

Interference of Extracts Obtained from *Baccharis Coridifolia* D. C. (Asteraceae-Astereae) Action of Antibiotics

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Abstract The family Asteraceae is the most numerous group within the Angiosperms. Plants from this family are studied for their chemical composition and biological activity. In these studies, the allelopathic, antimicrobial, cytotoxic and anti-inflammatory effects are highlighted. The aim of this study was to evaluate the action of the aqueous (AF1) and the oily (OF2) fractions obtained from the ethanol extract of *Baccharis coridifolia* D. C. on the antimicrobial activity of antibiotics used in clinical treatment. The drugs chloramphenicol (30 µg), norfloxacin (10 µg), sulfamethoxazole / trimethoprim (75/23 µg), vancomycin (30 µg) and amoxicillin (10 µg) were saturated with the fractions AF1 and OF2, and used pure for further comparison. The strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used. The results showed that both fractions interfered on the activity of the antibiotics tested. Synergistic, antagonistic or indifferent behavior was observed in the interactions, with various degrees of susceptibility to the antibiotics tested. These results show that the use of products derived from plants can, in some cases, interfere with the effectiveness of antibiotics during clinical therapy.

Keywords *Baccharis*, Antibiotics, Metabolites, Biological Activity

1. Introduction

Synthetic products started to appear more frequently in pharmacological treatment after the Industrial Revolution and the development of organic chemistry. Obtaining pure compounds became easier, with the development of structural change processes to produce safer and more active medicaments, and also because of the growing economic power of the large pharmaceutical companies. Even so, natural products have not lost their place in therapy, being considered by the population as safe medicines, as 25% of drugs prescribed worldwide are of natural origin[1].

The Asteraceae family is the most numerous systematic group within the Angiosperms, as it comprises about 1,100 genera and 25,000 species. These plants are extremely varied in appearance, mainly including small herbs or shrubs, and rarely trees[2]. About 98% of the genera are small plants, which are found in all types of habitats, but mainly in the mountainous tropical regions of south America[3].

Plants in this family are extensively studied for their

chemical composition and biological activity, with some supporting the development of new drugs, insecticides and other products[2,4,5].

The genus *Baccharis* (tribe Astereae) is represented by over 500 species largely distributed in the higher regions of Brazil, Argentina, Colombia, Chile and Mexico. In Brazil, 120 species of *Baccharis* have been described, with most of them located in the Southeast[6,7].

Baccharis species are generally shrubs and are in average 0.5 to 4 m tall. They have a high socio-economic value, with a wide dispersion in the Brazilian states of Santa Catarina, Paraná, São Paulo, Rio Grande do Sul, and other regions of the country. These plants are mainly consumed as teas, with indications for stomach, liver and prostate problems, as well as anemia, inflammations, diabetes, and body detoxification. In Brazil and Argentina, for example, *B. crispa* and *B. coridifolia* are used to heal wounds and inflammations. *B. trimera*, *B. uncinella* and *B. articulata* are also well-known in alternative medicine. *B. genistelloides* is a medicinal herb widely used in Brazil for a variety of diseases, including digestive and liver disorders, malaria, ulcers, diabetes, anemia, diarrhea, urinary tract inflammation, tonsillitis, verminosis and Hansen's disease[8].

Phytochemical studies on *Baccharis* species have reported the presence of flavonoids, diterpenes and triterpenes, as well as a greater accumulation of flavones, flavonols and labdane and clerodane diterpenes[2-10].

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About 120 *Baccharis* species have been studied chemically, with 30 of them investigated for biological activity. Generally, the most common compounds are the flavonoids and labdane and clerodane diterpenes, although kaurane diterpenes, triterpenes, germacrene, coumaric acids, trichothecene, sesquiterpenes and phenylpropanoids are also frequently found. Allelopathic, antimicrobial, anti-inflammatory and cytotoxic effects are highlighted in biological activity studies. The most researched species for chemical composition and/or biological activity include *B. megapotamica*, *B. incarum*, *B. trimera*, *B. trinervis*, *B. salicifolia*, *B. crispa*, *B. coridifolia*, *B. dracunculifolia*, *B. uncinella*, *B. grisebachii* and *B. tricuneata*[2-10].

The flavonoids quercetin, apigenin, hispidulin and eupatorin have already been isolated from *B. coridifolia*. In this species, the flavonoid rutin is higher in plants harvested during summer, when compared to plants harvested in winter and spring[11].

Some medicaments are made from flavonoids, in particular for the treatment of circulatory diseases and hypertension, acting as a vitamin C cofactor. Other studies suggest that some flavonoids show considerable antitumor action, and may also have antiviral, anti-hemorrhagic, hormonal, anti-inflammatory, antimicrobial and antioxidant effects[12].

Four flavonoids were isolated from the methanol extract of *B. coridifolia* leaves: 5,4'-dihydroxy-7-methoxy-flavone (genkwanin), 5,4'-dihydroxy-6,7-dimethoxy-flavone (cirsimaritin), 5,7,4'-trihydroxy-flavone (apigenin) and 5,7,4'-trihydroxy-6-methoxy-flavone (hispidulin). These compounds showed antimutagenic activity, indicating that they are responsible for such activity in the *B. coridifolia*[8]. Studies demonstrate that *B. coridifolia* has a potential antiaphidic effect, inhibiting proteolytic activity, hemorrhage, myotoxicity and edema induced by the venom of some *Bothrops* species[13-15].

The term "drug interaction" refers to the interference of one drug on the action of another, or of a food or nutrient on the action of a drug. It is important to remember that some drug interactions are beneficial or desirable in the treatment of concomitant diseases, reducing adverse effects and prolonging desired effects, preventing or delaying bacterial resistance, increasing adherence to treatment, increasing efficiency or enabling dose reduction. The undesirable interactions are linked to a reduced drug action or increased contrary results, incidence and range of adverse effects, as well as therapy costs, without increasing the therapeutic benefit. Interactions that lead to a reduced drug activity and, therefore, a loss in effectiveness, are difficult to detect and may be responsible for therapy failure or disease progression. Age, general health, kidney and liver function, alcohol consumption, smoking, diet, as well as genetic and environmental factors influence the susceptibility to drug interactions[16].

B. coridifolia (Figura 1) products are widely marketed in Brazil, and are being consumed in an irrational and indiscriminate manner together with other drugs,[17]. In

this context, this study aims to evaluate the effects of *B. coridifolia* ethanol extract on the antimicrobial activity of antibiotics used in clinical therapy.



Figure 1. *Baccharis coridifolia* D. C. – Asteraceae-Astereae

2. Material and Methods

2.1. Collection Site

The plant material was collected in the countryside in the town of Dois Vizinhos, Paraná, Brazil (25°46'23''S, 53°03'15''E; elevation 620m). The specimens were identified and stored in the collection at União de Ensino do Sudoeste do Paraná – UNISEP, Campus of Dois Vizinhos, PR. When the collections arrived at the laboratory, material was selected by separating the leaves, trunk and roots, which were dried in a stove for seven days at 60°C ± 4. The material was then ground and stored in paper bags in a dry environment. All of the collections were authorized by IBAMA under the permit number 13.234-2 (1 August, 2006).

2.2. Extraction by Maceration of the Plant Material

The 30% (w/v) hydroalcoholic extract was prepared by the maceration and trituration method, using a 70% ethanol solution as the extractor. The extract was filtered in absorbent cotton pads. After extraction, the material obtained was stored in its concentrated form. Two fractions were obtained: a water-soluble fraction (aqueous fraction or AF1) and a fraction soluble in organic solvent (oily fraction or OF2). Both fractions were stored in amber bottles for further analyses.

2.3. Antimicrobial Activity of the Fractions

The method used was based on the disk diffusion susceptibility test. In this method, a standardized solution of a specific microorganism is inoculated on agar plates. Then, paper disks impregnated with the substances investigated for antimicrobial activity are placed on the agar. Such substances diffuse in the culture medium and, if the sample in question exhibit inhibitory activity against the microorganism, a clear ring is formed around the disk. After

the incubation period, and under the specific conditions for the microorganism development, inhibition zones are measured around each disk[18-21].

2.4. Determination of the Minimum Inhibitory

2.4.1. Concentration Index (MIC)

The bacterial suspensions of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were inoculated onto plates containing Mueller-Hinton agar, with a Drigalski spatula. After this procedure, Whatman no.1 filter paper disks (6 mm diameter) previously prepared were transferred to the plates. The antibiotic disks, with and without the added extracts of *B. coridifolia*, were evaluated on each plate. Subsequently, the plates were incubated at $35 \pm 1^\circ\text{C}$ for 24 h. After this period, the plates were analyzed for the presence of inhibition zones (measured in mm). All assays were performed in triplicate. Inhibition zone diameters were interpreted according to the Clinical and Laboratory Standard Institute, recommended guidelines[22].

2.4.2. Preparation of Paper Disks

The fractions obtained were used for preparing disks of antimicrobial activity. The AF1 was dissolved in water, whereas OF2 was dissolved in DMSO (Dimethyl sulfoxide) to achieve concentrations of 100, 50, 25, 12.5, 6.25 and 3.12 mg/liter.

Two sets of disks were prepared with the obtained concentrations. The first set of disks were prepared with Whatman filter paper no.1 (6 mm diameter), saturated in AF1 and OF2, according to the previously mentioned concentrations. This procedure aimed to evaluate the antimicrobial activity of the aqueous and oily fractions and determine the Minimum Inhibitory Concentration (MIC).

The second set of disks was obtained commercially (Newprov®). These disks already contain a determined concentration of each antibiotic.

Chloramphenicol (30 µg), norfloxacin (10 µg), sulfamethoxazole/trimethoprim (75/23 µg), vancomycin (30 µg) and amoxicillin (10 µg) disks were saturated with both fractions by complete immersion in the dilutions, whose MIC had been determined. Subsequently, these disks remained in a laminar air flow system over 2 h for evaporating the excess solvent. Disks containing only antibiotics, without the oily and aqueous added fractions, were used as controls.

2.4.3. Preparation of the Culture Medium

The culture medium used for testing the antimicrobial activity was the Mueller-Hinton agar (MH - Merck), prepared from the dehydrated medium, according to the manufacturer's recommendation[18].

2.4.4. Preparation of The Inocula

E. coli ATCC 25922 (beta-lactamase negative) and *S.*

aureus ATCC 25923 were used. The strains were reconstituted according to supplier recommendations. The microbial culture was standardized at 10^8 cells/mL, which is compared to the 0.5 McFarland turbidity standard[23].

2.5. Benchmarks for Assessment

CLSI guidelines[21] were used to verify the antimicrobial susceptibility of the ATCCs. Synergistic effect was considered when a zone of inhibition with a diameter ≥ 2 mm formed by the combined application of the extract plus the antibiotic was observed, compared to the zone of inhibition formed by the action of the antibiotic alone. Antagonistic effect was considered when inhibition zones of smaller diameter were observed around the disks with antibiotic and extract, compared to that developed by the antibiotic alone. Indifferent effect was considered when there was a zone of inhibition surrounding the disks with antibiotic and extract, with a diameter equal to that resulting from the application of the antibiotic alone[24]. All activities were performed in triplicate.

3. Results and Discussion

The results shown in Table 1 were obtained by evaluation of the antimicrobial susceptibility and MIC determination in a Mueller-Hinton agar medium (MH-Merck®) in different AF1 and OF2 concentrations and measurement of inhibition zone formed.

In analyzing the data contained in Table 1, it appears that the MIC of AF1 for *E. coli* was 12.50 mg/L, whereas the MIC of OF2 was 6.25 mg/L. The MIC of both fractions for *S. aureus* was 12.50 mg/L.

The MIC values obtained served as a base to evaluate the effect of these extracts on a group of antibiotics used in clinical therapy. Thus, a MIC equal to 12.50 mg/L was used as a standard in the interference tests of the metabolites contained in the fractions AF1 and OF2, when associated to molecules of antibiotics used in clinical therapy.

Table 1. Susceptibility of *S. aureus* and *E. coli* to *B. coridifolia* extracts

| [] mg/L | <i>E. coli</i> | | <i>S. aureus</i> | |
|-------------|----------------|----------|------------------|----------|
| | AF1 | OF2 | AF1 | OF2 |
| 100.00 | 36.0±2.3* | 22.2±1.8 | 38.6±6.7 | 29.6±8.2 |
| 50.00 | 35.3±3.5 | 19.0±2.1 | 28.3±8.3 | 23.6±6.2 |
| 25.00 | 30.6±4.2 | 17.3±1.5 | 18.0±4.8 | 14.3±4.2 |
| 12.50 | 16.3±1.7 | 12.3±2.4 | 7.0±1.2 | 9.0±2.5 |
| 6.25 | 0.0 | 7.6±2.8 | 0.0 | 0.0 |
| 3.12 | 0.0 | 0.0 | 0.0 | 0.0 |

[] Concentrations mg/L

* Results are expressed as diameter (mm) of inhibition zones

Antimicrobial susceptibility tests with the antibiotics chloramphenicol (30 µg), norfloxacin (10 µg), sulfamethoxazole/trimethoprim (75/23 µg), vancomycin (30 µg) and amoxicillin (10 µg) (Newprov®), pure and with added AF1 and OF2, were carried out. Synergistic,

antagonistic and indifferent behaviors were observed when comparing the inhibition zones formed around antibiotic disks, pure and with added AF1 and OF2. These data are summarized in Tables 2 and 3.

The inhibition zones formed by the antibiotic disks when associated to OF2 indicate synergism on the activity of all antibiotics tested for *E. coli* - ATCC-25922, leading to an increase in bacterial susceptibility.

Synergistic behavior was observed between AF1 and the antibiotics amoxicillin, sulfamethoxazole / trimethoprim and vancomycin, through an increase in the average size of inhibition zones. Norfloxacin showed an antagonistic effect, because the inhibition zones were inferior when associated to AF, in comparison to its use alone. On the other hand, chloramphenicol show no change in its activity during the tests.

Table 2. Effects of *B. coridifolia* extracts on the action of antibiotics used in clinical therapy on *E. coli* ATCC-25922

| Antibiotics | <i>E. coli</i> - ATCC-25922 | | |
|-----------------|-----------------------------|---------------|---------------|
| | HA1 | OF2 | AF1 |
| Amoxicillin | 24 | 29.32±3.22(↑) | 29.33±5.67(↑) |
| Norfloxacin | 38 | 41.03±7.31(↑) | 31.34±6.24(↓) |
| Sulfa/Trim | 28 | 32.65±6.41(↑) | 37.36±5.95(↑) |
| Chloramphenicol | 23 | 26.36±8.58(↑) | 21.69±3.22(*) |
| Vancomycin | 18 | 24.62±2.36(↑) | 21.05±1.79(↑) |

(↑): synergistic effect; (↓): antagonistic effect; (*): indifferent effect.

HA1 - Inhibition zone obtained by isolated action of antibiotics on *E. coli*

The data in Table 3 show that the behavior of the studied antibiotics on *S. aureus* ATCC-25923 was modified when associated to OF2 and AF1. Inhibition of activity did not occurred in any case, and an indifferent behavior was observed with norfloxacin for both fractions (AF1 and OF2).

Table 3. Effects of *B. coridifolia* extracts on the action of antibiotics used in clinical therapy on *S. aureus* ATCC-25923

| Antibiotics | <i>S. aureus</i> - ATCC-25923 | | |
|-----------------|-------------------------------|---------------|---------------|
| | HA2 | OF2 | AF1 |
| Amoxicillin | 23 | 28.63±8.49(↑) | 28.35±9.44(↑) |
| Norfloxacin | 40 | 41.68±5.33(*) | 39.50±6.33(*) |
| Sulfa/Trim | 30 | 37.06±2.81(↑) | 35.60±8.24(↑) |
| Chloramphenicol | 23 | 26.09±2.65(↑) | 36.10±8.75(↑) |
| Vancomycin | 20 | 28.04±1.83(↑) | 20.43±2.88(*) |

(↑): synergistic effect; (↓): antagonistic effect; (*): indifferent effect

HA2 - Inhibition zone obtained by isolated action of antibiotics on *S. aureus*

Amoxicillin, sulfamethoxazole/trimethoprim and chloramphenicol, when associated with both fractions, showed synergistic behavior. Vancomycin had a synergistic behavior when combined with OF2, but showed indifferent behavior when associated to AF1. Therefore, it can be suggested that an interaction occurs between the antibiotics tested and the metabolites present in the AF1 and OF2 of *B. coridifolia*.

The present results corroborate Canton and Onofre[25],

who used the same methodology to assessed the interference of ethanol extracts obtained from *B. dracunculifolia* on 15 antibiotics, reporting activity changes that caused synergistic or antagonistic behaviors, and no alteration in some cases.

In a study on the extracts of *Alternanthera brasiliana* (L.) O. Kunt. (Amaranthaceae) and their effect on the activity of antibiotics used in clinical therapy,[26] found that when such extracts are associated to 18 inhibitors of protein synthesis, altered behavior and, consequently, synergism or antagonism, are observed. Indifferent behavior, though, was also observed.

It should be noted that the sale of medicinal plant products currently happens in pharmacies and health food stores, where they are marketed according to an industrialized labeling system. In general, these plant preparations have no certificate of quality and are produced from cultivated plants, which mischaracterize traditional medicine that uses almost always plants of the native flora.

The use of medicinal plants from the traditional Chinese and Hindu medicines, completely unknown to westerner civilizations, is increasingly common. These plants are marketed through advertisements that promise safe benefits, because they come from a natural source. However, often the supposed pharmacological properties advertised have no scientific validity, because they have not been investigated, or have not had their pharmacological actions proven through scientific tests in preclinical or clinical trials[24].

Media calls for the consumption of products made from natural sources increase every day in both developing and developed countries. Herbalists promise health and long life, based on the argument that plants used for millennia are safe for the population.

In Brazil, medicinal plants from the native flora are consumed with little or no evidence of their pharmacological properties, and their consumption is propagated by users or dealers. Frequently, these plants are even used for medicinal purposes different from those originally used by the native people. Compared to the drugs prescribed in conventional treatments, the toxicity of medicinal plants and herbal medicines may seem trivial, but this is not true. The toxicity of medicinal plants is a serious public health problem. Adverse effects of herbal medicines, possible adulteration and toxicity, as well as synergistic effect, occur commonly.

Research to evaluate the safe use of medicinal plants and herbal medicines is still incipient in Brazil, as well as the control of these products' sales by official bodies in public markets or health food stores.

In this sense, this work has contributed to the elucidation of the effects caused by the use of *B. coridifolia*, in relation to the metabolites present in two fractions, showing that such metabolites can interfere positively, causing synergism, or negatively, causing antagonism in the activity of antibiotics commonly employed in clinical therapy.

Multidisciplinary studies involving ethnobotanists, chemists, pharmacologists and agronomists are needed in order to expand the knowledge on medicinal plants. This would bring a better understanding on how their compounds

act, what are the toxic and adverse effects, how they interact with new allopathic medicines and what are the most appropriate strategies for production and quality control of herbal medicines, observing the regulatory agencies standards and resolutions.

Raising awareness in the professional team involved in the prescribing, dispensing and administering medications is important. Professionals from different healthcare areas must always question and warn their patients about the non-prescribed use of medicinal herbs.

4. Conclusions

This study shows that the oily (OF2) and aqueous (AF1) fractions from extracts of *B. coridifolia* can interfere on the activity of the antibiotics tested. Therefore, it is confirmed that the use of products of plant origin interfere with the effectiveness of some antibiotics during clinical therapy.

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Conflict of Interest

The authors declare no commercial or financial conflict of interest.

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