Antimicrobial Potential of Plant Extracts from the Pitangui-Mg Region, Brazil

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Abstract

Brazil is recognized at worldwide as one of the largest holders of biodiversity on planet, possessing more than 20% of all plant species in the world, with countless of them presenting great medicinal potential. The medicinal plants use throughout the world has reached very significant economic figures, although its usage in folk medicine has been recognized for hundreds of years in different regions of the planet. The aims of this work were to evaluate the antimicrobial potential of plant extracts of Cerrado and Atlantic Forest specimens that occurs at Pitangui City region in Minas Gerais state with emphasis on those with regional folk medicine reports. The plant material used were leaves obtained from specimens collected at EPAMIG Pitangui Experimental Fields and-Technical Institute of Agriculture and Cooperatives. The extracts obtained were submitted to experiments in Petri dishes and to the diffusion disc technique to determine their antimicrobial action through the inhibition zone. For evaluation of plant extracts effects on bacterial isolates and fungal isolate growth, it was possible to see a large responses variation in a scenario of species-specific dependence amid the tendency of slight decrease in the inhibitory effect for aqueous extracts. An average increase of 20% was observed in inhibition zone formation at the presence of ethanolic plant extract. For S. aureus, S. epidermidis and E. coli there was a higher number of leaf extracts in ethanolic solvent that inhibited the growth of those bacterial species, compared to the same plant extracts whose solvent was the aqueous. Exception for plant species, P. alata, in which leaf extracts only in aqueous solvent were effective against the species S. aureus and S. epidermidis. In both extracts, there was no antimicrobial effect detected for leaf extracts of B. forficata. The plant extracts that demonstrated greater amplitude of bacterial growth inhibition were: A. absinthium, S. cordifoliae, T. avellanedae, with emphasis on ethanolic extracts of these species. For fungal isolate tested, C. glabrata, there was no growth inhibition against any of plant extracts evaluated. Considering the species Baccharis retusa and Baccharis dracunculifolia, only ethanolic extracts were tested. Inhibition results were obtained for all organisms tested, except for Enterobacter cloacae in relation to ethanolic extract of B. dracunculifolia.

Keywords: Brazil medicinal plants; Bactericide potential; Bacteriostatic potential

Introduction

Brazil is recognized at worldwide as one of the largest holders of biodiversity on planet, possessing more than 20% of all plant species in the world, with countless of them presenting great medicinal potential [1]. The medicinal plants use throughout the world has reached very significant economic figures [2], although its usage in folk medicine has been recognized for hundreds of years in different regions of the planet. Thus, investment is justified in studies related to its active principles as well as new plant species with potential medicinal application, especially in Brazil, which, despite of its wide diversity of species already described, still presents large collection to be discovered and explored.

Proof of this is the enormous effort of Brazilian scientists to study the biodiversity of higher plants in the country in recent years, reflecting in the number of publications in international journals between 2011 and 2013, totaling 10000 publications in that period [3]. As another propellant factor, there is still growing interest in the use of phytotherapies and medicinal plants worldwide, especially in Brazil, where it was observed between 2013 and 2015 growth of 161% in the search for treatments based on medicinal plants and herbal medicines by the Unified Health System (SUS), notably for treatment of Burns, gastritis and ulcers, according to data from Ministry of Health, [3].
Although the pharmaceutical industries have produced an expressive number of new antibiotics in recent decades, the microbial resistance to these drugs has also increased. In general, bacteria have the genetic ability to acquire and transmit resistance to drugs used as therapeutic agents (Cohen, 1992). The use of plant and phytochemical extracts of known antimicrobial activity can guarantee significant importance in various therapeutic treatments. One of several examples and most emblematic that can be cited is the case of Boldo (Peumus Boldus), very employed in Brazil for gastrointestinal discomfort treatment. In recent years, studies have shown that extracts from that plant have important antimicrobial effects, due to antimicrobial peptides presence, as well as molecules with anti-inflammatory action [4].

Several studies are routinely developed in different countries to prove their efficacy [5-10]. Fungicides originated from plants have been used for centuries. The research involving the demand for fungicide obtained from plants, however, only gained momentum in recent years. It is also noteworthy the substances diversity that exist in plants in general and possibility of finding new ones with antifungal or antibiotic action, which could be used directly by producer, through the Plant cultivation, preparation and direct application of its extracts. Another possibility refers to identification of new substances in plant extracts with fungicide and/or antibiotic characteristics which would serve as a model to chemical synthesis of their active principles in a second moment [11].

Cerrado, an important Brazilian biome, contains more than 6,000 vascular plants [12], many of them with food and medicinal value [13], however there is a very limited studies number related to species identification that present medicinal properties, to obtain extracts, biological assessments, Pharmacological and toxicological studies, which provide subsidies for their safe use, especially, when considering the great existing biodiversity. On the other hand, the Atlantic Forest, another brazilian biome, is a forest formation of most threatened in Brazil, despite its ecological importance associated with exceptional biodiversity and high endemic species rates, information about the medicinal plants potential is rare. The medicinal plants extractivism presents great potential for rational use, since it does not imply the removal of forest and compared with other uses can generate lower environmental impacts [14] (Perreira 2012). The aims of this work were to evaluate the antimicrobial potential of plant extracts of Cerrado and Atlantic Forest specimens that occurs at Pitangui City region in Minas Gerais state, with emphasis on those with regional folk medicine reports.

**Material and Methods**

**Study area**

The plant material to be used were leaves obtained from specimens collected at EPAMIG Pitangui Experimental Fields and Technical Institute of Agriculture and Cooperatives, located at Pitangui municipality, Minas Gerais state (19° 42’ 18.4” latitude South and 44° 53’ 10.3” Longitude West), in Alto São Francisco region, bordering to the north by Pompéu city, to south with Conceição do Pará, to east with Jaguar of Pitangui and to east with Leandro Ferreira and Martinho Camposities. After collects, the specimens were identified, and their specimens exsiccates were deposited at/AG herbarium from Minas Gerais State Agriculture Research Company (EPAMIG). It is important to emphasize that access to genetic heritage was registered in SISGEN with registration code A3FD8E7, in October 2018, after the institutional register completion at State University of Minas Gerais on that platform also in October 2018.

**Preparation and partitioning of the plant extract**

To obtain aqueous extract from leaves, the infusion method was used, in which the dried leaves are placed in hot water (T > 90oC) (DIAS et al. 2000), in a mixture of 1g plant material for each 10 mL of water. The Maceration method was used to obtain ethanolic extract, in proportion of 1g material to 9 mL of alcohol at 70% (V/V). The next step involved the dilution of macerated material in water and DMSO (dimethyl sulfoxide) in proportion of 10:1 (Araújo et al. 2013).

**Antimicrobial and antifungal activity tests**

For the evaluation of bacteriostatic, bactericidal, fungicidal or fungicidal effects, the extracts obtained were submitted to experiments in Petri dishes and to the diffusion disc technique to determine their antimicrobial action through the inhibition halo. Experimental procedures will follow those described in CLSI [13].

**Result**

For evaluation of plant extracts effects on bacterial isolates and fungal isolate growth, it was possible to see a large responses variation in a scenario of species-specific dependence amid the tendency of slight decrease in the Inhibitory effect for aqueous extracts (table 1). An average increase of 20% was observed in inhibition halo formation at the presence of ethanolic plant extract. For S. aureus, S. epidermidis and E. coli there was a higher number of leaf extracts in ethanolic solvent that inhibited the growth of those bacterial species, compared to the same plant extracts whose solvent was the aqueous. Exception for plant species, P. alata Dryander, in which leaf extracts only in aqueous solvent were effective against the species S. aureus and S. epidermidis. In both extracts, there was no antimicrobial effect detected for leaf extracts of B. forficata. The plant extracts that demonstrated greater amplitude of bacterial growth inhibition were. absinthium, S. cordifoliae, T. avellanadeae, with emphasis on ethanolic extracts of these species. For fungal isolate tested, C. glabrata, there was no growth inhibition against any of plant extracts evaluated (data not shown). Considering the species Baccharis refuse and Baccharis dracunculifolia, only ethanolic extracts were tested. Inhibition results were obtained for all organisms tested, except for Enterobacter cloaceae in relation to ethanolic extract of B. Dracunculifolia. The positive and negative controls achieved the expected results by serving as a comparison parameter to treatments (data not shown) (Table 1 & 2).

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<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of Inhibition</th>
</tr>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1 2 3 4 5 6 7 8 9</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Enterobacter cloaceae</em></td>
<td>yes</td>
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<tr>
<td><em>Escherichia Coli</em></td>
<td>yes</td>
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**Aqueous Vegetable extract**

<table>
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<tr>
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<td>yes</td>
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</table>

**Ethanolic Vegetable extract**

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<tbody>
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</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Enterobacter cloaceae</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Escherichia Coli</em></td>
<td>yes</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>yes</td>
</tr>
</tbody>
</table>

Tests were also carried out with other extracts as follows in table below where antimicrobial potential was found in plant ethanolic extracts of 3 and 5 species, for *Klebsiella oxytoca*, *Staphylococcus epidermidis*, *Acinetobacter haemolyticus* organisms (Figure 1).

Table 2: Microbial growth inhibition results table in relation to ethanolic leaf plant extracts. 1. Hyptidendron canum (flower); 2. Hyptidendron canum (sheets); 3. Stryphnodendron sp; 4. Xylopia sp; 5. Gochnatia sp; 6. Braccharis sp.

<table>
<thead>
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<th>Zone of Inhibition</th>
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<tbody>
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</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter haemolyticus</em></td>
<td></td>
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<tr>
<td><em>Candida glabrata</em></td>
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Figure 1: Illustration of disc-diffusion test results in agar-nutrient showing inhibition halos of plants species extracts against *Staphylococcus aureus* ATCC 25923; *Staphylococcus epidermidis*; *Proteus mirabilis*; *Enterobacter cloaceae* e*Escherichia coli bacteria*.

Figure 1 illustrates the microbial growth inhibition results by disc technique diffusion, in front of plant extracts tested. Figure 2 shows, in turn, details of growth inhibition halo formation of bacterium S. epidermidis around the disc soaked with the solution containing the foliar extracts diluted in ethanolic solvent. In relation to halos’ rays observed diameter, those formed with leaf extract (P. alata Dryander) were that presented the largest dimension, with emphasis on ethanolic extract (Figure 1&2).
Discussion

The results obtained point to the presence of different plant components extracted in leaf extracts, especially secondary metabolites of a diverse nature, which would justify the differences in observed responses in relation to the various Microorganisms. In addition, the use of a polar solvent, water, compared to ethanol, more apolar, leads to the extraction of plant components with different degrees of polarities and different biological properties, such as anti-inflammatory, antioxidant, analgesic and Antimicrobial. Active compounds found in several plants demonstrate antiseptic action, such as Thymol and Carvacrol, Eugenol and Isoeugenol and Terpinenol-4. In some cases, the terpene of the essences, which are water soluble, have higher antibacterial power than others (Knobloch et al., 1989). Phenolic compounds, alkaloids, terpenes and sterols are considered the main family of secondary metabolites (Fumagali et al., 2008). Tannins are responsible for the astringency of many fruits and plant products, acting as antioxidant, antiseptic, cicatrizing and Vasocostrictor (Pereira, Cardoso, 2012). Flavonoids have many biological effects such as antioxidant, anti-inflammatory and antitumoral activity, power to reduce permeability and capillary fragility and inhibition of platelet aggregation [9]. Another characteristic is its antibiotic activity, probably related to the ability to form complexes with soluble and extracellular proteins and with the walls of bacterial cells [8].

In the studies by Truiti [16], the antibacterial property of C. nutansraised hypotheses that justify its popular use in the treatment of wounds contaminated by bacterial infections (Gram positive bacteria), being detected that the pure compound that Antibacterial activity was identified as 7-O-B-D-Glucopyranosyl-Nutanocumarina. The Oils essences also have a great value in the food and cosmetics industry, aiming its antiseptic and antimicrobial properties (Bakkali et al., 2008). In studies carried out with extracts from the root of C. Nutans, the bacterium S. aureus was considered susceptible and the bacteria E. coli and P. Aeruginosa were considered resistant to that extract [16]. Two important groups for the pharmacological area are the terpenoids and the flavonoids, the first are a part of a group of secondary metabolites, characteristic of essential oils, being their use based on their antimicrobial properties, very Used by the pharmaceutical industries. The flavonoids belong to numerous classes of chemical substances in a natural way, having several pharmacological activities acting in the biological system, favoring human health with its antioxidant and antimicrobial actions [12]. In our study it was observed that the leaf extracts of C. Nutans were effective in inhibiting the growth of P. mirabilis, E. coli and S. aureus isolates. These results are very promising in view of their action on Gram-negative and Gram-positive bacteria (S. aureus), having this last great importance in public health, especially due to the frequent association of this species with nosocomial infections. It is in the execution phase to perform phytochemical tests for the evaluation of secondary metabolites present in the crude extracts of that specimen to subsequently start the execution of the minimum inhibitory concentration (MIC) tests and Minimum bactericidal concentration (CBM), which will allow for more precise conclusions and associations between the secondary metabolites found and the antimicrobial effects observed.

Regarding the genus Passiflora, phytochemical assays previously performed with the hydroalcoholic extracts of the aerial parts (leaves, stems, epicarp, pulp and seeds) established that Passiflora Cincinnata has the following classes of metabolites Secondary: Condensate tannins, flohabanic, Flavones, Flavononoiis, Flavonois, Xantonas, Chalcones, Aurones, Flavones, Leucoanthocyanidine, catechins and alkaloids. These, because they are phytoconstituents derived from the secondary metabolism of vegetables, which almost always act in defense of itself against pathogens, can present interesting biological activities. Biological assays using isolated combinations reveal that the flavonoids exhibit a great action on biological systems demonstrating antimicrobial, antiviral, antiulcerogenie, cytotoxic, anticaner, antioxidant, Antifapetotic, antihypertensive, hydropipidemic, anti-inflammatory and antiplatelet (Djahanguiri et al. 1969). The activity demonstrated in the in vitro assays performed with the hydroalcoholic extracts of P. Cincinnata evidences a probable action of phenolic compounds present in all parts, confirming the antimicrobial activity attributed to this class of Secondary metabolites. The Association of natural products to antibiotics can exert direct activity against many bacterial species, modulating or even increasing the activity of a specific antibiotic, reversing the natural resistance of bacteria. The potentialization of the activity or the reversal of antimicrobial resistance allows the classification of these compounds as modifers of the antibiotic activity. The use of extracts becomes interesting because they present low possibility of microorganisms acquire resistance to their action, since they are complex mixtures, which hinders the microbial adaptability [17]. In our study, the growth inhibition of S. aureus and S. Epidermidis was observed in relation to aqueous extracts of Passiflora alata Dyander, demonstrating interesting results from the scientific point of view with few reports associated with the effects of those extracts on S. Epidermidis. As previously described, studies on the phytochemical composition of these extracts are executing in order to elucidate the secondary compounds present.

A. Absinthium L. (Asteraceae), commonly known as “Absinthe”, is a plant traditionally used as an anthelmintic, antiseptic, antispasmodic, for the treatment of cancer, bacillary dysentery and neurodegenerative diseases. Its essential oil has antimicrobial,
antiparasitic, insecticide and herbical properties [18]. Studies
developed by Sandra [16] showed that leaves of A. Absinthium
showed poor antimicrobial activity against Staphylococcus aureus
(7 mm halo), Staphylococcus Epidermidis (7 mm halo) and
Enterococcus faecalis (7 mm Halo). Previous studies [19] revealed
a potential activity of a. Absinthium versus S. Aureuscorroborating
the results obtained in this study in which the growth inhibition
of S. aureus was observed both for the ethanolic extract and for
the aqueous extract. It is important to emphasize that extracts of
A. Absinthiumobtained in this work demonstrated antimicrobial
activity for most of the microbial isolates evaluated, except P.
Mirabilis and E. Cloaca, demonstrating a broad spectrum of
performance and, thus, large Pharmacologic potential.

With regard to T. Avellanedae, popularly known as Purple ipê,
the bioactive compound Lapachol, I described for the first time in
1882 by Paternô [20] was already extracted. In popular use, it has
produced evident results in the treatment of diabetes and gastric
ulcers, besides being used as analgesic, anti-inflammatory and
even as anti-mutagênicos. It has also been used in hospitals, in
the form of fluid extract and powder or ointment [21]. More recent
studies have demonstrated the antimicrobial potential of T. Alba,
whose ethanolic extract obtained from its flowers demonstrated
bactericidal action against S. Epidermidis, while the ethanolic
extract of the leaf was moderately active with effect Bacteriostatic
on S. Epidermidis and S. aureus [14].

Sandra (2013) observed that the bark of T. Avellanedae showed
a very significant activity within the established criteria against
the microorganism S. Epidermidis, with an average of 10 mm
inhibitory halo, and, against S. aureus, E. Faecalis and B. Cereus
presented a little activity Significant. T. Avellanedae bark showed
significant antimicrobial activity against Staphylococcus aureus
(11 mm halo), B. Cereus (10 mm halo), S. Epidermidis (10 mm
halo) and E. Faecalis (11 mm halo), although there are reports of
studies about its activity against Helicobacter pylori [Saad et al,
2009], the analysis of the antimicrobial activity performed shows
that the results obtained are quite promising for the continuation
of the studies Antimicrobial activity against the microorganisms
analyzed. In our study, we observed expressive antimicrobial
activity of leaf extracts of T. Avellanedaein front of S. aureus, S.
Epidermidis and E. coli, demonstrating also a broad spectrum of
action whose biological components with antimicrobial action are
Studies of new antimicrobial drugs.

The plant species S. Cordifolia is native to tropical America
but is widespread in several regions of tropical and subtropical
climate of the world. It is used in folk medicine for the treatment
of Stomatitis, asthmatic bronchitis and nasal congestion. The anti-
inflammatory and analgesic effects were tested and confirmed
in the aqueous extract of the plant, proving its popular use for
that purpose [8]. In the microscopy of the white mawo powder,
fragments of several fibers were detected. In the phytochemical
prospecting of the white mawo observed the presence of alkaloids,
phenols and saponins. The polyphenols content was 0.84% and
the foam index was 100%. In phytochemical analysis of Leaves of
S. Cordifolia the presence of sympathomimetic amines, ephedrine
and pseudoephedrine, a potent vasoconstrictor, called Vasocinone
(22) was detected. There are few reports in the literature focused
on the evaluation of antimicrobial effects of leaf extracts of S.
Cordifolia, and the antimicrobial effect of extracts of that species
in the present study was observed in comparison with all microbial
isolates tested, involving in this case the two extractors used,
demonstrating a great antimicrobial potential of the components
of their foliar extract being in execution phytochemical studies in
the sense of elucidating them demonstrate their relationship with the
results raised.

Regarding the gender AIDS, in Waldilley Studies (2010) It was
observed that the ethanolic extract of S. Santaremensis (SSan-
EtOH) and Copaifera luetzelburgii (CL-ETOH), it can be concluded
that these species have anti-edematogênica activity evidenced in
the models of ear and paw edema. Within this perspective, there is
the need for studies in order to determine the possible mechanisms
of action of the extracts studied. Regarding the antimicrobial
activity of extracts of that genus, there are a few studies evaluating its
inhibitory effects. In the present study we detected inhibitory action
of leaf extracts of S. Cordifolia against S. aureus, S. Epidermidis and
E. coli, in different degrees, with emphasis on the ethanolic extracts
produced. This antimicrobial property represents poorly exploited
property and can be aggregated the biological activities already
elicited for extracts of that genus.

The genus Bidens is composed of about 240 species distributed
in the tropical and subtropical zones of the globe. In Brazil,
this species is native to Rio Grande do Sul and occurs in the
physiographic regions of Campos de Cima da Serra, Middle Plateau,
Central Depression, Serra do Sudeste and Campanha; Inhabiting
fields, forest margins, roadside ruderal, wasteland and agricultural
areas [23]. Bidens spp. It is used in traditional medicine for the
treatment of hepatitis, jaundice, fever, throat afflictions and coughs
[12]. In this sense, several studies have confirmed the medicinal
potential of Bidens spp. with anti-inflammatory actions [7]
Analgesics [18], Anti-hiperiglicêemia and antioxidant [3]. However,
the biological activities of B. Subalternans have not been evaluated
and the traditional uses of this species still need to be confirmed.
Species of the genus Bidens, in addition to the traditional effects,
have an important antimicrobial action. Deba et al. (2008) showed
that the essential oils of leaves and flowers of Bidens pilosa have a
significant activity against the Gram-positive bacteria Micrococcus
flavus, Bacillus subtilis, Bacillus cereus and Bacillus pumilus, as
well as against the rods Gram-negative E. coli and Pseudomonas
ovales. In addition, the extracts of leaves [24], Deba et al, 2008).
In our study we obtained a significant result of inhibition of
the growth of the bacterium E. Cloaca, both for the ethanolic
extract and for the aqueous extract. Considering the importance
of this bacterial species as an opportunistic and also nosocomial
pathogen, associated with the absence of reports in the literature
on the effects of leaf extracts of Bidens against that microbial
species [25-30], this result aroused interest in deepening studies in relation to the compounds eventually involved in that response. The phytochemical analysis of aqueous and ethanolic extracts obtained from specimens of Bidens SP is underway [31-35].

**Conclusion**

Based on the results obtained, it is possible to conclude the great antimicrobial potential of Cerrado and Mata Atlantica extracts from the region of Pitangui MG, Brazil. Extracts of B. forficata, A. absinthium, S. cordifolia, T. avellanedae, B. retusa and B. dracunculifolia showed interesting antibacterial and antifungal effects against several microbial species of medical importance. Complementary experiments are necessary to elucidate the constituents of those extracts responsible for the important results observed.

**Acknowledgement**

None.

**Conflicts of Interest**

No conflicts of interest.

**References**

27. Brazil, Ministry of Health. Use of herbal and phytotherapeutic plants rises 161%.