

Original Article**Antimicrobial Activity of *Indigofera suffruticosa***

Sônia Pereira Leite¹, Jeymesson Raphael Cardoso Vieira², Paloma Lys de Medeiros¹, Roberta Maria Pereira Leite³, Vera Lúcia de Menezes Lima⁴, Haroudo Satiro Xavier⁵ and Edeltrudes de Oliveira Lima⁶

¹Departamento de Histologia e Embriologia do Centro de Ciências Biológicas da Universidade Federal de Pernambuco, Recife-PE, Brazil, ²Mestrando em Patologia Geral do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, Recife-PE, Brazil, ³Doutoranda em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, Recife-PE, Brazil, ⁴Departamento de Bioquímica do Centro de Ciências Biológicas da Universidade Federal de Pernambuco, Recife-PE, Brazil, ⁵Departamento de Centro de Ciências Biológicas da Universidade Federal de Pernambuco, Recife-PE, Brazil and ⁶Departamento de Ciências Farmacêutica do Centro de Ciências da Saúde da Universidade Federal da Paraíba, João Pessoa-PB, Brazil

Various organic and aqueous extracts of leaves of *Indigofera suffruticosa* Mill (Fabaceae) obtained by infusion and maceration were screened for their antibacterial and antifungal activities. The extracts were tested against 5 different species of human pathogenic bacteria and 17 fungal strains by the agar-solid diffusion method. Most of the extracts were devoid of antifungal and antibacterial activities, except the aqueous extract of leaves of *I. suffruticosa* obtained by infusion, which showed strong inhibitory activity against the Gram-positive bacteria *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) of 5000 µg ml⁻¹. The MIC values to dermatophyte strains were 2500 µg ml⁻¹ against *Trichophyton rubrum* (LM-09, LM-13) and *Microsporum canis*. This study suggests that aqueous extracts of leaves of *I. suffruticosa* obtained by infusion can be used in the treatment of skin diseases caused by dermatophytes.

Keywords: antibacterial activity – antifungal activity – aqueous extract – *Indigofera suffruticosa*

Introduction

Plants are invaluable sources of pharmaceutical products (1) and Brazil, in particular, has yielded an incredible array of plant and animal products (2,3) that have drawn the attention of ethnopharmacologists from around the world. Many products, such as the one described in this paper, have traditional uses that are now being investigated to create an evidence base that will facilitate their inclusion in general medical practice. Belonging to the Fabaceae species, the *I. suffruticosa* Mill (Fig. 1) has been used as an infusion or decoction (flavor

extract by boiling 1 l of hot water per 5 g of leaves) (4). This plant is found in tropical and subtropical areas and is well adapted to growth in semi-arid regions and soils of low fertility (5–7). It occurs in the northeast countryside of Brazil and has intensive popular use in the treatment of inflammations (8,9) and other diseases such as epilepsy in humans (10) and in animal models (11–13). Recently, embryotoxic effects have been reported (14). A chemical investigation of extracts of leaves of *I. suffruticosa* in Natural Products Alert (NAPRALERT) (15) and Chemical Abstracts databases has revealed the presence of alkaloids, flavonoids, steroids, proteins, carbohydrates and indigo. Herbal folk medicines provide an interesting and still largely unexplored source for drug development with potential chemotherapeutic benefits.

However, up to date, little research has been done to investigate this traditionally used plant. Although numerous studies

For reprints and all correspondence: Sônia Pereira Leite, Laboratório de Cultura de Células II, Departamento de Histologia e Embriologia do Centro de Ciências Biológica da Universidade Federal de Pernambuco (UFPE). Cidade Universitária. Recife 50 670-420, Rua Alemanha 44 Apt 303 Imbiribeira Recife Pernambuco, Brazil. Tel: +55-81-21268515; Fax: +55-81-21268516; E-mail: spl@ufpe.br

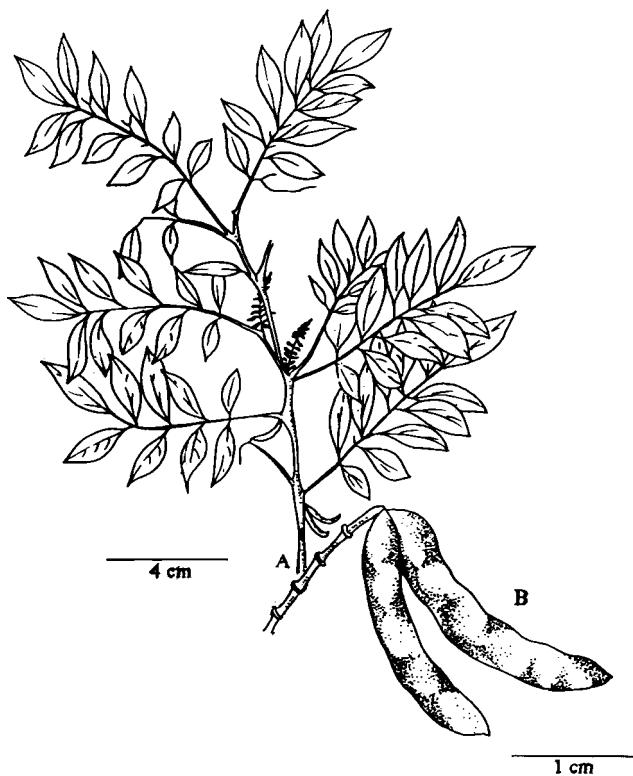


Figure 1. *Indigofera suffruticosa* Mill. (A) Aspect of blossomed branch. (B) Aspect of branch with seeds. (Erlize da Silvas).

have been carried out using natural extract products for screening antimicrobial activity (16,17), no attention was given to *I. suffruticosa* antimicrobial activity.

We report here our findings on some antibacterial and antifungal effects of various organic and aqueous extracts from leaves of *I. suffruticosa* obtained by infusion and maceration against a wide range of pathogenic bacteria and dermatophyte strains.

Materials and Methods

Plant Material

Leaves of *I. suffruticosa* were collected in June 2000 in São Caetano, State of Pernambuco, Brazil and authenticated by biologist Marlene Barbosa from the Department of Botany, Universidade Federal de Pernambuco (UFPE). A voucher specimen number 32 859 has been deposited at the herbarium of the above-cited department.

Preparation of Extracts

Two extracts were obtained by infusion and maceration from 150 g of the plant material. The material was weighed; chopped and extracted with solvents and water. The infusion was prepared with 75 g of fresh leaves in 2 × 200 ml of increasing polarity solvents (hexane, ethyl acetate and methanol) at 40°C for 10 min and removing solid matter by

filtration. After this preliminary step, the same plant material was extracted in boiling distilled water at the same conditions, and the maceration was obtained following the aforementioned process at room temperature (28°C) overnight. The solvents were removed by rotary evaporation. The yields (w/w) of the infusion and the maceration were, respectively, hexane (0.67 and 0.74%), ethyl acetate (0.39 and 0.34%) and methanol (3.90 and 1.88%) in terms of newly collected plant material. After lyophilization the aqueous extract yielded 4.20 and 1.75% and the dried material was stored at -20°C (18,19).

Preliminary Phytochemical Analysis

The aqueous, methanolic, hexane and ethyl acetate extracts of leaves of *I. suffruticosa* obtained by infusion were analyzed by qualitative method (thin layer chromatography on silica gel/UV detection at 365 nm) for presence of alkaloids, iridoids, saponins, carbohydrates, coumarins, flavonoids, phenol, terpenoides, indigo and sterol (20–22). Lectin activity was obtained from aqueous extracts of leaves of *I. suffruticosa* by infusion (23).

Antimicrobial Assays

Microorganisms Tested

Strains of human pathogen microorganisms used in this study were as follows. Three Gram-negative bacteria, *Klebsiella pneumoniae* (LM-49), *Pseudomonas aeruginosa* (LM-B6) and *Escherichia coli* (LM-209); two Gram-positive bacteria, *Staphylococcus aureus* (ATTCC-6538) and *Staphylococcus epidermidis* (ATCC-12228); 17 yeasts of fungi including 9 potentially pathogenic yeasts, *Candida albicans* (ATCC-76615, ICB-12, FCF-243), *Cryptococcus neoformans* (FFCF-19), *Cryptococcus guilliermondii* (LM-1), *Cryptococcus krusei* (FCF-1161), *Cryptococcus stellatoidea* (LM-19), *Cryptococcus parapsilosis* (CM-1), *Trichosporon inkin* (LM-267), and 8 filamentous fungi, *Trichophyton rubrum* (M-09, LM-13), *Trichophyton mentagrophytes* (LM-16), *Microsporum canis* (LM-828), *Aspergillus flavus* (LM-127), *Penicillium* sp (LM-131), *Geotrichum candidum* (LM-23) and *Fusarium* sp (LM-135). The microorganisms were originally obtained from the Mycology Laboratory of the Department of Pharmaceutical Science at Universidade de São Paulo (USP), Universidade Federal da Paraíba (UFPB) and Adolfo Lutz Institute in São Paulo, Brazil.

Antimicrobial Testing

Antimicrobial activity of the various organic and aqueous extracts from leaves of *I. suffruticosa* obtained by infusion and maceration was determined by the agar-solid diffusion method (24–28). Bacteria strains were cultured overnight at 37°C in Müller-Hinton agar, the filamentous and leveduriform yeasts in Sabouraud dextrose agar at 25°C overnight and at room temperature for a period of 14 days. The agar-solid diffusion method was used to determine antibacterial and

antifungal activities. The extracts, in concentrations of 10 000 and 625 $\mu\text{g ml}^{-1}$, were dissolved in dimethylsulfoxide (DMSO) for a final concentration of 4%.

Agar-solid Diffusion Method

Suspension of microorganisms (1 ml) with an optical density of McFarland 0.5 was prepared in physiological saline solution (0.9%) and it was adjusted to 90% of transmittance (530 nm) in a spectrophotometer. The antimicrobial spectrum of the extracts was determined qualitatively for the bacterial and fungal species in terms of zone sizes around wheels, cut in plates of agar Sabouraud and Müller-Hilton (supplemented as necessary) surface-inoculate with approximately 10^6 CFU of various microbial species containing 50 μl of the tested material dissolved in DMSO (equivalent to 5 mg of the dried extracts). The agar was melted (50°C) and the microorganism cultures were then added aseptically to the agar medium at 45°C in plates and poured into sterile Petri dishes to give a solid plate. All these experiments were performed in duplicate. The plates were incubated for 24–48 h, at 37°C for bacteria, and 10–14 days, at 30°C for filamentous fungi.

The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotics: chloramphenicol (30 μg) for bacteria, and ketoconazole (1000 μg) for fungi. They were used as positive control and the solvent DMSO as negative control.

The minimal inhibitory concentration (MIC) was applied to the aqueous extract that had proved to be highly effective against microorganisms by the agar-diffusion method. The aqueous extract of *I. suffruticosa* obtained by infusion (50 μl) was diluted in DMSO with decreasing concentrations (from 10 000 to 625 $\mu\text{g ml}^{-1}$). The strains were designated arbitrarily as sensitive or resistant and the zones were measured at the end of the incubation time. An inhibition zone of 10 mm or greater was considered to indicate good antibacterial and antifungal activities.

Results

Preliminary Phytochemical Analysis

The results of our assay on the aqueous, methanolic, hexane and ethyl acetate extracts of leaves of *I. suffruticosa* obtained by infusion are shown in Table 1. The aqueous extract showed

the presence of carbohydrates, glycoproteins, cinnamic derivatives, iridoids, leocyanidines and lectin activity but it was negative for alkaloids, flavonoids and coumarins. Alkaloids, sterols, triterpenes, indigo, flavonoids, carbohydrates, coumarins were detected in methanolic extract and it did not show the presence of iridoids and saponins. The hexane and ethyl acetate extracts showed the presence of β -sitosterol, β -amilina, triterpenes, sterols and azulene derivatives and they were negative for alkaloids, polar flavonoids, cinnamic derivatives, coumarins and phenylpropanoid glycosides.

Antimicrobial Activity of Extracts of *I. suffruticosa* Leaves

The aqueous, methanolic, hexane and ethyl acetate extracts of *I. suffruticosa* leaves obtained by infusion and maceration in a concentration of 10 000 $\mu\text{g ml}^{-1}$ were tested against 22 microorganisms (bacterial and fungal) by means of agar-solid diffusion method. From those extracts, only the aqueous obtained by infusion showed variable degrees of antibacterial and antifungal activities against one or more of the tested organisms. The aqueous extract of leaves of *I. suffruticosa* obtained by infusion exhibited results against *S. aureus*, *T. rubrum* (LM-09, LM-13) and *M. canis*, whereas the organic extracts showed no activity. An inhibition zone of 10 or greater was considered as good antimicrobial activity. The aqueous and the organic extracts by maceration did not show any effect against both Gram-negative and Gram-positive bacteria and fungi. The solvents used, as control exerted no effect against the microorganisms in broth medium.

The inhibitory bacterial and fungal growth by extract, indicated by their MIC values, is summarized in Table 2. The aqueous extract of leaves of *I. suffruticosa* obtained by infusion provided activity against *S. aureus* in a concentration of 5000 $\mu\text{g ml}^{-1}$ with an inhibition zone of 10 mm. The *T. rubrum* (LM-13, LM-9) showed an activity more significant in a concentration of 2500 $\mu\text{g ml}^{-1}$ with an inhibition zone from 12 to 14 mm, respectively, and *M. canis* at the same concentration with an inhibition zone of 12 mm.

Discussion and Conclusions

Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in

Table 1. Phytochemical screening of extract of leaves of *I. suffruticosa* by infusion

| Tested material | Yield (%) | Positive tests for | Negative tests for |
|-----------------------|-----------|--|---|
| Aqueous extract | 4.20 | Lectin, carbohydrates, glycoproteins, cinnamic derivatives, iridoids and leocyanidines | Alkaloids, flavonoids and coumarins |
| Methanolic extract | 3.90 | Alkaloids, sterols, triterpenes, indigo, flavonoids, carbohydrates and coumarins | Iridoids and saponins |
| Hexane extract | 0.67 | β -sitosterol, β -amilina, triterpenes, sterols and azulene derivatives | Alkaloids, polar flavonoids, cinnamic derivatives, coumarins and phenylpropanoid glycosides |
| Ethyl acetate extract | 0.39 | β -sitosterol, β -amilina, triterpenes, sterols and azulene derivatives | Alkaloids, polar flavonoids, cinnamic derivatives, coumarins and phenylpropanoid glycosides |

Table 2. Values of inhibition zone in (mm) by MIC evaluation of aqueous extracts of leaves of *I. suffruticosa* obtained by infusion against bacterial and fungal activities

| Microorganisms | Aqueous extract ($\mu\text{g ml}^{-1}$) | | | | | Microorganism growth without antimicrobians | Controls | |
|------------------------------------|---|------|------|------|-----|---|-------------------------------------|-----------------------------------|
| | 10 000 | 5000 | 2500 | 1250 | 625 | | Chloramphenicol (30 μg) | ketoconazol (1000 μg) |
| <i>Staphylococcus aureus</i> | 12 | 10 | 7 | 0 | 0 | + | 20 | 0 |
| <i>Trichophyton rubrum</i> (LM-09) | 20 | 17 | 14 | 10 | 7 | + | 0 | 20 |
| <i>T. rubrum</i> (LM-13) | 18 | 15 | 12 | 8 | 0 | + | 0 | 20 |
| <i>Microsporum canis</i> | 17 | 15 | 12 | 8 | 0 | + | 0 | 20 |

covering the basic health needs in developing countries, and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (29,30).

Results from our phytochemical analysis in methanolic (alkaloids, sterols, triterpenes, indigo, flavonoids, carbohydrates and coumarins), hexane and ethyl acetate extracts (β sitosterol, β amilina, triterpenes, sterols and azulene derivatives) obtained by infusion revealed no antimicrobial effects. However, the aqueous extract obtained by infusion (lectin, carbohydrates, glycoproteins, cinnamic derivatives, iridoids and leococyanidines) showed the presence of biologically active compounds, correlated to known substance that possess antimicrobial properties (31–34). Studies done with *I. linnaei* identified the amino acid indospicine as a natural toxic product (35). This observation provides strong circumstantial evidence that small protein/peptide plays an important role in a plant's antimicrobial defense system (36). The protein or peptide fractions from leaves of *I. oblongifolia* were reported (33). Biologically, activity detected in the aqueous extract of leaves of *I. suffruticosa* by infusion could be linked to lectins (23).

The aqueous and organic extracts of leaves of *I. suffruticosa* showed different activities against 22 microorganisms (bacterial and fungal). The aqueous extract of *I. suffruticosa* leaves by infusion showed an activity more significant on dermatophyte *T. rubrum* (LM-09, LM-13) and *M. canis*. The aqueous extract of *I. suffruticosa* obtained by infusion provided a zone of inhibition of 10 mm diameter (mm d^{-1}) for *S. aureus* and a zone of inhibition of 14 mm d^{-1} and 12 mm d^{-1} for *T. rubrum* (LM-09, LM-13) and of 12 mm d^{-1} for *M. canis* (filamentous fungal).

Our results concerning the aqueous extract of *I. suffruticosa* obtained by infusion corroborate those of Dahot (33) who showed that the aqueous extract of *I. oblongifolia* leaves has significant antimicrobial activity against both Gram-positive and Gram-negative bacteria and fungal species. Ethanolic extracts of the same species have been mentioned in the literature for their antibacterial activity on *S. aureus* and *P. aeruginosa* and antifungal activity on *Enterococcus faecalis*, *E. coli* (37).

Reports showed that the aqueous extract of *I. dendroides* has significant antimicrobial activity against the Gram-positive bacterial (*S. aureus*, *Bacillus subtilis*), Gram-negative bacterial (*K. pneumoniae*, *E. coli*) and fungal species (*Aspergillus niger*, *C. albicans*). (32).

The literature indicates that the antibacterial activity is due to different chemical agents in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other nature phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds (38).

Many plant extracts have been used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, gastrointestinal disorders, respiratory diseases, cutaneous affections, helmintic infections, parasitic protozoan diseases and inflammatory process (9,39–43). Elisabetsky has recently stressed that alstonine, a unique indole alkaloid is an example of richness of medicinal plants and traditional medical systems in the discovery of new prototypic drug (44).

From this study we can conclude that the traditional use of this plant for the treatment of infectious diseases is promising, mainly against bacteria and fungi. Purification of the bioactive component(s) from the extracts is underway and further investigations may improve our understanding of possible antimicrobial and antifungal activities.

Acknowledgments

We wish to express our appreciation and thanks to Dr Edeltrudes de Oliveira Lima (UFPB, Brazil) for providing us with the microorganisms and Dr Haroudo Satiro Xavier (UFPE, Brazil) for the partial support of this work. We are also grateful to CNPq for its financial support.

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Received March 22, 2005; accepted February 8, 2006



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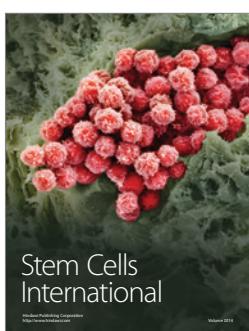
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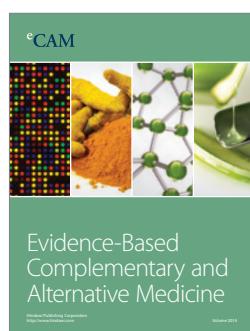
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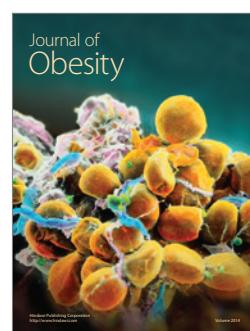
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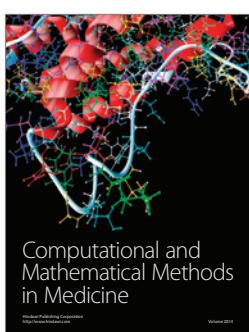
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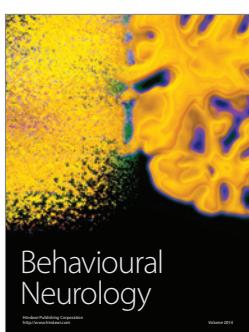
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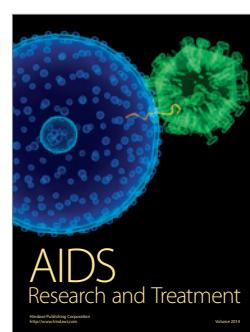
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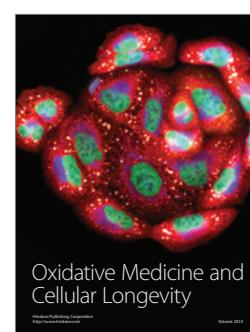
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