

**Statistical Analysis**

Data were analyzed by the MIXED procedure of Statistical Analyses System.[28] The model for MIC and MBC/MFC analysis considered each well of the microplates as an experimental unit. A Student t test was conducted to determine the differences between the mean values of four treatments (four plant extracts), the type of extracts (lyophilized and crude) and the interaction between treatment and extracts. Statistical significance was accepted at $P < 0.05$.




**A**

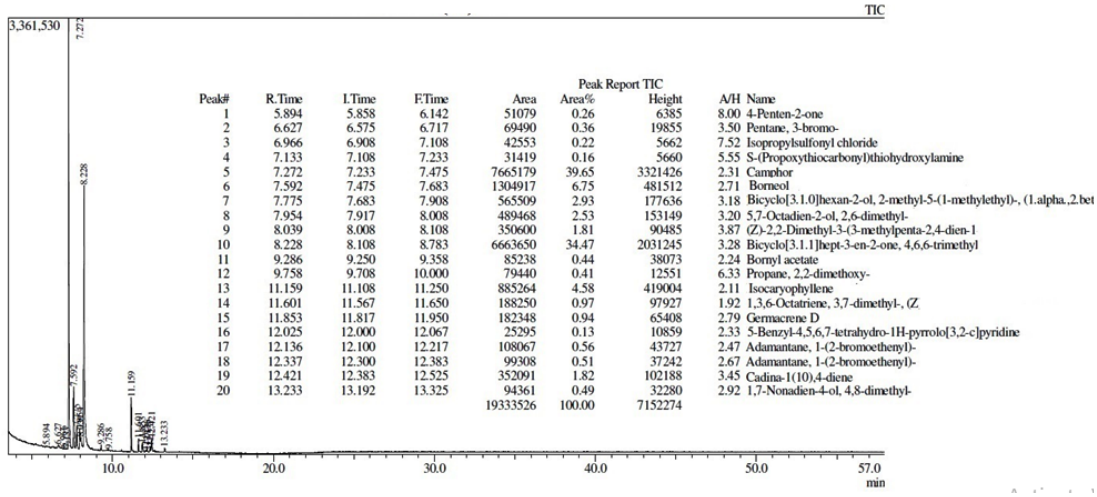



**B**

**Fig. 1: Chromatograms showing the major chemical compounds found in extracts from leaves of sweet passion fruit (A) and araçá (B)**

**Results**

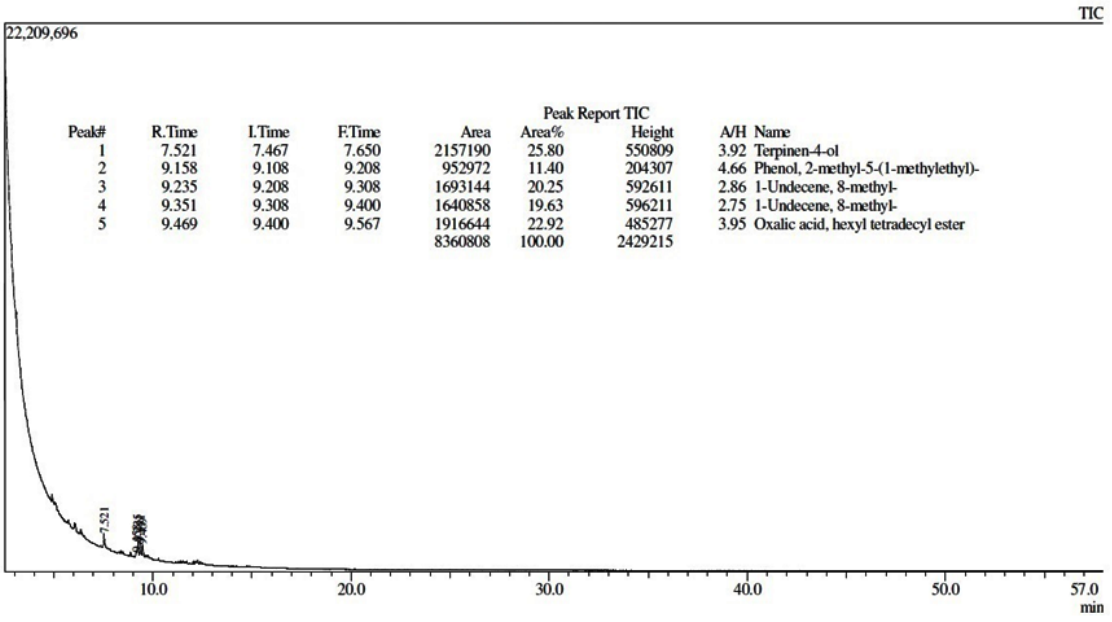
The major compounds found in the GC-MS analysis of crude extracts from each plant were compared with NIST 14 standards, and the results are described in the chromatograms of the Brazilian native plants (Fig. 1) and seasonings (Fig. 2). The majority of compounds found were classified as terpenoids, although some hydrocarbons have also been identified. The compound with highest percentage in sweet passion fruit (Fig. 1A) was the hydrocarbon isononane (31.3%), followed by 3-ethyl-2-methylhexane (16.8%), methyl-2-methylundecanoate (12.9%) and heptanenitrile (11.4%). The chromatograms of araçá extract (Fig. 1B) indicated that caryophyllene




Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | A/H | Name |
|---|---|---|---|---|---|---|---|---|
| 1 | 5.894 | 5.858 | 6.142 | 51079 | 0.26 | 6385 | 8.00 | 4-Penten-2-one |
| 2 | 6.627 | 6.575 | 6.717 | 69490 | 0.36 | 19855 | 3.50 | Pentane, 3-bromo- |
| 3 | 6.966 | 6.908 | 7.108 | 42553 | 0.22 | 5662 | 7.52 | Isopropylsulfonyl chloride |
| 4 | 7.133 | 7.108 | 7.233 | 31419 | 0.16 | 5660 | 5.55 | S-(Propoxythiocarbonyl)thiohydroxylamine |
| 5 | 7.272 | 7.233 | 7.475 | 7665179 | 39.65 | 3321426 | 2.31 | Camphor |
| 6 | 7.592 | 7.475 | 7.683 | 1304917 | 6.75 | 481512 | 2.71 | Borneol |
| 7 | 7.775 | 7.683 | 7.908 | 565509 | 2.93 | 177636 | 3.18 | Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.bet |
| 8 | 7.954 | 7.917 | 8.008 | 489468 | 2.53 | 153149 | 3.20 | 5,7-Octadien-2-ol, 2,6-dimethyl- |
| 9 | 8.039 | 8.008 | 8.108 | 350600 | 1.81 | 90485 | 3.87 | (Z)-2,2-Dimethyl-3-(3-methylpenta-2,4-dien-1 |
| 10 | 8.228 | 8.108 | 8.783 | 6663650 | 34.47 | 2031245 | 3.28 | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl |
| 11 | 9.286 | 9.250 | 9.358 | 85238 | 0.44 | 38073 | 2.24 | Bornyl acetate |
| 12 | 9.758 | 9.708 | 10.000 | 79440 | 0.41 | 12551 | 6.33 | Propane, 2,2-dimethoxy- |
| 13 | 11.159 | 11.108 | 11.250 | 885264 | 4.58 | 419004 | 2.11 | Isocaryophyllene |
| 14 | 11.601 | 11.567 | 11.650 | 188250 | 0.97 | 97927 | 1.92 | 1,3,6-Octatriene, 3,7-dimethyl-, (Z |
| 15 | 11.853 | 11.817 | 11.950 | 182348 | 0.94 | 65408 | 2.79 | Germacrene D |
| 16 | 12.025 | 12.000 | 12.067 | 25295 | 0.13 | 10859 | 2.33 | 5-Benzyl-4,5,6,7-tetrahydro-1H-pyrrolo[3,2-c]pyridine |
| 17 | 12.136 | 12.100 | 12.217 | 108067 | 0.56 | 43727 | 2.47 | Adamantane, 1-(2-bromoethenyl)- |
| 18 | 12.337 | 12.300 | 12.383 | 99308 | 0.51 | 37242 | 2.67 | Adamantane, 1-(2-bromoethenyl)- |
| 19 | 12.421 | 12.383 | 12.525 | 352091 | 1.82 | 102188 | 3.45 | Cadina-1(10),4-diene |
| 20 | 13.233 | 13.192 | 13.325 | 94361 | 0.49 | 32280 | 2.92 | 1,7-Nonadien-4-ol, 4,8-dimethyl- |
| | | | | 19333526 | 100.00 | 7152274 | | |

**A**




Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | A/H | Name |
|---|---|---|---|---|---|---|---|---|
| 1 | 7.521 | 7.467 | 7.650 | 2157190 | 25.80 | 550809 | 3.92 | Terpinen-4-ol |
| 2 | 9.158 | 9.108 | 9.208 | 952972 | 11.40 | 204307 | 4.66 | Phenol, 2-methyl-5-(1-methylethyl)- |
| 3 | 9.235 | 9.208 | 9.308 | 1693144 | 20.25 | 592611 | 2.86 | 1-Undecene, 8-methyl- |
| 4 | 9.351 | 9.308 | 9.400 | 1640858 | 19.63 | 596211 | 2.75 | 1-Undecene, 8-methyl- |
| 5 | 9.469 | 9.400 | 9.567 | 1916644 | 22.92 | 485277 | 3.95 | Oxalic acid, hexyl tetradecyl ester |
| | | | | 8360808 | 100.00 | 2429215 | | |

**B**

**Fig. 2: Chromatograms showing the major chemical compounds found in extracts from leaves of rosemary (A) and oregano (B).**

(28.9%) and eucalyptol (11.7%) are the main compounds, followed by naphthalene (7.6%), gamma muurolene (7.2%), humulene (5.9%), 2,6,10,10-tetramethylbicyclo [7.2.0] undeca-2,6-diene (5.8%), allo-aromadendrene (5.4%) and cycloheptane (5.0%). Rosemary extracts (Fig.1C) showed a predominance of camphor (39.7%), verbenone (34.5%) and borneol (6.7%), which are all terpenoid compounds. The major compounds in oregano extracts (Fig. 1D) were terpinen-4-ol (25.8%), oxalic acid, isohexyl neopentyl ester (22.9%), 8-methylundec-1-ene (20.3%) and carvacrol (11.4%).

Table 1 presents the *in vitro* antibacterial activities of crude and lyophilized extracts against S. *aureus* (ATCC 29213). The MIC or MBC values obtained in the broth microdilution technique are not absolute numbers, since each one of them represents an interval between two values. Therefore, the actual MIC or MBC values should be considered as a point between the lowest concentration that inhibits the micro-organism growth and the next lowest test concentration.[22] Extracts from sweet passion fruit had no antibacterial activity against the S. *aureus* strain tested, since its MIC and MBC values could not be determined ( >14.15 and > 20 mg/ml for crude and lyophilized extracts, respectively). However, crude and lyophilized extracts from leaves of araçá (P. *cattleianum*), rosemary (R. *officinalis*) and oregano (O. *vulgare*) inhibited S. *aureus*. Compared with other extracts, araçá had the lowest ($P < 0.05$) MIC or MBC values. In general, MIC or MBC values of crude and lyophilized extracts from the same plant were not different ($P > 0.05$), except for MIC of rosemary and MBC of araçá ($P < 0.05$).

**Table 1: Mean values of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of plant extracts against Staphylococcus aureus (ATCC 29213)**

| Plant Extract | MIC | MBC |
|---|---|---|
| Crude extracts | | |
| Sweet passion fruit (P. *alata*) | > 14.15 [a] | > 14.15 [a] |
| Araçá (P. *cattleianum*) | 0.39 [c] | 0.78 [c] |
| Rosemary (R. *officinalis*) | 0.84 [b] | 1.68 [b] |
| Oregano (O. *vulgare*) | 0.65 [bc] | 1.30 [bc] |
| Standard error | 0.12 | 0.24 |
| Lyophilized extracts | | |
| Sweet passion fruit (P. *alata*) | > 20.00 [a] | > 20.00 [a] |
| Araçá (P. *cattleianum*) | 0.45 [c] | 0.90 [b] |
| Rosemary (R. *officinalis*) | 0.57 [c] | 1.15 [b] |
| Oregano (O. *vulgare*) | 1.23 [b] | 1.84 [b] |
| Standard error | 0.11 | 0.30 |

[a-c] Mean values within each column with no common superscript differ significantly ($P < 0.05$).
MIC: minimum inhibitory concentration.
MBC: minimal bactericidal concentration.

The antifungal activities of the evaluated plant extracts against A. *parasiticus* are presented in table 2. MIC values could be determined only for crude and lyophilized extracts from araçá, crude extract from rosemary and lyophilized extract from oregano. However, MFC values could not be determined for any plant extract because all the dilutions presented fungal growth on the plates. Similarly to results obtained in the antibacterial assays, both types of extracts from araçá had lowest ($P < 0.05$) MIC values than rosemary, although the results did not differ ($P > 0.05$) from the lyophilized extract from oregano.

## Discussion

Several studies conducted with plant extracts and essential oils have demonstrated efficacy of some compounds with antimicrobial activities against bacteria[29–31] and fungi.[32–34] These compounds are secondary metabolites of plants with variations in their structure and chemical composition resulting in different antimicrobial effects.[35,36] There are three main categories of secondary metabolites: terpenes, phenolics and nitrogen.[11] They are classified according to their structure and main mechanisms of action. The GC-MS analysis revealed that the main compounds identified in the extracts of this study were eucalyptol, camphor and carvacrol (Fig. 1 and 2), which have reportedly antimicrobial and antioxidant actions.[13,37,38] In our study, ethanolic extraction was used to achieve an efficient and easy way of extraction, aiming future applications in food products. Moreover, according to previous studies, ethanolic extracts had higher antimicrobial activities than aqueous extracts,[39,40] possibly because of the higher ability of water-like polar solvents to extract the compounds, compared with water. This extraction methodology does not require much equipment, and

the technique is simpler when compared with other procedures such as extraction of essential oils.

All the plant extracts were separated in two types, crude and lyophilized, and their antimicrobial activities were evaluated separately. Results for crude or lyophilized extracts from sweet passion fruit (P. *alata*) were inconclusive, because the highest concentration tested had bacterial or fungal growth. Araçá (P. *cattleianum*) extracts, crude or lyophilized, demonstrated the higher antimicrobial activities against S. *aureus* (ATCC 29213) and A. *parasiticus* (NRRL 2999), with the lowest MIC found in the experiment. The antibacterial effects of lyophilized extracts from rosemary (R. *officinalis*) were similar to araçá extracts (Table 1), while the lyophilized extract from oregano (O. *vulgare*) had inhibitory effects against A. *parasiticus* (Table 1). The MBC values obtained for the 4 types of plants against S. *aureus* followed the same pattern as reported for MIC values. However, the antifungal activity was not expressive as the antibacterial effects.

According to Elisha,[41] there is no consensual classification about the effective MIC values for plant extracts. However, other researchers proposed MIC values of ≤ 0.5 mg/mL, 0.5 > MIC ≥ 1.5 mg/mL and > 1.5 mg/mL as indicative of strong, moderate and weak antimicrobial activities, respectively.[42] By using these criteria, both types of extracts from P. *cattleianum* could be considered as strong inhibitors for S. *aureus*, while R. *officinalis* and O. *vulgare* extracts would be classified as moderate inhibitors. Regarding the antifungal activity, all extracts were labeled as a weak inhibitor. Alternatively, others considered MIC values ≤ 1 mg/ml as satisfactory and promising.[43,30] Considering this parameter, the inhibitory effects results obtained with crude extracts from P. *cattleianum*, R. *officinalis* and O. *vulgare* were characterized as satisfactory against S. *aureus*. However, none of the plant extracts showed satisfactory results against A. *parasiticus*, according to the recommendations of other studies.[42,43,30]

In this study, none of the P. *alata* extracts had significant inhibitory activities against the micro-organisms tested. Passiflora genus is already explored for preparations of several phytochemicals including alkaloids, flavonoids, saponins, essential oils and carotenoids, as well as minerals, fibers and vitamins.[44,45] However, none of the predominant compounds found in the GC-MS analysis of this extracts was described in the literature as microbial inhibitors, which is consistent with the low antimicrobial activity described in this work. Our results differ who found antimicrobial activity of P. *alata* extracts against 27 different micro-organisms,[34] including S. *aureus* and A. flavus. The authors hypothesized that variable cropping conditions promote differences in bioactive compounds, leading to different concentrations of tannins and/or phenols. In a recent survey, produced four Passiflora species *in vitro* and identified, which variety of P. *alata* had saponins as major compounds in their leaves,[44] besides flavonoids. After testing the extracts against several Gram-positive and Gram-negative bacterial species, the authors found inhibitory effects against *Bacillus thuringiensis* and *Streptococcus pyogenes*, but no effect against S. *aureus*, similarly to our findings.

**Table 2: Mean values of minimal inhibitory concentration (MIC) of plant extracts against *Aspergillus parasiticus* (NRRL 2999)**

| Type of plant | Crude extract | Lyophilized extract |
|---|---|---|
| Sweet passion fruit (P. *alata*) | > 14.15 [a] | > 20 [a] |
| Araçá (P. *cattleianum*) | 3.125 [b] | 10 [b] |
| Rosemary (R. *officinalis*) | 16.12 [a] | > 20 [a] |
| Oregano (O. *vulgare*) | > 3.225 [b] | 8.33 [b] |
| Standard error | 0.44 | 0.31 |

[a-c] Mean values within each column with no common superscript differ significantly (P < 0.05).

P. *cattleianum* extracts were considered the most promising as antimicrobial agents, since they showed the lowest MIC results against S. *aureus* and A. *parasiticus*. In a similar study carried out by other researchers,[40] the ethanolic extract of P. *cattleianum* had a higher MIC value (3.125 mg/mL) against S. *aureus*. The extract presented tannins, flavonoids and terpenoids as major compounds, hence confirming that the antimicrobial action of P. *cattleianum* is associated with low molecular components in its extracts. Phenolic compounds are low molecular substances found in many plants that can inactivate essential enzymes and/or form complexes with metallic ions. In another studies[46] observed that P. *cattleianum* extracts with high contents of phenolic compounds had significant antimicrobial activity, which was attributed to the effects of those compounds on the bacterial cell membrane stability and on the respiratory mechanism. There were also identified several phenolic compounds in araçá fruits,[47] with the highest percentage (22.5%) of sesquiterpene caryophyllene. This compound was also identified in the araçá extract evaluated in the present study, comprising 28.9% of the total compounds. Therefore, the antimicrobial activity as demonstrated by the araçá extract in this study could be attributable to this compound, as wells as to a possible synergistic action among the major components found in the extract, as already proposed by other studies.[12]

R. *officinalis* and O. *vulgare* also showed interesting results against S. *aureus*, thus confirming their great potential for food applications, since they are worldwide used for cooking. A study comparing methanolic and aqueous extraction procedures for rosemary indicated important differences between the concentrations of active compounds in the extracts from this plant.[48] The authors observed that the inhibitory action of methanolic extract was greater than aqueous extract for the same plant. However, methanol is a toxic compound, which promotes a number of side effects such as mucosal and nervous system irritation, eye injuries, nausea, vomiting.[48] Our study demonstrated that ethanol is a suitable solvent choice for the extraction of major active compounds from rosemary. It was also observed that ethanolic extracts presented lower MIC values, when compared with the corresponding aqueous extracts.[18] Rosemary is a plant from the Lamiaceae family that presents camphor and verbenone as major components in its extract determined by GC/MS.[49,50] It was confirmed that these two compounds were responsible for the antibacterial effect[51] against S. *aureus* and Escherichia coli. In this study, we confirmed the action of these two compounds as main responsible for the antimicrobial action of rosemary. There were found the same composition of rosemary as described in our study, with possible variations of compounds according to the environment in which the plant is cultivated.[52] In a study with rosemary essential oil against A. *parasiticus* (NRRL2999), the main components found were piperitone, $\alpha$-pinene, limonene and 1.8-cineole.[53] These findings might explain the differences in the antifungal MIC value obtained in the present study (16.12 mg/mL) and that reported by the others as1.75 mg/ml.[53] Moreover, although a fungistatic effect was observed, both studies had no fungicidal activity on the strain studied. The mechanism of antibacterial action of rosemary extracts has also been associated with a decrease in cytoplasmic pH ($pH_{int}$) and cell wall disruption in Gram-positive and Gram-negative strains.[18] Therefore, the reduction in the internal pHint caused by compounds such as thymol and carvacrol is also a strong indicative of antibacterial activity.[54] Carvacrol, which is classified as a phenolic compound, was the major component found in oregano essential oil.[19,55]

In our study, oregano extracts had large concentrations of monoterpenes, but the proportion of carvacrol (11.4%) was relatively small when compared with the other compounds. This may be one of the possible explanations for the relative low antibacterial effect of oregano extract found in this work. However, the major compound identified in the oregano extracts was terpinen-4-ol (25.8%), which has been reported56 as responsible for the inhibitory action of oregano extracts against S. *aureus* ATCC 29213, the same strain tested in the present study. In a study performed by Barra *et al.*,[57] terpinen-4-ol also showed good activity against A. flavus, which is consistent with our results showing the activity of oregano extract on A. *parasiticus*, although the inhibitory concentrations were neither satisfactory nor promising. There was also described good fungistatic activity using oregano extracts,[33] hence indicating the possible use of oregano extracts in food preservation systems with the aim of eliminating

or preventing the growth of micro-organisms. Oregano is already a seasoning widely used in Brazilian cuisine, which helps in its acceptance with this objective.

**Conclusion**

In the present study, the seasonings plants studied showed some antimicrobial action as expected. Sweet passion fruit showed no action against any of the micro-organisms tested. However, crude and lyophilized extracts from Araçá had the highest antimicrobial effects against two microorganisms of public health importance, S. *aureus* and A. *parasiticus*. This preliminary screening study indicated that araçá, rosemary and oregano are interesting alternatives as antimicrobial agents in food substrates, although further studies are needed to develop commercial formulations based on field trials.

**Conflict of Interest**

The authors declare they have no conflicts of interest.

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