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BRIEF ARTICLE

Antibacterial activity of *Bauhinia splendens* leaves (Leguminosae)

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Resumen: Varios extratos foliares de *Bauhinia splendens*, una planta medicinal utilizada en Brasil como remedio contra infecciones, se investigaron como agente antibacteriano con el método de difusión y el cálculo de la cantidad mínima inhibitoria (MIC), contra bacterias patógenas Gram-positivas y Gram-negativas. Los extractos menos polares fueron efectivos contra todas las bacterias ensayadas. Los extractos en DCM y en EA produjeron zonas de inhibición de aproximadamente 20 mm (2.5 mg/disco) mientras que el extracto AQE resultó inactivo. Los valores de MIC contra *Salmonella typhimurium* fueron 0.7 mg/ml para el extracto en DCM y 0.8 mg/ml para el extracto en EA. Cuando se ensayaron contra *Staphylococcus aureus*, ambos extractos mostraron valores de MIC de 0.4 mg/ml. Estos efectos sustentan el uso popular de *B. splendens* en la medicina folclórica para el tratamiento de enfermedades infecciosas.

Key words: *Bauhinia splendens*, folk medicine, antibacterial activity, diffusion method, minimum inhibitory concentration, phenolic compounds.

Bauhinia splendens (Leguminosae) is a native medicinal plant, which occurs in several regions of Brazil, and is known as cipó-escada, escada-de-jabuti or escada-de-macaco. Its leaves and bark are used in traditional medicine against several disorders, including infections, inflammations, affections of the urinary tract, etc. (Pio Correia 1984, Cirilo 1993).

We have recently demonstrated that some extracts obtained from leaves, bark and roots of this plant were active as analgesic when evaluated in several experimental models of pain on mice (Breviglieri *et al.* 1995; Cechinel Filho *et al.* 1995).

Phytochemical studies carried out with the barks of *B.splendens* revealed the presence of b-sitosterol, stigmasterol, stearic acid and a new flavone denoted bausplendin (Laux *et al.* 1985) whereas some flavonoids, such as rutin, quercetin and quercetrin, and gallic acid ethyl ester were isolated from leaves of this plant (Breviglieri *et al.* 1995; Cechinel Filho *et al.* 1995).

In the present study, we have examined the possible antibacterial effects of some extracts obtained from leaves of *B.splendens* against several pathogenic microorganisms, by the agar diffusion method (Bauer *et al.* 1966) and a minimum inhibitory concentration (MIC) method (Mitscher *et al.* 1972).

Plant material and chemical extraction: The leaves of *B.splendens* were collected in Urussanga (state of Santa Catarina - BRAZIL) in June 1995 and the vouchers were deposited in FLOR Herbarium (UFSC). The hydroalcoholic extract was obtained in usual manner and it was then partitioned successively with dichloromethane (DCM) and ethyl acetate (EA). The water phase was called aqueous extract (AQE).

Bacteriological Assays

Diffusion Method: The extracts of *B.splendens* were investigated by the agar diffusion method (Bauer *et al.* 1966) against *Salmonella sp* and *Streptococcus sp*, both strains isolated clinically, and against *Staphylococcus aureus* ITAL SFCO 001, obtained from ITAL (Instituto de Tecnologia de Alimentos) in Campinas, São Paulo, BRAZIL. Tetraciclin (30 mg) was used as a standard for control which produced inhibition zones of 30-40 mm.

Minimum Inhibitory Concentration (MIC) Method: The extracts were investigated by determining the MIC in the presence of *Salmonella typhimurium* (ITAL-ESLML-(X)1) and *Staphylococcus aureus* (ITAL - SFCO-001). The MIC values were determined by a technique previously described (Mitscher *et al.* 1972).

Tables 1 and 2 show the antibacterial activity of different extracts obtained from leaves of *B.splendens*, using diffusion method and minimum inhibitory concentration (MIC) method, respectively. As can be observed, the results indicated in Table 1 show that DCM and EA extracts exhibit interesting antibacterial activity in all concentrations employed against *Salmonella sp*. Inhibition potential as measured by the inhibition zone halo for DCM were 19, 25 and 29 mm, in the concentrations of 1.25, 2.5 and 3.75 mg, respectively. In the same concentrations, the inhibition zone for EA was 20, 25 and 26 mm, respectively. Zero values for inhibition halos were obtained for the AQE. This fact suggest that the less polar chemical constituents which are present in leaves of *B.splendens* are responsible, at least in part, for the effects above observed. On the other hand, DCM extract was also active against *Streptococcus sp* and *Staphylococcus aureus*, with inhibition zone of 26 and 18 mm, respectively, in the concentration of 2.5 mg/disk. The EA extract was active against *Streptococcus*, with inhibition zone of 24 mm (2.5 mg/disk), but was inactive against *S.aureus*, indicating different kinds of active compounds. As demonstrated for *Salmonella*, AQE was also inactive against both strains, confirming the view that less polar substances exert antibacterial activity, in contrast with more polar compounds. Control experiments were carried out involving the same microorganisms and the same solvent.

Table 2 shows the MIC obtained for the extracts of *B. splendens* The results indicated that DCM and EA exhibited effect against both microorganisms tested. The MIC values determined for DCM against *S.typhimurium* was 0.7 mg/ml and against *S.aureus* was 0.4 mg/ml, whereas EA exhibited MIC values of 0.8 and 0.4 mg/ml, respectively. Both strains were resistant until 1.0 mg/ml of AQE, confirming the lack of activity of this more polar extract, shown by diffusion method.

These effects may be related with the phenolic compounds previously isolated from EA extract of this plant (Breviglieri *et al.* 1995; Cechinel Filho *et al.* 1995). However, considering the high effect presented by DCM, we

TABLE I

Antibacterial activity of different extracts obtained from B. splendens. *The results are expressed as average inhibition zone diameter (mm) in diffusion assays*

Extract	Concentration *	Zone of Inhibition (mm) *Salmonella* sp.	*Streptococcus* sp.	*S. aureus*
DCM	1.25	19	NT**	NT
	2.50	25	26	18
	3.75	29	NT	NT
EA	1.25	20	NT	NT
	2.50	25	24	0
	3.75	26	NT	NT
AQE	1.25	0	0	0
	2.50	0	0	0
	3.75	0	0	0

* Amount (mg) contained in each disk solubilized in DMSO (DCM and EA) or EtOH 50 %, (AQE); ** Not tested

TABLE 2

Minimum inhibitory concentration (MIC) in mg/ml for extracts obtained from B. splendens

Extract	MIC (mg/ml) *Salmonella typhimurium*	*Staphylococcus aureus*
DCM	0.7	0.4
EA	0.8	0.4
AQE	> 1.0	> 1.0

believe that other active compounds less polar should be present in leaves of *B.splendens*. The chemical and biological studies are now in progress in our laboratories in order to determine the active constituents responsible for the antibacterial activity shown in this study.

In conclusion, our results demonstrated that some extracts obtained from the leaves of *B.splendens*, mainly the less polar, exhibit antibacterial properties against pathogenic bacteria, which may consist in new therapeutic possibilities. Futhermore, these results confirm the popular use of this plant against infections and suggest that besides its analgesic effects (Cechinel Filho *et al.* 1995), *B. splendens* may be effective as antibacterial agent.

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