

# Bioactive Hexane Extracts from *Piper aduncum* and *Xylopia aromatica* Against Bacterial Strains which Cause Food Poisoning

## Extratos Hexânicos Bioativos de *Piper aduncum* e *Xylopia aromatica* Contra Cepas Bacterianas que Causam Intoxicação Alimentar

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Plants and their extracts have been used as natural antimicrobial agents in food products as a strategy to mitigate food contamination. Therefore, this study aimed to determine the chemical composition of hexane extracts from *Piper aduncum* leaves and inflorescences (HE-PL and HE-PI, respectively) and from *Xylopia aromatica* leaves (HE-XA) by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID). Antibacterial activities of HE-PL, HE-PI and HE-XA were evaluated in terms of their minimum inhibitory concentration (MIC = µg/mL) and minimum bactericidal concentration (MBC = µg/mL) values by the broth microdilution method in 96-well microplates. Major constituents found in HE-PL were germacrene B (33.1%), valencene (14.5%), β-cadinene (9.1%) and α-humulene (8.1%) while the ones found in HE-PI were sesquiterpenes caryophyllene oxide (20.5%), spathulenol (15.2%) and germacrene B (9.7%). Spathulenol (41.2%), α-copaene (16.6%) and β-phellandrene (7.9%) were the major constituents in HE-XA. Since the extracts under study exhibited excellent MIC and MBC values, they showed high bioactive potential against *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli*, *Brochothrix thermosphacta*, *Clostridium botulinum* and *Pseudomonas fluorescens*. Besides, this study showed, for the first time, that both *P. aduncum* and *X. aromatica* have antimicrobial properties against these microorganisms, a fact that reveals their biotechnological potential in food preservation.

**Keywords:** Natural bactericide; non-polar extracts; monkey pepper; food spoilage

## 1. Introduction

Since foodborne illnesses have struck several people worldwide, strategies must be developed to mitigate and prevent them.<sup>1</sup> They result from intake of food contaminated with pathogenic microorganisms, harmful agents, chemical compounds and toxic compounds naturally found in food and have led to hospitalization, deaths and irreversible complications.<sup>1</sup>

Epidemiological studies carried out in Europe and North America identified the main etiological agents of foodborne toxoinfections: *Salmonella* spp, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, and *Escherichia coli*.<sup>2</sup> Thus, preventing the illnesses is fundamental, since they represent one of the main causes of death and loss of productivity worldwide.<sup>2</sup> The following bacteria are some pathogens that may be involved in toxoinfections and disorders related to the use of contaminated food: *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli*, *Brochothrix thermosphacta*, *Clostridium botulinum*, and *Pseudomonas fluorescens*. They were the focus of this study.

Studies of application of plants with pharmacological properties to the development of new pharmaceuticals and products for food industries have broadened their fields and encouraged researchers to discover such properties in plants that have yet to be investigated. In this respect, the search for new knowledge in the area of natural products has increased. Plants are rich in bioactive secondary metabolites (tannins, terpenoids, alkaloids, coumarins, iridoids, lignans, steroids, saponins, xanthenes and flavonoids) which exhibit important antimicrobial activity against several pathogenic microorganisms.<sup>3</sup> Another fact that justifies constant search for new antimicrobial agents extracted from plants is microbial resistance to synthetic antimicrobials that have already been used commercially.<sup>4</sup>

Plants targeted by this chemical and biological study were *Piper aduncum* (Piperaceae) and *Xylopia aromatica* (Annonaceae). The former has been highlighted in the field of chemistry

and biotechnological prospection because it can produce essential oils and bioactive extracts that may be successfully applied to both health and agrochemical areas.<sup>5</sup> The latter also stands out for being rich in active metabolites and several activities, such as cytotoxic, trypanocidal, antimalarial, anti-obesity, anti-inflammatory and antimicrobial ones, which the literature has already reported.<sup>6</sup>

This study aims to continue the work carried out by the research group that investigates species that exhibit antimicrobial activity against foodborne pathogens and spoilage bacteria.<sup>7</sup> For the first time, we describe *in vitro* activities exhibited by hexane extracts from *P. aduncum* and *X. aromatica* against foodborne pathogens and spoilage bacteria and their chemical characterization.

## 2. Experimental

### 2.1. Plant material

*Piper aduncum* leaves and inflorescences and *X. aromatica* leaves were collected on August 14th, 2021, at 9 am, on the campus of the Universidade de Rio Verde (UniRV) in Rio Verde, Goiás (GO), Brazil (17°47'22.776"S and 50°57'56.894"W). Plants were identified by the botanist Luzia Francisca de Souza and a sample was deposited at the Herbarium Jataiense Professor Germano Guarim Neto at exsiccate numbers HJ 7872 (*P. aduncum*) and HJ 7873 (*X. aromatica*). Finally, they were ground, placed into a sealed container and stored in a refrigerator up to the preparation of crude hexane extracts (HE-PL, HE-PI and HE-XA). Access to the botanical material was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) under the code AEACDCA.

### 2.2 Preparation of hexane extracts (HE-PL, HE-PI and HE-XA)

*Piper aduncum* leaves (300 g) and inflorescences (300 g) and *X. aromatica* leaves (300 g) were air-dried and milled by a Wiley mill. Subsequently, they were exhaustively cold-extracted with hexane. Every resulting extract was filtered and concentrated under reduced pressure. Finally, 5.0 g crude hexane extract from *P. aduncum* leaves (HE-PL), 4.3 g from *P. aduncum* inflorescences (HE-PI) and 5.6 g from *X. aromatica* leaves (HE-XA) were obtained.

### 2.3. Chemical identification of HE-PL, HE-PI and HE-XA constituents

HE-PL, HE-PI and HE-XA were dissolved in ethyl ether and analyzed by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) with the use of Shimadzu QP5000 Plus and GCMS2010 Plus (Shimadzu Corporation,

Kyoto, Japan) systems. The temperature of the column in GC-FID was programmed to rise from 60 to 240 °C at 3 °C/min and was held at 240°C for 5 min; the carrier gas was H<sub>2</sub> at the flow rate of 1.0 mL/min. The equipment was set to operate in the injection mode; the injection volume was 0.1 µL (split ratio of 1:10) while injector and detector temperatures were 240 and 280°C, respectively. Relative concentrations of components were reached by normalizing peak areas (%). Relative areas consisted of the average of triplicate GC-FID analyses. GC-MS conditions and identification have been previously reported.<sup>8</sup> Identification of volatile components in HE-PL, HE-PI and HE-XA (Tables 1 and 2) was based on their retention indices on an Rtx-5MS (30 m × 0.25 mm; 0.250 µm) capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>20</sub>). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 and their fragmentation patterns were compared with literature data.<sup>9</sup>

### 2.4. Antibacterial activity of HE-PL, HE-PI and HE-XA: MIC and MBC determination

HE-PL, HE-PI and HE-XA were assayed against six bacterial strains that were provided by the American Type Culture Collection (ATCC): *Bacillus cereus* (ATCC 10987), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Brochothrix thermosphacta* (ATCC 11509), *Clostridium botulinum* (ATCC 19397) and *Pseudomonas fluorescens* (ATCC 17397). Minimum inhibitory concentration (MIC), which is defined as the lowest concentration of a sample that can inhibit bacterial growth, was determined by microdilution on 96-well microplates in triplicate.<sup>10</sup> HE-PL, HE-PI and HE-XA were assayed at concentrations ranging from 20 to 400 µg/mL. Positive controls were penicillin and streptomycin (both from Sigma-Aldrich, St. Louis, MO, USA) for Gram-positive and Gram-negative bacteria, respectively, at concentrations ranging from 0.0115 to 5.9 µg/mL. Final dimethylsulfoxide (DMSO) content in the sample was 5% (v/v); the same concentration was employed as the negative control. Inoculated wells containing the microorganism were only included to control bacterial growth. Noninoculated wells (without any microorganisms) were also employed to ensure broth sterility. Inoculum cell concentration was adjusted to 5 × 10<sup>5</sup> colony-forming unit (CFUs)/mL on the basis of the absorbance read at 625 nm by a Nanodrop spectrophotometer (Thermo Scientific). Bacteria were incubated at 37°C under aerobic conditions for 20 h. Resazurin (0.02%; Sigma-Aldrich) was employed to detect bacterial growth.<sup>11</sup> The methodology described by Leandro *et al.*,<sup>12</sup> for aerobic bacteria was used for determining minimum bactericidal concentration (MBC), which was defined as the lowest concentration of a sample at which there was no bacterial growth.

### 3. Results and Discussion

#### 3.1. Chemical composition

Chemical compositions of HE-PL and HE-PI were identified by GC-MS and quantified by GC-FID. Major constituents found in hexane extract from leaves were germacrene B (33.1%), valencene (14.5%),  $\beta$ -cadinene (9.1%),  $\alpha$ -humulene (8.1%) and  $\beta$ -cubebene (6.5%) (Table 1). Hexane extract from inflorescences exhibited the following constituents at high concentrations: caryophyllene oxide (20.5%), spathulenol (15.2%), germacrene B (9.7%), valencene (6.4%) and  $\alpha$ -cubebene (8.2%) (Table 1).

The analysis of studies of hexane extracts from *P. aduncum* leaves showed that there are few reports

in the literature. No study of hexane extracts from its inflorescences was found. Both facts reinforce the novelty of this study. On the other hand, *P. aduncum* has had its bioactive essential oils deeply investigated.<sup>13,25-26</sup>

Hexane extract from *P. aduncum* leaves exhibits several constituents, such as apiol (90.7%),  $\beta$ -caryophyllene (3.6%),  $\beta$ -selinene (1.5%) and myristicin (1.7%).<sup>14</sup> The authors highlighted the chemical importance of extracts from *P. aduncum* in agriculture, mainly against *Anticarsia gemmatilis* and *Spodoptera frugiperda*. Another study was carried out by Santos *et al.*,<sup>15</sup> who stated that the extracts are predominantly composed of sesquiterpenes (caryophyllene,  $\alpha$ -calacorene,  $\gamma$ -elemene, *cis*- $\gamma$ -cadinene, germacrene D, linalool oxide, nerolidol,  $\beta$ -elemene,  $\delta$ -cadinene and  $\alpha$ -caryophyllene). Monoterpene (linalool) and fatty alcohol (falcariol) were also characterized as their main components.

**Table 1.** Volatile composition of hexane extracts from *P. aduncum* leaves (HE-PL) and inflorescences (HE-PI)

| Compound                   | RT (min) | RI <sub>exp</sub> | RI <sub>lit</sub> | % RA        |             |
|----------------------------|----------|-------------------|-------------------|-------------|-------------|
|                            |          |                   |                   | HE-PL       | HE-PI       |
| $\alpha$ -Cubebene         | 33.57    | 1350              | 1351              | 0.9         | 8.2         |
| $\beta$ -Elemene           | 34.59    | 1373              | 1375              | 1.9         | 2.3         |
| Isoledene                  | 34.76    | 1376              | 1377              | 0.5         | —           |
| $\beta$ -Cubebene          | 35.33    | 1389              | 1390              | 6.8         | —           |
| $\beta$ -Caryophyllene     | 36.43    | 1416              | 1418              | —           | 3.7         |
| $\gamma$ -Elemene          | 36.78    | 1425              | 1423              | 5.0         | —           |
| Aromadendrene              | 37.10    | 1438              | 1439              | 2.1         | 1.0         |
| $\alpha$ -Selinene         | 37.71    | 1449              | 1451              | —           | 2.6         |
| $\alpha$ -Humulene         | 37.94    | 1455              | 1455              | <b>8.1</b>  | —           |
| Alloaromadendrene          | 38.12    | 1459              | 1460              | —           | 6.0         |
| $\beta$ -Cadinene          | 38.58    | 1471              | 1472              | <b>9.1</b>  | 5.2         |
| $\gamma$ -Muurolene        | 38.76    | 1475              | 1476              | 1.3         | 1.0         |
| $\beta$ -Selinene          | 38.88    | 1483              | 1485              | 6.5         | —           |
| Valencene                  | 39.38    | 1491              | 1491              | <b>14.5</b> | 6.4         |
| Bicyclogermacrene          | 39.56    | 1493              | 1493              | 2.9         | 3.5         |
| $\alpha$ -Cadinene         | 41.15    | 1537              | 1538              | 1.0         | —           |
| Germacrene B               | 41.95    | 1559              | 1560              | <b>33.1</b> | <b>9.7</b>  |
| Spathulenol                | 42.48    | 1574              | 1575              | —           | <b>15.2</b> |
| Isoaromadendrene oxide     | 42.71    | 1578              | 1579              | —           | 2.0         |
| Caryophyllene oxide        | 42.89    | 1583              | 1581              | 0.8         | <b>20.5</b> |
| Viridiflorol               | 43.16    | 1589              | 1590              | 0.6         | —           |
| Humulene epoxide           | 43.93    | 1610              | 1609              | —           | 0.9         |
| Isospathulenol             | 44.23    | 1630              | 1631              | 1.8         | —           |
| $\alpha$ -Muurolol         | 45.19    | 1645              | 1645              | —           | 1.1         |
| Aromadendrene oxide        | 45.91    | 1667              | 1668              | —           | 1.8         |
| Alloaromadendrene oxide    | 47.03    | 1696              | 1697              | —           | 0.7         |
| Sesquiterpene hydrocarbons |          |                   |                   | 93.7        | 49.6        |
| Oxygenated sesquiterpenes  |          |                   |                   | 3.2         | 42.2        |
| <b>Total</b>               |          |                   |                   | <b>96.9</b> | <b>91.8</b> |

RT = Retention time; RI<sub>exp</sub> = Retention index relative to *n*-alkanes (C<sub>8</sub>–C<sub>20</sub>) on the Rtx-5MS column; RI<sub>lit</sub> = Kovats retention index (values from the literature<sup>9</sup>). %RA = Relative abundance. Bold numbers mean that the volatile constituents were considered major constituents of the extracts.

In addition, a recent study described the larvicidal potential of hexane, dichloromethane and ethanolic extracts from *P. aduncum* dry leaves against *A. aegypti* larvae.<sup>27</sup>

Chemical composition of hexane extract from *X. aromatica* leaves (HE-XA) was also determined by GC-MS and GC-FID. Only three major constituents were identified in it: spathulenol (41.2%),  $\alpha$ -copaene (16.6%) and  $\beta$ -phellandrene (7.9%) (Table 2).

Researchers who previously studied extracts from the species *X. aromatica* reported low concentration of kaurene-type diterpenes by comparison with other species that belong to the same genus.<sup>16</sup> A recent study of hexane extracts from *X. aromatica* leaves identified different classes of compounds, such as phenolic acids, flavonoids and alkaloids, by HPLC-DAD.<sup>17</sup> The authors attributed the promising biological potential of *X. aromatica* to those compounds. Another recent study reinforces its antimicrobial, antimalarial and antitumoral potential.<sup>18</sup>

Essential oils from both *P. aduncum* and *X. aromatica* have been investigated and reported in the literature. Regarding its volatile constituents, it should be highlighted that the chemical composition of HE-XA is similar to the one of essential oils from leaves of this species.<sup>19</sup> It confirms that HE-XA exhibits volatile constituents which are also found in the essential oil, a fact that may be explained by the low polarity of their molecules.

### 3.2. Antibacterial activity

HE-PL, HE-PI and HE-XA proved to be highly promising against all target bacteria investigated by this study. Biological analyses showed excellent values of MIC and MBC. The literature reinforces that natural products with MIC values between 10 and 100  $\mu\text{g/mL}$  are considered

the ones that exhibit good activity while values between 100 and 500  $\mu\text{g/mL}$  refer to the ones with moderate activity.<sup>7</sup>

HE-PL exhibited better antibacterial activity against *Pseudomonas fluorescens* and *Bacillus cereus* with MIC and MBC values below 100  $\mu\text{g/mL}$  (Table 3) and moderate activity against the other bacteria under investigation. HE-PI showed good antibacterial activity against all bacteria under study since MIC and MBC values were below 100  $\mu\text{g/mL}$  (Table 3). HE-XA also exhibited satisfactory antibacterial activity against all bacteria with MIC and MBC values below 100  $\mu\text{g/mL}$  (Table 3).

It should be highlighted that this study is the first one to evaluate antibacterial activity of HE-PL, HE-PI and HE-XA against *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli*, *Brochothrix thermosphacta*, *Clostridium botulinum* and *Pseudomonas fluorescens*. A recent review reported that crude extract from *P. aduncum* leaves exhibited significant antibacterial activity against *Bacillus subtilis* and *Micrococcus luteus*. In addition, it also showed *in vitro* inhibitory activity against *Mycobacterium intracellulare* since the MIC value is above 100  $\mu\text{g/mL}$ . It also exhibited moderate/poor antimicrobial activity against *E. coli* and *S. aureus*.<sup>20</sup>

Good antibacterial activity of HE-PL against *Pseudomonas fluorescens* and *Bacillus cereus* and its moderate activity against the other bacteria may be explained by the chemical constituent germacrene B. A high content of the sesquiterpene germacrene B was already identified in essential oil from *Parentucellia latifolia* and results showed good antibacterial activity.<sup>21</sup> Besides, HE-PI and HE-XA exhibited good antibacterial activity against all bacteria under evaluation, a fact that may be explained by high concentrations of spathulenol, caryophyllene oxide and  $\alpha$ -copaene found in the extracts. Spathulenol has already

**Table 2.** Volatile composition of hexane extracts from *X. aromatica* leaves (HE-XA)

| Compound                          | RT (min) | RI <sub>exp</sub> | RI <sub>lit</sub> | % RA        |
|-----------------------------------|----------|-------------------|-------------------|-------------|
| $\alpha$ -Pinene                  | 10.08    | 938               | 939               | 5.9         |
| $\beta$ -Pinene                   | 12.80    | 979               | 980               | 5.3         |
| $\beta$ -Phellandrene             | 15.96    | 1030              | 1031              | <b>7.9</b>  |
| $\alpha$ -Cubebene                | 33.49    | 1349              | 1351              | 1.8         |
| $\alpha$ -Copaene                 | 34.57    | 1375              | 1376              | <b>16.6</b> |
| $\alpha$ -Gurjunene               | 36.11    | 1408              | 1409              | 6.0         |
| Aromadendrene                     | 37.21    | 1438              | 1439              | 4.6         |
| Alloaromadendrene                 | 38.11    | 1459              | 1460              | 3.2         |
| Germacrene D                      | 38.89    | 1478              | 1480              | 4.2         |
| Bicyclogermacrene                 | 39.42    | 1492              | 1493              | 3.2         |
| Spathulenol                       | 42.47    | 1575              | 1575              | <b>41.2</b> |
| <i>Sesquiterpene hydrocarbons</i> |          |                   |                   | 58.8        |
| <i>Oxygenated sesquiterpenes</i>  |          |                   |                   | 41.2        |
| <b>Total</b>                      |          |                   |                   | <b>100</b>  |

RT = Retention time; RI<sub>exp</sub> = Retention index relative to *n*-alkanes (C<sub>8</sub>–C<sub>20</sub>) on the Rtx-5MS column; RI<sub>lit</sub> = Kovats retention index (values from the literature<sup>9</sup>). % RA = Relative abundance. Bold numbers mean that the volatile constituents were considered major constituents of HE-XA.

**Table 3.** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of HE-PL, HE-PI, HE-XA and positive controls (Penicillin; Streptomycin), expressed as µg/mL, against foodborne pathogenic bacteria and food spoilage bacteria

| Bacteria                             | MIC and MBC (µg/mL) |           |           |            |              |
|--------------------------------------|---------------------|-----------|-----------|------------|--------------|
|                                      | HE-PL               | HE-PI     | HE-XA     | Penicillin | Streptomycin |
| <i>B. cereus</i> <sup>a</sup>        | 62.5/62.5           | 50/50     | 62.5/62.5 | 5.9/5.9    | Not rated    |
| <i>S. typhimurium</i> <sup>b</sup>   | 100/100             | 62.5/62.5 | 20/20     | Not rated  | 1.25/1.25    |
| <i>E. coli</i> <sup>b</sup>          | 400/400             | 20/20     | 62.5/62.5 | Not rated  | 5.9/5.9      |
| <i>B. thermosphacta</i> <sup>a</sup> | 150/150             | 62.5/62.5 | 50/50     | 1.47/1.47  | Not rated    |
| <i>C. botulinum</i> <sup>a</sup>     | 200/200             | 32.5/32.5 | 32.5/32.5 | 5.9/5.9    | Not rated    |
| <i>P. fluorescens</i> <sup>b</sup>   | 50/50               | 50/50     | 50/50     | Not rated  | 1.25/1.25    |

<sup>a</sup>Gram-positive bacteria; <sup>b</sup>Gram-negative bacteria; Positive controls: Penicillin and Streptomycin; HE-PL: hexane extract from *P. aduncum* leaves; HE-PI: hexane extract from *P. aduncum* inflorescences; HE-XA: hexane extract from *X. aromatica* leaves.

been isolated from *Azorella compacta* and used in assays of antibacterial (MIC) and bactericidal (MBC) activities with promising results.<sup>22</sup> Essential oil from *S. hydrangea* flowers, which is rich in caryophyllene oxide, exhibited potential activity against different bacteria; thus, its biological activity may be due to the chemical constituent.<sup>23</sup> Finally, the chemical constituent  $\alpha$ -copaene has been widely found in oils extracted from different plant species; the interesting fact is that all oils that have  $\alpha$ -copaene exhibit antibacterial activity. It suggests that copaene (either individually or synergically with other chemical constituents) may be responsible for such good activity.<sup>24</sup>

## 4. Conclusions

Hexane extracts from *P. aduncum* (leaves and inflorescences) and *X. aromatica* (leaves) were highly active against bacteria under investigation with excellent MIC and MBC values. Both HE-PI and HE-XA exhibited high antibacterial activity against a larger spectra of bacteria than HE-PL. Constituents found at the highest concentrations in HE-PL and HE-PI were germacrene B and caryophyllene oxide, respectively, while spathulenol was the sesquiterpene found at the highest concentration in HE-XA. Even though results of this study reinforce and confirm the *in vitro* biological potential of *P. aduncum* and *X. aromatica*, further *in vivo* studies are needed to support their popular use against severe infections caused by intake of food contaminated with pathogenic microorganisms.

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## References

1. Abu-Zaid, A. A.; Al-Barty, A.; Morsy, K.; Hamdi, H.; *In vitro* study of antimicrobial activity of some plants seeds against bacterial strains causing food poisoning diseases. *Brazilian Journal of Biology* **2022**, 82, e256409. [[Crossref](#)] [[PubMed](#)]
2. Mostafa, A. A.; Al-Askar, A. A.; Almaary, K. S.; Dawoud, T. M.; Sholkamy, E. N.; Bakri, M. M.; Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences* **2018**, 25, 361. [[Crossref](#)] [[PubMed](#)]
3. Atwaa, E. S. H.; Shahein, M. R.; Radwan, H. A.; Mohammed, N. S.; Aloraini, M. A.; Albezrah, N. K. A.; Alharbi, M. A.; Sayed, H. H.; Daoud, M. A.; Elmahallawy, E. K.; Antimicrobial activity of some plant extracts and their applications in homemade tomato paste and pasteurized cow milk as natural preservatives. *Fermentation* **2022**, 8, 428. [[Crossref](#)]
4. Silva, M. G.; Nunan, E. A.; Rocha, M. P.; Estudo químico e de atividade antibacteriana de extratos e óleo essencial de *Citrus x limonia*. *Brazilian Journal of Health and Pharmacy* **2019**, 1, 11. [[Link](#)]
5. Rodrigues, D. W.; Oliveira, J. A. S.; Óleos essenciais de *Piper* L. (Piperaceae) e sua aplicação biotecnológica na agricultura: uma revisão da literatura. *Arquivos do Mundi* **2021**, 25, 100. [[Link](#)]
6. Junqueira, J. G. M.; Nascimento, M. N. G.; Costa, L. G.; Romualdo, L. L.; Aquino, F. W. B.; Abubakar, M. N.; Terezan, A. P.; Cunha, G. O. S.; Severino, V. G. P.; *In vivo* and *in vitro* volatile constituents of the flowers of *Xylopia aromatica* by HS-SPME/GC-MS. *Journal of the Brazilian Chemical Society* **2021**, 32, 1111. [[Crossref](#)]
7. Dias, A. L. B.; Fernandes, C. C.; Souza, J. H.; Martins, C. H. G.; Moreira, F. F.; Crotti, A. E. M.; Miranda, M. L. D.; Antibacterial activity of essential oils from Brazilian plants and their major constituents against foodborne pathogens and spoilage bacteria. *Journal of Essential Oil Research* **2022**, 34, 195. [[Crossref](#)]
8. Cabral, F. V.; Fernandes, C. C.; Dias, A. L. B.; Ribeiro, A. B.; Squarisi, I. S.; Tavares, D. C.; Crotti, A. E. M.; Moreira, F. F.; Miranda, M. L. D.; Hexane extract from *Tradescantia pallida* (Rose) D. R. Hunt (Commelinaceae): its volatile constituents and *in vitro* antifungal and cytotoxic activities. *Brazilian Archives of Biology and Technology* **2022**, 65, e22210621. [[Crossref](#)]
9. Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, Illinois, USA: Allured Publishing Corporation.
10. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria

- That Grow Aerobically. Approved standard M7-A9. Wayne, PA: CLSI, 2012; 32.
11. Sarker, S. D.; Nahar, L.; Kumarasamy, Y.; Microtitre platebased antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* **2007**, *42*, 321. [[Crossref](#)] [[PubMed](#)]
12. Leandro, L. F.; Moraes, T. S.; Oliveira, P. F.; Alves, J. M.; Senedese, J. M.; Ozelin, S. D.; Resende, F. A.; Grandis, R. A.; Varanda, E. A.; Bastos, J. K.; Tavares, D. C.; Martins, C. H. G.; Assessment of the antibacterial, cytotoxic and mutagenic potential of the phenolic-rich hydroalcoholic extract from *Copaifera trapezifolia* Hayne leaves. *Journal of Medical Microbiology* **2016**, *65*, 937. [[Crossref](#)] [[PubMed](#)]
13. Monzote, L.; Scull, R.; Cos, P.; Setzer, W. N.; Essential oil from *Piper aduncum*: chemical analysis, antimicrobial assessment, and literature review. *Medicines* **2017**, *4*, 49. [[Crossref](#)] [[PubMed](#)]
14. Lucena, D. C.; Vargas, L. R. B.; Silva, W. C.; Machado, A. F.; Lopes, T. S.; Moura, S.; Barros, N. M.; Biological activity of *Piper aduncum* extracts on *Anticarsia gemmatilis* (Hubner) (Lepidoptera: Erebididae) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Anais da Academia Brasileira de Ciências* **2017**, *89*, 1869. [[Crossref](#)] [[PubMed](#)]
15. Santos, M. L.; Magalhães, C. F.; Rosa, M. B.; Santos, D. A.; Brasileiro, B. G.; Carvalho, L. M.; Silva, M. B.; Zani, C. L.; Siqueira, E. P.; Peres, R. L.; Andrade, A. A.; Antifungal activity of extracts from *Piper aduncum* leaves prepared by different solvents and extraction techniques against dermatophytes *Trichophyton rubrum* and *Trichophyton interdigitale*. *Brazilian Journal of Microbiology* **2013**, *44*, 1275. [[Crossref](#)]
16. Melo, A. C.; Cota, B. B.; Oliveira, A. B.; Braga, F. C.; HPLC quantitation of kaurane diterpenes in *Xylopia* species. *Fitoterapia* **2001**, *72*, 40. [[Crossref](#)] [[Crossref](#)]
17. Gomes, I. N. F.; Silva, A. G.; Lima, G. F. F.; Longatti, T. R.; Carmo, L. F.; Villar, J. A. F. P.; Araujo, A. A. C.; Tome, R. G.; Santos, H. B.; Ribeiro, R. I. M. A.; Alkaloid and phenolic compounds of *Xylopia aromatica* inhibits tumor growth down-regulation matrix metalloproteinase-2 (MMP-2) expression. *Pakistan Journal of Pharmaceutical Sciences* **2021**, *34*, 599. [[Crossref](#)] [[PubMed](#)]
18. Boni, T. S.; Azevedo, J. P.; Rodriguez, A. O.; Maltoni, K. L.; *Xylopia aromatica*: crescimento inicial e status nutricional de mudas em solo degradado condicionado com resíduos. *Research, Society and Development* **2022**, *11*, e59211628582. [[Crossref](#)]
19. Silva, L. E.; Reis, R. A.; Moura, E. A.; Amaral, W.; Sousa Jr, P. T.; Plantas do gênero *Xylopia*: composição química e potencial farmacológico. *Revista Brasileira de Plantas Medicinais* **2015**, *17*, 814. [[Crossref](#)]
20. Taher, M.; Amri, M. S.; Susanti, D.; Kudos, M. B. A.; Nor, N. F. A. M.; Syukri, Y.; Medicinal uses, phytochemistry, and pharmacological properties of *Piper aduncum* L. *Sains Malaysiana* **2020**, *49*, 1829. [[Crossref](#)]
21. Badalamenti, N.; Bruno, M.; Formisano, C.; Rigano, D.; Effect of germacrene-rich essential oil of *Parentucellia latifolia* (L.) Caruel collected in Central Sicily on the growth of microorganisms inhabiting historical textiles. *Natural Product Communications* **2022**, *17*, 1. [[Crossref](#)]
22. Dzul-Beh, A. J.; García-Sosa, K.; Uc-Cachón, A. H.; Bórquez, J.; Loyola, L. A.; Barrios-García, H. B.; Peña-Rodríguez, L. M.; Molina-Salinas, G. M.; *In vitro* growth inhibition and bactericidal activity of spathulenol against drug-resistant clinical isolates of *Mycobacterium tuberculosis*. *Brazilian Journal of Pharmacognosy* **2019**, *29*, 798. [[Crossref](#)]
23. Ghavam, M.; Manca, M. L.; Manconi, M.; Bacchetta G.; Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of *Salvia hydrangea* DC. Ex Benth. *Scientific Reports* **2020**, *10*, 15647. [[Crossref](#)] [[PubMed](#)]
24. Turkez, H.; Çelik, K.; Togar, B.; Effect of copaene, a tricyclic sesquiterpene, on human lymphocytes cells *in vitro*. *Cytotechnology* **2014**, *66*, 597. [[Crossref](#)] [[PubMed](#)]
25. Durofil, A.; Radice, M.; Blanco-Salas, J.; Ruiz-Téllez, T.; *Piper aduncum* essential oil: a promising insecticide, acaricide and antiparasitic. A review. *Parasite* **2021**, *28*, 42 [[Crossref](#)] [[PubMed](#)]
26. Morais, V. P.; Cabral, F. V.; Fernandes, C. C.; Miranda, M. L. D.; Brief review on *Piper aduncum* L., its bioactive metabolites and its potential to develop bioproducts. *Brazilian Archives of Biology and Technology* **2023**, *66*, e23220314. [[Crossref](#)]
27. Ridzuan, P. M.; Alaina, H. Y.; Atika, A. R.; Nazira, C. M.; Afrina, A.; Alya-Syarafina, M. S.; Mujahid, M. S.; Halim, S.; The efficacy of *Piper aduncum* extracts as natural larvicides against *Aedes aegypti* larvae. *International Journal of Medical Toxicology & Legal Medicine* **2019**, *22*, 154. [[Crossref](#)]