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Antimicrobial potentials of some plant species of the bignoniaceae family

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Summary

The methanol extracts of the leaves and stem bark of four Bignoniaceae plants *Jacaranda mimosifolia* D. Dol., *Tecoma stans Linn.*, *Tabebuia rosea* (Bertol) D.C., and *Crescentia cujete* Linn. were studied for their antimicrobial activity using a wide range of Gram-positive and Gram-negative bacteria and fungi. Extracts of both the leaves and stem bark of majority of plant species studied showed variable but remarkable broad spectrum antimicrobial activity. However, methanol extracts of *Tecoma stans* leaves was found to be effective against only *Candida albicans* at the concentrations employed. It was observed that the extracts of stem bark generally showed better antimicrobial activity than those of the leaves and some organisms were selectively more sensitive to the extracts than others. Preliminary phytochemical screening of these plants revealed the presence of tannins, flavonoids, alkaloids, quinones and traces of saponins. The antimicrobial activity observed are discussed in relation to the chemical constituents reportedly isolated from these plants and their traditional uses.

Résumé

Les estraits méthanoliques des feuilles et des écorces des tiges de quatre plantes bignoniaceae à savoir *le Jacaranda, mimosifolia* D. Dol., *le Tecoma stans* Linn *le Tabebuia rosea* (Bertol) d.C. et *le Crescentia cujete* Linn, sont étudiés à cause de leur activité antimicrobique utilisant une large rangee de bacteria et de funge de Gramme-positif et de Gramme negatif. Les extraits des deux feuilles et écorces de tige de la majorité des plantes relevées et étudiées ont montré une variable mais remarquable spectre activité antimicrobique. Pourtant les estraits methanoliques des feuilles *Tecoma stans* semble être effectiffs pour une seul *Candida albecans* de la concentration entamude. On a observé que les extraits des écorces de tige ont generallement montré une activité antimicrobique plus efficace que ceux des feuilles et quelque organismes étaient plus sensibles aux extraits que les autres. L'abritage phytochimique préliminaire de ces plantes a revelé la presence de tannines, de flavonoides, d'alkaloides, de quinones et de traces de saponines. L'activité antimicrobique observée est discuté en relation avec les constituants chimiques tirés des plantes et leur usages traditionnels.

Introduction

The family Bignoniaceae is a family of Tropical trees, shrubs or lianes of about 120 genera and 65 species. Some of the genera in this family include *Jacaranda, Catalpha, Tecoma, Crescentia, Kigelia, Amphicome* and *Newbouldia* among others[1]. Many of these plants have been reportedly used traditionally for the treatment of various bacterial infections such as bronchitis, pneumonia, tonsillitis, enteritis, syphillis and others[2].

Information on *in-vitro* antimicrobial activity of these plants is however not available in the literature, as of now, hence this study was designed to identify their antimicrobial potentials and justify scientifically their antimicrobial relevance in traditional medicine.

Materials and methods

Plant material: Authenticated samples of leaves and stem bark of *Tecoma stans, Crescentia cujete, Tabebuia rosea* and *Jacaranda mimosifolia* plants collected from the Botanical Garden of the University of Ibadan during the raining season, were oven dried at 50°C and pulverised.

Preparation of plant extracts: Coarsely powdered material weighing 10 g was soxhlet extracted with methanol for 24 hours. The extracts were concentrated to dryness under vacuum and the

residue was resuspended in the same extracting solvent to a concentration of 10 mg/ml and stored at 4°C until used.

Organisms: The microorganisms employed in this study consisted of gram-positive and gram-negative bacteria and two fungi listed in Table 1.

Table 1: List of microorganisms used to assess the antimicrobial activity of the plant extracts

	Organisms	Relevant properties	Source
1.	*Staphylococcus aureus* NCTC 6571		Dept. of Pharmacy, University of Strathclyde, Glasgow
2.	*S. aureus* UCH 7059	Sensitive to all antibiotics	Clinical isolate
3.	*S. aureus* UCH 7127	Resistant to P and Te	Clinical isolate
4.	*S. aureus* UCH 7148	Resistant to P, Te and C	Clinical isolate
5.	*S. aureus* UCH 502	Resistant to Cro	Clinical isolate
6.	*S. aureus* UCH 486	Resistant to Gn and Cro	Clinical isolate
7.	*Escherichia coli* Enteroreference 36004		Dept. of Pharmacy, University of Strathclyde, Glasgow
8.	*E. coli* UCH 7185	Resistant to Am, Te, and S & T	Clinical isolate
9.	*Pseudomonas aeruginosa* NCTC 6750		Dept. of Pharmacy, University of Strathclyde, Glasgow
10.	*Pseudomonas aeruginosa* UCH 7110	Resistant to Py	Clinical isolate
11.	*Pseudomonas aeruginosa* UCH 495	Resistant to Py and Cro	Clinical isolate
12.	*Pseudomonas aeruginosa* UCH 490	Resistant to Py, Gn and Cro	Clinical isolate
13.	*Pseudomonas aeruginosa* UCH 7093	Resistant to Py and Gn	Clinical isolate
14.	*Bacillus subtilis*		Laboratory stock
15.	*Bacillus cereus*		Laboratory stock
16.	*Corynebacterium diphtheriae*		Laboratory stock
17.	*Streptococcus pyogenes*		Laboratory stock
18.	*Klebsiella edwardsiella* NTCT 10896		Laboratory stock
19.	*Proteus mirabilis* ATCC 14273		Laboratory stock
20.	*Candida albicans*		Laboratory stock
21.	*Aspergillus niger*		Laboratory stock

Gn Gentamycin; Py, Carbenicillin (pyopen); Cro Rocephine (Ceftriazone); P, Penicillin; Te, Tetracycline; C, Chloramphenicol; Am, Ampicillin; S & T, Septrin (Cotrimoxazole).
ATCC : American Type Culture Collection, NCTC: National Collection of Type Cultures
UCH : University College Hospital Collection.

Media: Nutrient broth No. 2 pH 7.4, nutrient agar pH 7.4, Malt extracts broth pH 5.6 and sabouraud dextrose agar pH 5.4, all products of Oxoid Laboratories, England were used in this study.

Antimicrobial agents: The following chemotherapeutic agents were included in the test as controls; Gentamicin sulphate 1 µg/ml. (Nicholas Laboratories Limited, England). Tetracycline hydrochloride 0.1 µg/ml (Pfizer Inc. Nigeria) and Tioconazole 1% $^{w}/_{w}$ (Pfizer Inc. New York).

Phytochemical Screening: The qualitative chemical analysis of the powders were carried out for the

presence of alkaloids, volatile oils, tannins, saponins, steroid, cardiac glycosides, cyanogenetic glycosides, anthraquinones and flavonoids using the methods adopted in similar surveys[3,4].

Antimicrobial activity determination: A 0.1 ml of a 1 in 100 dilution of overnight broth culture of each bacteria (equivalent to 10^6 - 10^7 cells/ml) was used to seed sterile molten nutrient agar medium maintained at 45°C. Sterile Sabouraud dextrose agar plate was similarly seeded with the fungi. They were allowed to set and wells (6 mm in diameter) were made on them using a sterile standard cork borer. A 0.2 ml of a 100 mg/ml solution of each test sample dissolved in methanol was added into each well. The bacterial seeded plates were incubated at 37°C for 24 hours and the fungal seeded plates were incubated at 25°C for 3 days after which zones of inhibition were measured. Each plate has wells filled with each of methanol, gentamicin and tetracycline, if seeded with bacteria and filled with methanol and tioconazole cream if seeded with fungi as control.

Results

Phytochemical screening showed in Table 2 that the most common constituents found in these plants are tannins, flavonoids saponins and quinones. One of them, *Tecoma stans* contained mainly alkaloids.

Table 2: Phytochemical screening of some Bignoniaceae plants

Botanical source	Part	Phytochemical groupings						Volatile oils	Cyanogenetic glycosides	Cardiac glycosides
		Alkaloids	Flavonoids	Tannins	Saponins	Anthraquinones	Steroids			
Tabebuia rosea	L	—	++	+++	+	+	+	—	—	—
	SB	—	++	++	+	+	++	—	—	—
Jacaranda mimosifolia	L	+	+++	+++	++	++	++	—	—	—
	SB	—	+++	+++	++	++	++	—	—	—
Tecoma stans	L	+++	+	++	—	+	+	—	—	—
	SB	+++	+	++	—	++	++	—	—	—
Crescentia cujete	L	—	+++	+++	+	++	++	—	—	—
	SB	—	++	++	—	+	++	—	—	—

Constituents — = Not detectable + = low concentration ++ = Medium Concentration
+++ = High concentration ± = Traces
L = Leaf SB = Stem bark.

Results of antimicrobial activity is shown in Table 3. All extracts of both the leaves and stem bark of each plant showed variable but remarkable broad spectrum inhibitory activity against most bacterial species tested and *Aspergillus niger*. Only the methanolic extract of leaves of *Tecoma stans* showed slight inhibitory activity against *Candida albicans* at the concentration used. Of all the extracts from these plant species, *Jacaranda mimosifolia* stem bark extract and *Tabebuia rosea* leaves extract were the most active against the organisms. The most susceptible organisms to majority of these extracts were *Corynebacterium diphtheriae, Klebsiella edwardsiella* and *Aspergillus niger*. The extracts of stem bark generally showed better antimicrobial activity than those of the leaves.

It is interesting to note that some strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* which were resistant to both tetracycline and gentamicin were found to be sensitive to some of the extracts under test. Methanol used to resuspend the extracts was tested and found to have no appreciable inhibitory activity against any of the organisms.

Table 3: Antimicrobial activity of methanolic extracts of some Bignoniaceae plants

Test microorganisms number[a]	*Tabebuia rosea*		*Jacaranda mimosifolia*		*Tecoma stans*		*Crescentia cujete*		Gentamicin	Tetracycline	Tioconazole
	SB	L	SB	L	SB	L	SB	L			
1	+	+	+	—	+	+	+	—	++++	+++	NT
2	—	+	+	—	+	—	+	+	—	—	NT
3	+	+	+	++	+	+	+	++	++++	—	NT
4	+	+	+	+	+	+	+	+	++	—	NT
5	—	+	+	+	+	+	+	+	++++	—	NT
6	+	+	+	+	+	+	+	+	—	—	NT
7	+	+	+	+	+	—	+	+	++++	+++	NT
8	—	—	—	—	—	—	—	—	—	—	NT
9	—	—	++	++	++	+	++	++	++	—	NT
10	+	+	++	+	+	+	++	++	++++	—	NT
11	—	+	—	—	+	—	+	—	++++	—	NT
12	+	+	+	+	++	++	+	+	—	—	NT
13	—	—	+	+	++	++	+	+	—	—	NT
14	+	+	++	—	—	++	++	++	++++	++++	NT
15	+	+	+	—	+	—	+	—	++++	++++	NT
16	++	+++	++++	+++	++++	+++	+++	+++	++++	++++	NT
17	—	+++	++++	+	+	+	++	++	++++	++++	NT
18	—	+++	++++	+	++	+	++	+++	++++	+++	NT
19	—	+++	++++	+	+	—	+	+	++++	+	NT
20	—	—	—	—	—	+	—	—	NT	NT	++++
21	—	+++	++++	++++	++	+	++	++	NT	NT	++++

a. Each organism number refers to the corresponding one in Table 1
Inhibition zones + = 8-10 mm ++ = 11-13 mm, +++ = 14-17 mm ++++ = 18 mm
— = no zone NT = not tested

Discussion

The results obtained in this study may justify the scientifically undetermined reason for the use of the plants in the family bignoniaceae in treatment of various kinds of bacterial related infections such as bronchitis, pneumonia, enteritis and syphyllis. Their antimicrobial activity appeared broad spectrum since both Gram-positive and Gram-negative organisms and filamentous fungi were sensitive. Although the zones of inhibition of the antibiotics control included, the effectiveness of the extracts even against pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which in some cases were resistant to gentamicin and tetracycline is interesting.

Antimicrobial property exhibited by these plants could be traced to the possession of tannins, flavonoid, quinones and traces of saponins which are known to have antimicrobial activity. Lapachol, a naphthoquinone in the heart wood and barks of many Bignoniaceae plants especially the tribe of Tecomeae has been shown to have antimicrobial and antineoplastic properties[5]. The possible presence of this compound or others related to it could explain the higher microbial inhibitory activity observed in the stem bark. Extracts of various medicinal plants containing flavonoids have been reported to possess antimicrobial activity[6]. The antibacterial activities of isoflavonoids and flavonoids[7,8] have been reported. Tannins have also been implicated with antimicrobial properties[9]. The presence of these groups of compounds may therefore account for their activity. Flavonoids have been reported in the leave of *Jacaranda mimosifolia* [10].

Since the leaves and barks of these plants have been used in different African communities including Nigeria for various ailments with no reported adverse or toxic effects, phytochemical studies are in progress to isolate specific compounds in these plants responsible for the antimicrobial activity.

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