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Research Article

Antimicrobial Activity of *Sideroxylon obtusifolium* (Roem. and Schult) T.D. Penn. (Sapotaceae)

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Abstract

Sideroxylon obtusifolium (Sapotaceae), popularly known as 'quixaba', is used in folk medicine for the treatment of gastritis, ulcer, inflammation and hyperglycemia. The present deals with the determination of antimicrobial action *in vitro* of *S. obtusifolium* bark extract. The ethanolic extract and different polarities fractions have been subjected to detect potential antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* using a disc diffusion method. The results showed that the *S. obtusifolium* bark extract and fractions exhibited antimicrobial activity against tested strains, except *E. coli*.

Keywords: *Sideroxylon obtusifolium; In vitro Test; 'Quixaba'; Antimicrobial Activity*

Introduction

Sideroxylon obtusifolium (Roem. and Schult) T.D. Penn. (Sapotaceae), popularly known as 'quixaba', is a widespread plant in the Northeast of Brazil. The bark has been used in folk medicine for the treatment of gastritis, ulcer, inflammations and hyperglycemia [1-6].

Pharmacology studies show that *S. obtusifolium* bark has anti-inflammatory activity [7,8], which may be caused by an antioxidant effect, due to a peroxyl radical scavenging activity ($ROO^{\cdot}$) and an inhibition of lipid peroxidation in mice [8]. The phytochemical screening of the extract revealed the presence of several classes, including phenols; tannins, flavonoids, catechins and alkaloids [5].

According to Almeida and coworkers [7] the component of an ethanol extract of the bark bassic acid may be responsible for its anti-inflammatory activity.

Other studies showed that *S. obtusifolium* bark has a hypoglycemic effect in normal and alloxan-induced diabetic rats [7,9] and suggest that bassic acid can be responsible for this hypoglycemic activity [9].

Phytochemical investigations of the plant led to the identification of triterpenes (taraxeron, taraxerol and eritridiol), triterpenic acid (bassic acid) and steroids [2]. Previous preliminary phytochemical screening of the material showed the presence of flavonoids, tannins and saponins.

Despite its popular use as a medicinal plant, there is no sufficient scientific literature about all the ethnomedicinal uses related, including the antimicrobial effect.

The aim of the present work is to determinate a preliminary phytochemical screening of the dried extract and to evaluate the antimicrobial activity of 70% ethanolic bark extract of *S. obtusifolium* and different crescent polarities fractions by the disc diffusion method.

Material and Methods

Microorganisms

Four microbial species were used and taken from international collections, which included *Bacillus subtilis* (ATCC 9372), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 64550).

Plant Material

Dried plant material of *Sideroxylon obtusifolium* (Roem. and Schult) T.D. Penn. (Sapotaceae) were purchased at herbal market Casa das Raízes, Goiânia, Brazil followed by finding of the supplier and have been kept in our laboratory for future reference.

Preparation of Extracts

The ground plant material was extracted by maceration in ethanol 70°GL (200 g/200 mL) for six hours and subjected to percolation with the same solvent (1,25L) at room temperature in duplicate. After exhaustive extraction, the extract was concentrated under reduced pressure at 40 - 45oC. The supernatants were filtered and evaporated on a rotary evaporator under vacuum to dryness, which, on phytochemical screening they showed saponins, tannins and flavonoids (Table 1).

Fraction	Amount (g)	(%)
FEB	1.8970	94.8500
FD	0.4208	2.1040
FE	3.2061	16.0300
FB	0.4016	3.0080
FF	12.5000	62.5000

Table 1: *S. obtusifolium bark contents.*

For microbiological assays, a weighed amount of the dried extract was fractionated three times with solvents in series dichloromethane, ethyl acetate and n-butanol to produce crescent polarities fractions designated FEB (aqueous initial fraction), FD (dichloromethane fraction), FE (ethyl acetate fraction), FB (n-butanol fraction) and FF (aqueous final fraction) which were evaporated until completely dry and resuspended in ethanol 70°GL to a final concentration of 100 mg of the extract/mL.

Disc Diffusion Method

The microorganisms were inoculated in Brain Heart Infusion (BHI) liquid medium and incubated at a temperature of 37ºC for 24 hours. After growth, the disc diffusion method was performed according Bauer and coworkers [10]. An aliquot of 20 µL suspension containing 10^8 cells/mL was spread on the surface of Mueller-Hinton agar plates through of the BHI culture. The resuspended fractions were sterilized by filtration through 0.45 µm Millipore filters and100 µL were impregnated on the discs at the concentration of 100 mg/mL. After totally dried, the discs were placed on the surface of each inoculated plate. The plates were incubated at 37ºC for 24 hours for bacteria and for 48 hours for *C. albicans*. The zones of growth inhibition around the discs were measured and the diameter of inhibition zone equal to or greater than 7 mm were considered susceptible to the tested extract [11].

The negative control was the solvent used (ethanol 70°GL) and the positive control was ciprofloxacin (5 µg/disc) for bacteria and nystatin (2.4 µg/disc) for *C. albicans*. All determinations were carried out in triplicate.

Results and Discussion

In 1991, the World Health Organization, finally, recognized the importance of natural products as therapeutic agents under the regulations for the use of traditional medicines. This fact had an origin in the higher consumption of medicinal products and natural therapeutic products and consequently it needs a better quality control by the producers [12-25].

Popular medicine is an important root of potentially useful new compounds for the development of therapeutic agents because they are available at low cost and easy access. The screenings for new antimicrobial agents are especially important due to growing resistance of the microorganisms against commons antimicrobials. In this scenario, many studies have been carried out aiming to obtain new antimicrobial compounds with activity against bacteria and yeasts around the world [16,26-35].

However, several medicinal plants commercialized have not any scientific research to prove their efficacy in Brazil. So, it is necessary to develop quality control and analytical methods to prove their efficacy to improve public health as well the harvest, production and commercialization of these products [36,37].

Motivated by the anti-inflammatory action from *Sideroxylon obtusifolium* (Sapotaceae), the present work determined the antimicrobial activity from bark extract and its fractions.

Flavonoids, saponins, and tannins were detected on preliminary phytochemical screening of the dried extract of *S. obtusifolium*. They can be responsible for the determined activity of FE and FB fractions against tested microorganisms.

The results of the antimicrobial activity assay of *S. obtusifolium* bark extract and fractions are shown in table 2. It was revealed that the growth of *Bacillus subtilis, Staphylococcus aureus* and *Candida albicans* were affected by the extracts and fractions by forming clear inhibition zones between 8 and 12 mm of diameter. The ethyl acetate and n-butanol fractions showed the highest percentages of antimicrobial activity, especially against the Gram-positive bacteria *B. subtilis* and *S. aureus*. There was no activity from the plant extract or their fractions against *Escherichia coli*, a Gram-negative bacterium. The control ethanol did not inhibit any of the microorganisms tested. Figure 1 shows the distribution of impregnated discs on agar medium. The growth inhibition can be visualized in figure 2.

Fraction	**Microbial species**			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Candida albicans***
FEB	0	0	8.0	0
FD	0	10.0	0	8.0
FE	0	11.4	10.0	8.0
FB	0	10.5	11.0	8.0
FF	0	0	8.0	0
Ethanol	0	0	0	0
Ciprofloxacin	13.7	33.0	24.0	NT
Nystatin	NT	NT	NT	30.0

Table 2: *Diameter of inhibition zone (mm) of ethanolic extract and fractions of 100 mg/mL S. obtusifolium.*

FEB: Aqueous Initial Fraction; FD: Dichloromethane Fraction; FE: Ethyl Acetate Fraction; FB: n-Butanol Fraction; FF: Aqueous Final Fraction; NT: Not Tested.

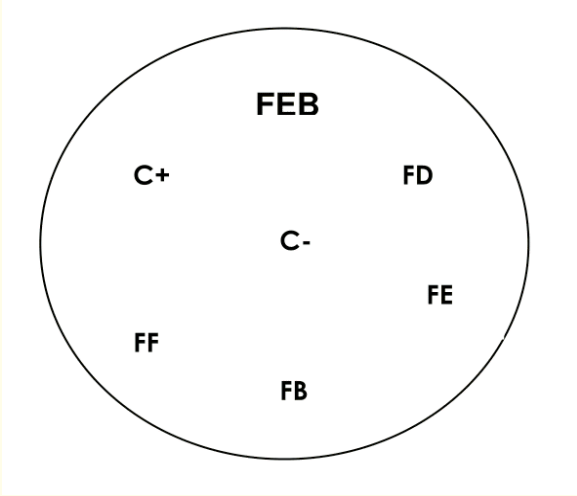

Figure 1: *Distribution of impregnated discs on agar medium to determine antimicrobial activity of S. obtusifolium extract and fractions.*

FEB: Raw Extract; FD: Dichlorometane Fraction; FE: Ethyl Acetate Fraction; FB: n-Butanol Fraction; FF: Final Fraction; C+: Positive Control (ciprofloxacin for bacteria; nystatin for Candida); C-: Ethanol

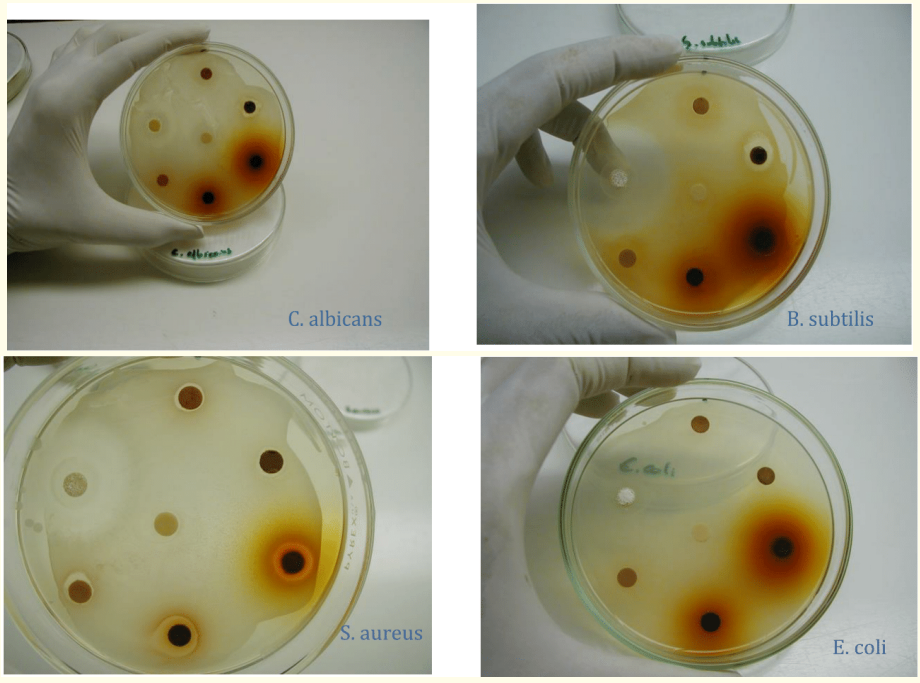

Figure 2: *Antimicrobial activity of S. obtusifolium extract and fractions against C. albicans, B. subtilis, S. aureus and E. coli.*

As the literature reports, these results suggest that the antimicrobial activity may be due to the presence of tannins and flavonoids which mechanism of action could be associated with the inhibition of microbial enzymes [38-41].

After all, like the allopathic pharmaceutical products quality control [42], the standardization of the natural therapeutic products is definitively necessary to assure the minimal quality of the therapeutic drug because they could substitute or counterfeit herbal materials often found in the commercial market.

Conclusion

The results indicate the existence of antimicrobial compounds in the ethanolic extract of the plant, and show a good correlation between the reported uses of *S. obtusifolium* in folk medicine against infectious diseases with consequent inflammation and the experimental data of such extract and fractions towards the most common pathogens. This research characterizes the folk use of *S. obtusifolium* plant extracts and indicates that they can be effective potential candidates for the development of new strategies to treat antibacterial infections.

Further phytochemical studies are required to establish which constituents are responsible for the bioactivity of *Sideroxylon obtusifolium* and if these agents can be used as new antimicrobial drugs for therapy of infectious diseases.

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