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Chemical profiles and antimicrobial activities of plants utilized in Brazilian traditional medicine

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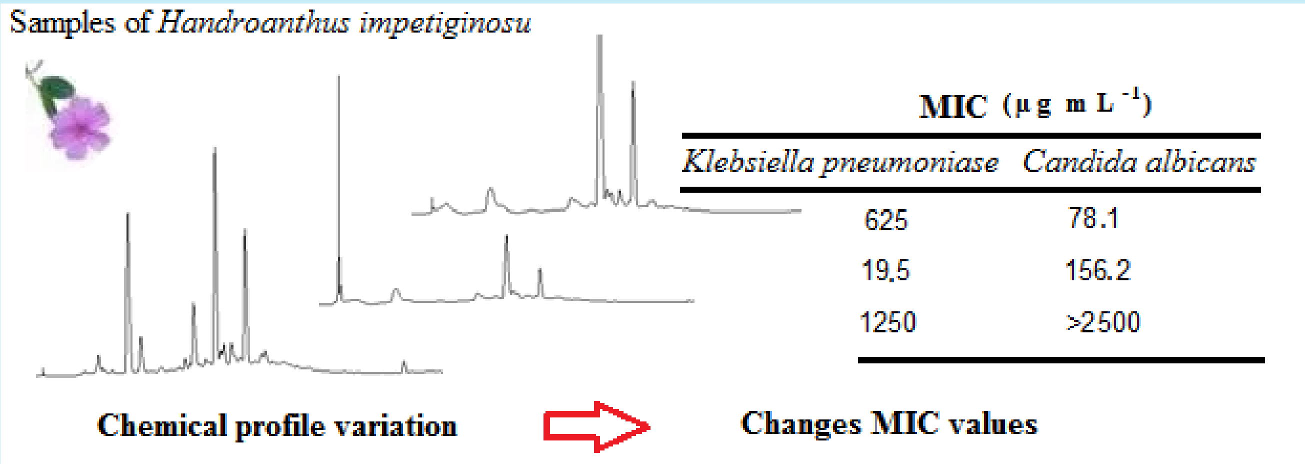
ABSTRACT: Medicinal plants are used for primary health care in many countries. In Brazil, there are hundreds of street markets selling a wide variety of herbs for medicinal purposes without quality control or scientific evidence; instead, their purported efficacy is based exclusively on empirical ethnobotanical knowledge. The present study evaluated the effectiveness of five medicinal plants widely utilized in Brazil to treat infections, as well as determined their chemical profiles. The results revealed that the five plants investigated (*Anacardium occidentale* L., *Handroanthus impetiginosus* Mart., *Bumelia sartorum* Sw., *Zornia brasiliensis* Vogel and *Cnidosculus urens* Pohl) demonstrated moderate to strong antimicrobial activity against most fungi and bacteria tested, principally for infections caused by gram-negative bacteria. The extracts of four plants exhibited MIC of 19.5 µg mL^{-1} against the bacterium *Escherichia coli.* The results also confirmed that these five traditional medicinal plants are efficient and inexpensive alternative sources of substances to treat infections. The samples of the commercially marketed plants did not have consistent chemical compositions in at least one type of HPLC, GC/MS, UV or ^{1}H NMR analysis.



1. Introduction

Since the beginning of human civilization, plants have been utilized for a wide array of purposes[1]. It is estimated that around 80% of the global population relies on medicinal plants for primary health care[1-3]. It is also estimated that more than 65% of commercial pharmaceutical preparations contain active ingredients from natural sources, 32% being natural compounds or derivatives[4]. Over a period of 30 years (1981 to 2014), 43.5% of the medicines in the world approved for the treatment of infections caused by bacteria, fungi, parasites and viruses were obtained from natural products[5]. Morphine, isolated from *Papaver somniferum* L., penicillin, obtained from fermentative processes of *Penicillium chrysogenum* Thom, and taxol, isolated from *Taxus brevifolia* Nutt., are among drugs developed from natural sources[6]. Despite the advantages of using medicinal plants, such as their lower cost, fewer side effects, greater protection and easier accessibility, there are still considerable risks to consumers due to problems of self-dosing, variability in the chemical standards and inflated marketing claims regarding herbs[7].

Brazil has the largest biodiversity in the world and 50% of plant species of the family Leguminosae have been reportedly used in folk medicine[8]. Hence, it is not surprising that street markets flourish selling myriad species of herbal and plant cures for an array of common ailments. Moreover, 66% of the Brazilian population has no full access to commercial medicines[9]. Indeed, medical anthropologists have verified the existence of time-tested ethnobotanical knowledge among cultures worldwide[10], including in Brazil[11,12]. There is no doubt about the importance of medicinal plants for the treatment and prevention of diseases. As part of the systematic study of phytochemicals and biological activity, previously were demonstrated the antidiarrheal property of *Solanum paniculatum*, L. roots, used in Brazil for culinary purposes and to treat gastric dysfunctions[13]. However, a lack of information about the origin, taxonomic identification, storage and chemical standardization of plants is a potential drawback to their safe consumption, posing possible health risks to users, particularly those in poor health[14]. Hence, the present study aimed to determine the chemical profile and antimicrobial potential of five medicinal plants (*Anacardium occidentale* L., *Handroanthus impetiginosus* Mart., *Bumelia sartorum* Sw., *Zornia brasiliensis* Vogel and *Cnidosculus urens* Pohl) popularly used in Brazil for the treatment of infections.

A. occidentale, known in Brazil as purple cashew, used in the form of tea and juice, is indicated for treatment of infections via oral administration and for wound healing by topical application[15,16]. The stems and flowers of *H. impetiginosus*, known as purple Ipê, are used to make poultices or concentrated as tea for the treatment of diseases caused by microorganisms[17-19]. Stems of *B. sartorum*, known as *quixaba*, are used in Northeast Brazil to prepare for treatment of various conditions, such as diabetes mellitus, inflammation, genital sores, colic, bruises, ulcers and others[20]. Extracts of leaves of *Z. brasiliensis*, known as *urinana*, are used for their molluscicidal, spasmolytic, muscle relaxant and anticonvulsant properties and reportedly possess antioxidant, antibacterial, cytotoxic, antinociceptive, anti-inflammatory and antitumor activities[21]. Extracts from roots of the white nettle, *C. urens*, are indicated for the treatment of cancer, inflammation, infection and dermatological lesions, besides being used for their antiseptic, expectorant, tonic, antispasmodic, diuretic, sedative and hemostatic activities[22].

2. Experimental

2.1 Plant material

2.1.1 Plant selection

The plant species were selected based on professed traditional ethnobotanical knowledge. Ten suppliers selling herbs at the São José market in the city of Recife (the state capital of Pernambuco, population 1,645,727; 2020) were interviewed by researchers and asked to identify the medicinal plants most frequently purchased by their customers for treating common infectious diseases. *Anacardium occidentale* (stems), *Handroanthus impetiginosus* (stems), *Bumelia sartorum* (stems), *Zornia brasiliensis* (leaves) and *Cnidosculus urens* (roots) were the plant species most frequently indicated by the herb vendors and thus, were selected for the study. Samples of these five plant species were purchased from three different medicinal plant suppliers in March 2018.

2.1.2 Obtaining extracts

The five selected plants were dried at 50 °C for 48 h. The plants were milled and extracted separately with ethanol (3 × 100 mL). The extract obtained was concentrated under reduced pressure at 45 ºC to yield the crude extract (Tab. 1). The extracts were subjected to chromatographic and spectrometric analysis (HPLC, GC/MS, UV-VIS e ^{1}H NMR).

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Table 1. Yields values of extracts obtained with ethanol from the five plants selected.

Samples		Dried plants (g)	Crude extracts (g)	Yielding (%)
A. occidentale	1Ao	32.3	0.7	2.0
	2Ao	19.1	0.6	3.0
	3Ao	40.3	0.8	2.0
H. impetiginosus	1Hi	43.4	1.0	2.2
	2Hi	32.2	2.1	6.7
	3Hi	26.7	1.4	5.3
B. sartorum	1Ba	56.4	1.2	2.2
	2Ba	29.9	0.9	2.8
	3Ba	36.4	2.6	7.3
Zornia sp	1Zs	18.9	0.5	2.7
	2Zs	17.3	0.5	3.2
	3Zs	23.5	0.65	2.7
C. urens	1Cu	20.5	0.5	2.9
	2Cu	24.0	0.3	1.1
	3Cu	21.5	0.2	1.0

2.2 Instruments

GC/MS analysis were carried out using a Perkin Elmer, model Clarus 589/Clarus SQ 8S capillary column (cross linked 5% phenyl methyl silicone, 0.25 mm i.d. x 30 m, Palo Alto, CA, USA), with oven temperature programmed from 100 to 250 °C at 10 ºC min^{-1} rate and a carrier gas (helium) flow rate of 1 mL min^{-1}. IR spectra were measured in KBr pellets with a Varian infrared spectrometer. The VIS-UV analyses were carried out in an Agilent 8453 UV-Vis spectrophotometer in the interval from 200 to 480 nm, using 10-mm quartz cuvettes. ^1H NMR analyses were recorded at 300 MHz using a Varian Unity Plus equipment. Samples were dissolved in $CDCl_3$ with TMS as the internal standard. HPLC analyses of extracts and pure compounds were performed in a Shimadzu LC 20AT instrument using a Luna C_{18} reverse phase column (250 × 4.6 mm × 5 µm, Phenomenex) and eluted in gradient mode starting with 0.001 % formic acid/methanol (3:7) for 5 min, rising to 90% formic acid after 30 min, with diode array detector. TLC was performed on pre-coated silica gel 60 F_{254} plates. Spots were visualized under UV light (254 and 365 nm).

2.3 In vitro assay for antimicrobial activity

The antimicrobial assay with crude extracts of the five plants studied was evaluated against the gram-positive bacteria *Bacillus subtilis* (UFPEDA 86), *Enterococcus faecalis* (UFPEDA 138), the gram-positive bacteria *Escherichia coli* (UFPEDA 224) and *Klebsiella pneumoniae* (UFPEDA 396), as well as against the fungi *Candida albicans* (ATCC 1007) and *Candida krusei* (UFPEDA 1002). The microorganisms were maintained in nutrient agar (NA), stored at 4 °C. The antimicrobial activity evaluation was performed by determination of the values of minimum inhibitory concentrations (MIC), as previously reported[23].

3. Results and discussion

The chemical profiles of the ethanolic extracts of *A. occidentale* (stems), *H. impetiginosus* (stems), *B. sartorum* (stems), *Z. brasiliensis* (leaves) and *C. urens* (roots) marketed as medicinal plants in Recife, Pernambuco, Brazil and indicated for the treatment of common infectious diseases were obtained by HPLC, TLC, GC/MS, UV and ^1H NMR analysis. Based on the interpretations of the spectroscopic and chromatographic analyses (see Supplementary Material, Figures S1-S15), some plant samples showed qualitative and quantitative differences in the chemical profiles in at least one type of analysis. The chemical profiles of *B. sartorum* specimens had the greatest chemical similarity among the five plants analyzed. The ^1H NMR spectra of the ethanolic extracts of *B. sartorum* samples revealed signals from the region of 0.9 to 5.5 ppm as bassic acid. Previous isolates from *B. sartorum* root bark and bassic acid have demonstrated anti-inflammatory activity[24]. Despite the small variation in the chemical profiles among the *B. sartorum* samples, the antimicrobial potential of *B. sartorum* showed a variation of MIC values, especially for sample **3Ba**, which presented lower activity against the bacterium *Escherichia coli* and the fungus *Candida albicans* (Tab. 2).

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Table 2. Minimum inhibitory concentrations (MIC) values in µg mL^{-1}.

Plants	Samples	Gram-positive bacteria		Gram-negative bacteria		Fungi	
		E. faecalis	*B. subtilis*	*E. coli*	*K. pneumoniae*	*C. krusei*	*C. albicans*
A. occidentale	1Ao	312.5	312.5	156.2	19.5	312.5	1250
	2Ao	156.2	78.1	78.1	19.5	625	2500
	3Ao	312.5	312.5	19.5	19.5	78.1	1250
H. impetiginosus	1Hi	1250	625	19.5	1250	2500	>2500
	2Hi	1250	625	156.2	625	2500	78.1
	3Hi	78.1	19.5	78.1	19.5	2500	156.2
B. sartorum	1Ba	1250	625	19.5	625	39.0	>2500
	2Ba	625	625	19.5	39.0	39.0	39.0
	3Ba	1250	625	1250	312.5	>2500	625
Zornia sp	1Zs	1250	1250	625	625	>2500	>2500
	2Zs	1250	625	625	625	>2500	2500
	3Zs	1250	312.5	625	625	>2500	78.1
C. urens	1Cu	1250	625	625	2500	>2500	>2500
	2Cu	1250	625	19.5	625	19.5	625
	3Cu	1250	625	19.5	625	19.5	625

The chemical profile of *H. impetiginosus* sample **1Hi** presented a difference when compared to the chemical profiles of samples **2Hi** and **3Hi**, mainly in the HPLC and UV analyses. *H. impetiginosus* exhibited strong antimicrobial activity with MIC of 19.5 µg mL^{-1} against the bacteria *E. coli*, *B. subtilis* and *K. pneumonia*, but the MIC values varied among the samples due to their different chemical profiles.

The chemical profiles of ethanolic extracts of the *Z. brasiliensis* samples showed differences when analyzed by HPLC, UV and ^{1}H NMR, especially sample **2Zs**. It was observed in the ^{1}H NMR spectrum of the *Z. brasiliensis* extract the presence of doublets at δ 7.6 and 7.30 ppm, an intense singlet from the methoxy group at δ 3.9 ppm, while the UV spectrum showed absorption in the 380 to 560 nm. These signals indicate the presence of flavonoids in the extract. Flavonoids as chalcones and flavones have been previously reported of *Z. brasiliensis* tissues, and flavone 7-methoxyflavone isolated from the aerial exhibited antinociceptive activity[25]. Among the species of plants studied here, *Z. brasiliensis* presented the least antimicrobial potential against all microorganisms tested.

The chromatograms obtained by HPLC of the three *C. urens* extract samples showed a major peak at t_r 40 min. The chemical profiles showed qualitative and quantitative differences, mainly the chromatogram of the **1Cu** sample. It was inactive against the fungi *C. albicans* and *C. krusei*, as well as presenting weak activity against all bacteria tested. The samples **2Cu** and **3Cu,** on the other hand, showed better results with strong activity against the gram-negative bacterium *K. pneumoniae* and the fungus *C. krusei*, with MIC of 19.5 µg mL^{-1}. The ^{1}H NMR spectra of *C. urens* extracts did not indicate the presence of hydrogen signals for aromatic compounds but showed standard signals of triterpenoids between δ 5.0 and 0.8 ppm, identical to the triterpenoid signals previously obtained for ethanolic extracts of *C. urens*[26].

Extracts from the *A. occidentale* samples exhibited strong antimicrobial activity against the bacteria tested, mainly against the gram-negative bacteria *K. pneumonia* and *E. coli*, corroborating previous studies which demonstrated the antimicrobial potential of *A. occidentale*[27,28]. The ^{1}H NMR analysis of *A. occidentale* samples revealed chemical signals characteristic of flavonoids and benzoic acid derivatives, such as quercetin, kaempferol, rhamnetin and 2-hydroxy-6-pentadecylbenzoic acid, previously observed in extracts of *A. occidentale* tissues[29,30].

4. Conclusions

In summary, the five medicinal plants popularly indicated for treating common infectious diseases in Recife, Brazil, showed moderate to strong antimicrobial activity against most of the fungi and bacteria tested, principally gram-negative bacteria, responsible for most infection-related deaths.

The results confirmed that these five traditional medicinal plants are efficient low-cost sources of extracts to treat infections, especially for the 5.1 million of Brazilians living in abject poverty[31] who have limited access to conventional medicines, as well as for other people seeking natural cures. The study also revealed that samples of the commercially marketed plants failed to have a consistent chemical

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composition in at least one type of HPLC, GC/MS, UV or ^{1}H NMR analysis. Another drawback noted was the lack of information about the authenticity of herbs on the packaging. The samples' variability suggests a need for more rigorous quality control of informally marketed herbal medicines in this setting to avoid potential risks to consumers' health.

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