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ANTIBACTERIAL ACTIVITY OF EXTRACTS OBTAINED FROM *SENNA CORYMBOSA* AND *TIPUANA TIPU*

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Abstract

Ethereal, dichloromethane and ethyl acetate extracts from aerial parts of *Senna corymbosa* Lam. and *Tipuana tipu* (Benth.) Kuntze (Fabaceae/Leguminosae) were analyzed for *in vitro* antimicrobial activity by broth microdilution assay against bacterial organisms like *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* CLIP 74910, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 at the concentrations of 8000 to 125 µg/ml. Ethyl acetate and dichloromethane extracts of both species inhibited growth of *Staphylococcus* and *Listeria* with MIC values from 250 - 500 µg/ml. The MIC values of ethereal extract of *T. tipu* was 2000 µg/ml and 1000 µg/ml against *S. aureus* and *L. monocytogenes* respectively, while the ethereal extract of *S. corymbosa* only showed activity against *Listeria* (MIC= 4000 µg/ml). The ethyl acetate extracts of both plants inhibited *E. coli* (MIC= 4000 µg/ml). These results suggest that both species could be considered as new source of natural antibacterial principles.

Key words: Antibacterial activity; broth microdilutions; *Senna corymbosa*; *Tipuana tipu*

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Introduction

The plants have been shown to be a potential source for the new antimicrobial agents (1, 2, 3, 4). In the last three decades has taken place a growing interest in the investigation of natural plant products for the discovery of new antimicrobial agents (5, 6, 7). Many plants synthesize secondary active metabolites with powerful antimicrobial activities, some of which have formed the basis for application in pharmaceuticals, alternative medicine and natural therapies (8, 9).

Senna corymbosa (Lam.) H.S. Irwin & Barneby, commonly known as "sen del campo", "rama negra" or "mata negra" (ingl. "Buttercup bush", "Argentine Senna") is a shrub to small tree up to 15 ft. high, unarmed, semi-evergreen, native to South America (Brazil, Paraguay, Argentina and Uruguay). Various species of *Senna* are reported to have laxative, purgative, antidiabetic, anti-inflammatory, antimicrobial, antifungal, hepatoprotective, antimalarial, antipyretic, antiasthmatic, antiviral and wound healing properties (10, 11, 12). The main phytochemical components founded from *S. corymbosa* are anthraquinone glycosides, naphthoquinones and flavonoids (13). It has been reported previously that flavonoids and saponins have antibacterial activity (3, 14, 15, 16, 17).

Tipuana (Fabaceae/Leguminosae: Faboideae, Tribe Dalbergieae) is a monotypic genus represented by a single South American species, *Tipuana tipu* (Benth.) Kuntze. This is a big tree up to 75 ft. high, unarmed, umbrella-shaped, native to northern and northeastern Argentina, Bolivia, Paraguay, Uruguay and Brazil, vulgarly known as "Tipa", "Tipa blanca", "Tipu" or "Palo rosa". This plant is extensively used as a laxative, also by the high content of tannins is used as an astringent. The resin is used by cicatrizant and uterine anti-inflammatory (11). From their leaves, branches and flowers have been isolated lupeol and -amyrin (18).

The objective of the present study was to test the antibacterial activity of dichloromethane, ethyl acetate and ethereal extracts prepared from aerial parts of *Senna corymbosa* and *Tipuana tipu.*

Materials and Methods

Plant Material

The aerial parts (leaves, stem, and fruits) of *Senna corymbosa* and *Tipuana tipu* were collected in San Luis province (Argentine) in summer 2011 and were authenticated by two of the authors. The vouchers specimen (UNSL 535 - *Tipuana tipu* and UNSL 536 - *Senna corymbosa*) were deposited in the Herbarium of UNSL.

Preparation of Extracts

A 10 g of air-dried plant powder (leaves, stem, and fruits) was soaked in 100 ml of organic solvents, viz., petroleum ether, dichloromethane and ethyl acetate separately for 72 h in a round bottomed flask at room temperature. Extracts were filtered through the Whatman filter paper N°1. The ethereal (E), dichloromethane (DMC) and ethyl acetate (EA) extracts were obtained. The extracts were concentrated to dryness under reduced pressure at 37°C using a rotary evaporator. Condensed extracts were weighed and stored in tight containers at 4° C until their use for the antimicrobial tests.

Microorganisms

Antibacterial activity of the extracts was tested individually on gram-positive and gram-negative bacterial strains. The gram-positive bacterial strains used were *Staphylococus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* CLIP 74910 and gram-negative bacterial strains used were *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. Bacterial strains were maintained on Tryptycase soy broth supplemented with 20% glycerol at -80 ° C until use.

Antibacterial Activity

Determination of Minimal Inhibitory Concentration (MIC)

The MICs of *S. corymbosa* and *T. tipu* E, DCM and

EA extracts were determined by micro-well dilution in tripticase soy broth supplemented with 0.01% (W/V) of 2,3,5,-triphenyltetrazolium chloride as visual indicator of bacterial growth (19). The inoculum used was a suspension of microorganisms of 10^7 cfu/ml. The extracts were dissolved in destilled water to the highest concentration to be tested (8000 µg/ml), and then serial two-fold dilutions were made in concentrations ranges from 8000 to 125 µg/ml. The 96-well microplates were prepared by dispensing into each well 95 µl of culture medium, 5 µl of the inoculum and 100 µl serial dilution in base two (8000 to 125 µg/ml) of different extracts. The final volume in each well was 200 µl. The plates were incubated at 37°C for 24 h and read visually. MIC was defined as the lowest concentration of the extracts in the medium in which there was no visible growth after incubation. Media, extract and strains controls were included. The test was performed in duplicate.

Results and Discussion

The antibacterial activity of the extracts from *Senna corymbosa* and *Tipuana tipu* was assessed *in vitro* against Gram positive and Gram negative bacteria. Results are showed in Table 1.

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The best growth inhibitory activity was observed with EA extracts of both plants towards *S. aureus* and *L. monocytogenes* with MIC values of 250 to 500 µg/ml, while these extracts showed less activity against *E. coli* and *P. aeruginosa.* The DCM extracts of both plants were active against all Gram-positive microorganism tested (MIC= 500 µg/ml) but showed weak activity against *Pseudomonas* (MIC=4000 µg/ml) and showed no activity against *E. coli*. The MIC values of E extract of *T. tipu* was 2000 µg/ml and 1000 µg/ml against *S. aureus* and *L. monocytogenes,* respectively, but resulted less active against *P. aeruginosa* (MIC=4000 µg/ml).

The E extract of *S. corymbosa* only showed weak activity against *L. monocytogenes* and *P. aeruginosa* (MIC=4000 µg/ml). As shown in table 1, only the EA extracts of both plants inhibited *E. coli* (MIC= 4000 µg/ml).

In general, Gram positive bacteria were more sensitive to the tested extracts. These results are consistent with some studies finding that Gram positive bacteria are more sensitive to plant extracts than Gram-negative bacteria, which, it has been suggested, may be due to the relatively impermeable outer membrane that surround Gram-negative bacteria (20).

Kansoh et al (21) have reported that leaf extract and volatile oil of flowers of *Tipuana tipu* growing in Egypt showed a broad spectrum antimicrobial effect, while pods extract showed obvious activity on *E. coli*. The MIC of the methanolic and chloroformic extracts of leaves and pods were ranged from 200-600 µg/ml.

The antibacterial activity of *S. corymbosa* found in this work can be attributed to flavonoids or saponins whose presence was reported in previous studies (22).

To our knowledge, in Argentina, there is no scientific information relating to the antimicrobial activity of the plants tested in this study. Further studies of separation and identification of bioactive principles could clarify the properties of these plants. Is also necessary to determine the degree of toxicity of extracts. The results of this study also support the use of these plants for human disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of new bioactive substances.

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Microorganism	*Senna corymbosa* (MIC, µl/ml)			*Tipuana tipu* (MIC, µl/ml)		
	EA	DCM	E	EA	DCM	E
S. aureus ATCC 43300	500	500	NA	500	500	2000
S. aureus ATCC 25923	250	500	NA	500	500	2000
E. coli ATCC 35218	4000	NA	NA	4000	NA	NA
L. monocytogenes CLIP 74910	250	500	4000	250	500	1000
P. aeruginosa ATCC 27853	4000	4000	4000	4000	4000	4000

Table 1: Antibacterial activity of extracts from *S. corymbosa* and *T. tipu*

EA: ethyl acetate;
DCM: dichloromethane;
E: ethereal;
NA: no activity