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Journal of Ethnopharmacology 87 (2003) 163–167
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Biological activity of extracts from *Catalpa bignonioides* Walt. (*Bignoniaceae*)

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Received 19 February 2002; received in revised form 17 March 2003; accepted 21 March 2003

Abstract

Catalpa bignonioides Walt. (*Bignoniaceae*) is a species that belongs to a tropical family but has been introduced in many countries as ornamental. Although this plant is consumed by indigenous cultures of South America for medical uses, experimental studies of the biological properties of *Catalpa bignonioides* are lacking. The aim of this work was to study the biological activity of crude extracts from either pods, seeds or leaves of *Catalpa bignonioides* which were collected in Spain. Ethyl ether, butanolic and aqueous fractions of the pod extract were also prepared and studied. We have examined the antimicrobial activity against five bacteria and one yeast, the cytotoxic activity against HepG2 cells and the anti-inflammatory and antinociceptive effects in rodents. A preliminary phytochemical analysis of the extracts and fractions was also conducted. Results showed no antimicrobial or antitumoral effects, but prominent anti-inflammatory and antinociceptive actions of the extracts. These last activities may be a result of the presence of either of saponins, sterols or phenols, mainly found in the leaves and pods of the plants.

Keywords: *Catalpa bignonioides*; Extracts; Biological activities

1. Introduction

Species belonging to the *Bignoniaceae* are widely used for many different purposes in medicinal practice by the indigenous cultures of South America (Rasadah and Houghton, 1998). Some of these species, like *Jacaranda micranta* Cham., *Tabebuia caraiba* (Mart.) Bureau, *Tecoma sambucifolia* Kunth and *Tecoma stans* (L.) Juss. ex Kunth are known for the anti-inflammatory, antirheumatic, antinociceptive, narcotic or antisyphilitic activity of their extracts. The *Catalpa* Scop. genus, which belongs to this family, comprises 11 species of trees and shrubs native to East Africa and North and South America. Outside the tropics, several species are found in cultivation as greenhouse ornamentals. Some extracts from the *Catalpa* species have shown interesting biological properties: extracts from *Catalpa ovata* G. Don fructus have mutagenic activity towards *Salmonella typhimurium* (Nozaka et al., 1989), while its stem-bark extracts have antitumoral activity (Fujiwara et al., 1998).

Regarding properties and uses of *Catalpa bignonioides*, commonly known as Bean-tree, which were already mentioned in the physiomedical dispensatory of Cook (1869), the bark of *Catalpa bignonioides* is cited as a stimulating tonic in syrup form, the decoction of the pods as a demulcent with relaxant and stimulant properties and the leaves are described as useful for irritable ulcers. *Catalpa bignonioides* has also been described for the treatment of respiratory diseases (decoction of pods and seeds), irritable scrofulous ulcers (cataplasms of bruised leaves), strumous

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0378-8741/03/$ – see front matter © 2003 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S0378-8741(03)00111-9

ophthalmia, cutaneous affections (juice of leaves and roots), scrofulous maladies and helmintic infections (decoction or powder of barks). Despite all these potential beneficial effects, experimental studies of the biological properties of *Catalpa bignonioides* extracts are lacking.

Phytochemical studies (phytochemDB) show the presence of several classes of compounds in extracts of *Catalpa bignonioides*, which could be linked to some of the traditional uses of *Bignoniaceae*. These include fats, sugars, terpenes, alkaloids, tannins and, particularly, flavonoid and phenolic compounds, which were detected in high amounts. Previously Rau (1870; King's American Dispensatory) made an examination of the inner bark and found it to contain tannin and a nauseating matter soluble in ether. Sugar, tannin, resin and fixed oil are constituents of the seeds.

In this paper we report a preliminary phytochemical analysis and biological screening on bactericidal, cytotoxic, anti-inflammatory and antinociceptive activities of aqueous extracts from leaves, pods and seeds of *Catalpa bignonioides*. These activities were chosen in part on the basis of previous findings of our team regarding the biological activity of *Tecoma sambucifolia*, another *Bignoniaceae* plant (Alguacil et al., 2000). The pod extract was further fractionated in order to approach the isolation of the possible active principles.

2. Methodology

2.1. Plant material

Catalpa bignonioides specimens were collected in Boadilla del Monte (Madrid, Spain) and identified by Dr. Galán de Mera, USP (San Pablo University), Madrid. A voucher specimen was deposited in the USP herbarium under the number 250399. Collected material was dried under darkroom conditions.

2.2. Plant extracts

Crude extracts of leaves, pods, and seeds were prepared by decoction of 10 g of each pulverized material in 200 ml of water for 30 min. The resultant extracts were then filtrated and concentrated to dryness under reduced pressure. The yield was 22.9% (w/w) for pods, 14.1% (w/w) for leaves and 5.3% (w/w) for seeds.

The concentrated aqueous extract of pods was fractionated by successive extraction with ethyl ether (3 × 100 ml) and butanol (3 × 100 ml). An ethyl ether (P-E, 3.94% w/w), a butanol (P-B, 28% w/w) and an aqueous fraction (P-A, 55.2% w/w) were obtained.

2.3. Preliminary phytochemical analysis

Plant materials were screened for the presence of saponins, tannins, total phenols, anthraquinones, flavonoids and sterols by using the methods previously described by Tona et al. (1998).

2.4. In vitro cytotoxicity assay

The cell line used was human hepatoma cell line (HEpG2) obtained from the American Type Culture Collection (ATCC, HB 8065). The cell line was grown on Dulbeco's Mem medium supplemented with 10% fetal bovine serum (FBS), and incubated at 37 °C in an atmosphere of 5% CO_2 in air (95% humidity). At a log phase of their growth cycle, the cells were treated in triplicate with various concentrations of the extracts (between 25 and 0.1 mg/ml) with a final dimethylsulfoxide (DMSO) concentration of 0.1%. This concentration of DMSO did not affect cell viability.

The assay was performed using a 96-well plate which contained 2500 cells per well incubated with the corresponding extract, during 72 h, as previously described. Test wells containing the cells alone, free of plant extracts were used as controls. The MTT assay, according to Borenfreund et al. (1988), was used to obtain the effective dose that inhibits 50% growth after the incubation period (IC_{50}).

2.5. Antimicrobial assays

The bacterial growth inhibition assays were performed using cultures of *Escherichia coli* (ATCC 35219), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 24213), *Enterococcus faecalis* (ATCC 29212), *Salmonella tiphymurium* (ATCC 13311), and the yeast *Candida albicans* (ATCC 10231). Bacteria strains were maintained on Mueller-Hinton broth and the yeast on Sabourand's dextrose agar.

The diluted extract suspension was homogenized and the screening was then performed according to the liquid dilution method (Vanden Berghe and Vlietinck, 1991). Minimum inhibitory concentration (MIC) was determined by incorporating various amounts (1–200 mg/ml) of reconstituted extract solution into the medium.

The MIC was interpreted as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the control.

2.6. In vivo pharmacological assays

2.6.1. Animals and sample preparation

Male Sprague–Dawley rats (200–250 g; San Pablo-CEU University breeding) and male OF1 mice (25–30 g, Iffa-Credo, France) were used. The animals were housed in cages with water and food available ad libitum, kept in a controlled environment (temperature, 20–22 °C; dark/light, 12 h/12 h, humidity, 45–55%) and randomly assigned to the different experimental groups. The aqueous extracts were dissolved in physiological saline for i.p. administration (10 ml/kg) at a dose of 1 g/kg. The doses of the fractioned

pod extracts were calculated to provide an amount of the different substances equivalent to that of the crude pod extract, i.e. 40 mg/kg for P-E, 300 mg/kg for P-B and 555 mg/kg for P-A.

2.6.2. Acetic acid writhing in the mouse

The test was performed as described by Koster et al. (1959). Nociception induced by acetic acid was characterized by the presence of abdominal constrictions, consisting of the contraction of the flank muscles associated with inward movements of the hind limbs or with whole body stretching. Animals were injected with the extracts from *Catalpa bignonioides*, saline or indomethacin (3 mg/kg; Sigma, Spain), which was used as a reference compound. Thirty minutes after treatment, all the animals received 2% acetic acid i.p., and 10 min afterwards, the number of abdominal constrictions was recorded for 15 min by visual observation of the animals.

2.6.3. Carrageenan-induced hind paw edema in the rat

Carrageenan-induced inflammation was initially described by Winter et al. (1962) and has become a widely used model for screening anti-inflammatory agents. At the beginning of the study, the baseline paw volume was determined by submerging the right hind paw up to the tibiotarsal joint into a water cell of a plethysmometer (Digital Pletysmomet, L37500; Letica). The volume of displacement, which is equal to the paw volume, was then read on a digital display. Each determination was the average of three repeated measures. After baseline determination, the rats were injected with the extracts from *Catalpa bignonioides*, saline or indomethacin (5 mg/kg; Sigma), which served as a positive control. One hour afterwards, the rats were subcutaneously injected with 0.1 ml of 1% lambda carrageenan (Sigma) into the surface of the right hind paw, and the effect on paw volume was studied 1, 2 and 3 h post injection.

3. Statistical analysis

Statistical analysis was performed with ANOVA followed by multiple range tests (least-squares difference test, LSD). Differences were considered significant at $P < 0.05$.

4. Results

4.1. Phytochemical study

The results of our assay on the crude extracts and fractions are shown in Table 1. All crude extracts showed the presence of saponins (triterpenic and steroidic), sterols and phenols and were negative for anthraquinones. Regarding pod fractions, saponins were detected in P-A, sterols in P-A and P-B and flavonoids in P-B and P-E.

Table 1
Phytochemical screening and cytotoxic activity, against HEpG2 cells, of crude aqueous extracts and fractions from *Catalpa bignonioides*

	Anth.	Sap.	Este.	Tan.	T. Ph.	Flav.	IC_{50} (mg/ml)
Crude extract							
Seeds	−	+	+	−	+	−	28
Leaves	−	+	+	−	+	−	23.6
Pods	−	+	+	+	+	+	10.9
Pod fractions							
P-A	−	+	+	−	+	−	3.5
P-B	−	−	+	+	+	+	0.68
P-E	−	−	−	−	−	+	3.58

Anth.: anthraquinones, Sap.: saponins, Este.: sterols, Tan.: tannins, T. Ph.: total phenols, Flav.: flavonoids. P-A: aqueous fraction, P-B: butanol fraction, P-E: ethyl ether fraction.

4.2. Cytotoxicity assay

The IC_{50} (mg/ml) values obtained in the cytotoxicity assay against the HepG2 cell line are shown in Table 1. A very low toxicity was found in all extracts and fractions. The highest toxicity was found in the crude extract of pods and fraction P-B.

4.3. Antimicrobial activity

The inhibitory bacterial growth by extracts, indicated by their MIC values, is summarized in Table 2. None of the extracts examined showed a significant bactericidal activity, since the lower MIC (showed by pods against *Salmonella tiphymurium*) was 3.12 mg/ml. No effects were observed in yeast analysis, as the MIC obtained was higher than 200 mg/ml in all cases.

4.4. Anti-inflammatory activity

The results of this study are shown in Table 3. The extracts of leaves and pods exhibited a significant anti-inflammatory activity of similar magnitude 2 and 3 h after carrageenan injection, while the extract of seeds was devoid of significant effects during all the periods considered. Although the three pod fractions exhibited some activity in the test, only P-A provided a marked and sustained effect throughout the evaluation period considered. P-E only produced a transient decrease of paw edema, and P-B also provided a slight effect that achieved statistical significance 3 h post injection.

4.5. Antinociceptive activity

The effects obtained in the antinociception experiments were qualitatively similar to those of the anti-inflammatory study (Fig. 1). Although the three crude extracts exhibited some pharmacological activity, the extract of seeds was the least potent and the extracts of pods and leaves behaved similarly. Concerning the pod fractions, P-A was again the

Table 2
Antibacterial activity of crude aqueous extracts and fractions from *Catalpa bignonioides* (MIC, mg/ml)

	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Salmonella tiphymurium*
Crude extract					
Seeds	100	100	50	50	50
Leaves	6.25	100	12.5	12.5	6.25
Pods	6.25	50	12.5	12.5	3.12
Pod fractions					
P-A	25	50	25	25	6.25
P-B	>200	>200	100	>200	>200
P-E	>200	>200	100	100	>200

P-A: aqueous fraction, P-B: butanol fraction, P-E: ethyl ether fraction.

Table 3
Anti-inflammatory effect of crude aqueous extracts and fractions from *Catalpa bignonioides* on carrageenan-induced hind paw inflammation in the rat

Treatment	*n*	Paw volume (ml) after carrageenan injection			
		Baseline	1 h	2 h	3 h
Saline	15	1.53 ± 0.02	2.00 ± 0.03	2.64 ± 0.10	2.91 ± 0.10
Indomethacin	14	1.55 ± 0.02	1.79 ± 0.03*	2.18 ± 0.04*	2.43 ± 0.04*
Crude extract					
Seeds	6	1.56 ± 0.01	1.92 ± 0.07	2.62 ± 0.20	2.86 ± 0.25
Leaves	6	1.59 ± 0.04	1.93 ± 0.03	2.15 ± 0.10*	2.31 ± 0.22*
Pods	16	1.61 ± 0.02	1.92 ± 0.04	2.10 ± 0.11*	2.25 ± 0.11*
Pod fractions					
P-A	7	1.51 ± 0.04	1.77 ± 0.06*	2.15 ± 0.13*	2.37 ± 0.15*
P-B	9	1.53 ± 0.03	1.88 ± 0.04	2.35 ± 0.10	2.56 ± 0.12*
P-E	7	1.48 ± 0.02	1.81 ± 0.04*	2.33 ± 0.07	2.68 ± 0.12

P-A: aqueous fraction, P-B: butanol fraction, P-E: ethyl ether fraction.
* $P < 0.05$ vs. saline.

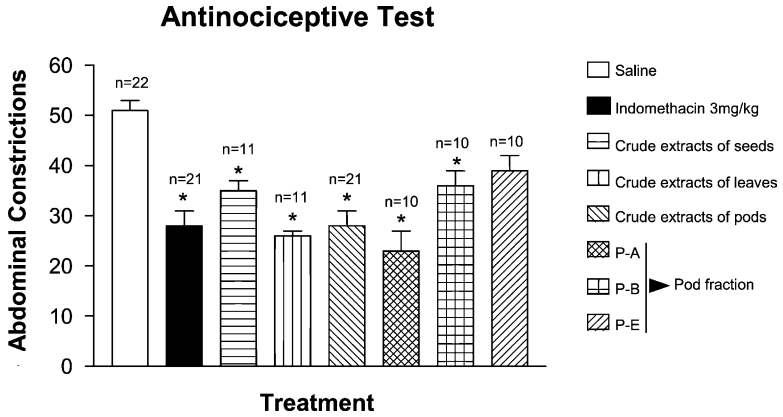

Fig. 1. Effect of aqueous extracts of *Catalpa bignonioides* on abdominal constrictions produced by acetic acid. Bars are means ± S.E.M. * $P < 0.05$ vs. saline. (P-A: aqueous fraction, P-B: butanol fraction, P-E: ethyl ether fraction).

most active; on the other hand, the effect of P-E injection did not reach statistical significance.

5. Discussion and conclusions

The antitumoral activity of species such as *Catalpa ovata* (Kingston and Rao, 1982; Fujiwara et al., 1998) has been related to phenolic compounds like naphtoquinones. Since the phytochemical analysis of aqueous extracts from *Catalpa bignonioides* show the presence of phenolic compounds, the cytotoxic effect observed is considerably low and has little relevance against the human hepatoma cell line (HEpG2), it has been suggested that the reputed antimicrobial activity of some species of *Bignoniaceae* like *Kigelia pinnata* (Jacq.) DC (Akunyili et al., 1991), *Jacaranda mimosifolia* D. Don, *Tecoma stans*, *Crescentia cujete* L. (Binutu and Lajubutu, 1994) *Tabebuia spectabilis* (Planch. and Linden)

G. Nicholson, and *Oroxylum indicum* (L.) Vent. (Rasahad and Houghton, 1998) could be linked to another kind of substances, the iridoids. Although these compounds have previously been reported in *Catalpa bignonioides* (Iwagawa et al., 1991; McDaniel, 1992), the isolation of iridoids was not favored in our study and therefore would explain the very low effect obtained.

All these results indicate that the potential antitumoral and antimicrobial activities of *Catalpa bignonioides* extracts appear to be of lesser therapeutic value than those of other *Bignoniaceae*; however, this conclusion can be strictly applied only to the Spanish specimen of this species and we cannot rule out that other tropical plants might contain higher amounts of active substances or even different compounds of interest.

In contrast, the anti-inflammatory and antinociceptive effects of the extracts studied were quite significant. These findings are coherent with the similar pharmacological profile of the extracts obtained from other *Bignoniaceae*, such as *Tecoma sambucifolia* (Alguacil et al., 2000). The crude extracts of pods and leaves were more active with respect to those of seeds. This result, combined with the phytochemical analysis, suggests that the biological activity can be attributed either to saponins, sterols or phenolic compounds, while the contribution of flavonoids does not seem probable. On the other hand, the aqueous fraction of the pods is the one that retains the highest pharmacological activity. In this sense, the substances that could be expected to play a significant role in the case of *Catalpa bignonioides* are steroidic saponins, which possess hydro-aromatic ring systems similar to those of steroids (Gupta et al., 1969).

In summary, neither antitumoral nor antimicrobial activities were detected in crude aqueous extracts of *Catalpa bignonioides* but it has an anti-inflammatory and antinociceptive potential, which is probably related to the saponins, sterols or phenols contained in its leaves and pods. Further fractioning experiments are in progress to isolate the active principles responsible for these biological activities.

Acknowledgements

We wish to express our gratitude to Linda Hamalainen for her linguistic help.

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