BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Limitations on the ethno-directed approach as a tool for the selection of medicinal plants with antimicrobial activity

[Limitaciones en el uso del enfoque etnodirigido como herramienta para la selección de plantas antimicrobianas]

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Section Ethnobotany

Received: 16 July 2023
Accepted: 19 October 2023
Accepted corrected: 23 June 2024
Published: 30 January 2025

Citation:
Corrêa ALC, Andrade BA, Neri PMS, Castro VTNA, Araújo RO, Sena KXFR, Machado KS, Araújo TAS, Sobrinho TJSP, Amorim ELC. Limitations on the ethno-directed approach as a tool for the selection of medicinal plants with antimicrobial activity
Bol Latinoam Caribe Plant Med Aromat
24 (1): 33 - 46 (2025)
https://doi.org/10.37360/blacpma.25.24.1.3

Abstract: In this work, we evaluate the antimicrobial properties of three groups of plants selected by the ethnopharmacological method, reported as having antimicrobial and/or anti-inflammatory properties by a rural community in the Brazilian State of Pernambuco. The samples were divided into groups of seven plants reported as having antimicrobial properties (GI), another seven as having anti- inflammatory properties (GII) and eight plants reported to have both (GIII). The antimicrobial properties of these groups were compared using the disc-diffusion method for nine microorganisms: Gram-positive and Gram-negative bacteria, acid-alcohol resistant bacillus (BAAR) and yeast. Among the samples of GI, 28.6% demonstrated activity against the micro-organisms tested, compared with 57.1% for the GII plants and 100% of GIII. This study shows, then, that the selected species should be looked more carefully that greater care should be taken in selecting species recommended by ethnopharmacological reports for studies of antimicrobial properties, since plants reported to have anti-inflammatory properties may be more active than those reported as being antimicrobial.

Keywords: Antiinflammatory x antimicrobial activity; Ethnopharmacology; Caatinga; Medicinal plants; Phenolic compounds

Resumen: Evaluar la actividad antimicrobiana de tres grupos de plantas seleccionadas por el método etnodirigido, citadas como antimicrobianas y/o antiinflamatorias por una comunidad rural del estado de Pernambuco, Brasil. Las muestras de las especies seleccionadas fueron divididas en grupos de siete plantas citadas como antimicrobianas (GI), siete plantas citadas como antiinflamatorias (GII) y otro grupo con ocho plantas citadas para ambas situaciones (GIII). Se realizó una comparación de las actividades antimicrobianas de estos grupos mediante el método de difusión en disco frente a nueve microorganismos: Gram-positivos, Gram-negativos, bacilos ácido-alcohol resistentes (BAAR) y levadura. De las plantas citadas como antimicrobianas (GI), el 28,6% mostró actividad frente a los microorganismos probados, mientras que de las plantas pertenecientes al GII, el 57,1% presentó actividad y todas las plantas citadas para ambas situaciones (GIII) fueron activas en un 100%. Se necesita tener cuidado en la selección de especies provenientes del enfoque etnodirigido para estudios que buscan actividad antimicrobiana, ya que las plantas citadas como antiinflamatorias fueron más activas que las citadas como antimicrobianas.

Palabras clave: Antiinflamatorio x actividad antimicrobiana; Etnofarmacología; Caatinga; Plantas medicinales; Compuestos fenólicos.

INTRODUCTION

There are about 50,199 plant species along various biogeographical zones of Brazil, including the Amazon Rainforest, the Pantanal, the Cerrado, the Caatinga and the Atlantic Forest (Flora e funga do Brasil, 2023). Considering such a diversity, one of the current difficulties for development of new drugs is the adequate selection of medicinal species that have a greater chance of success in discovery and the production of novel drugs, especially in the case of Brazilian endemic plants (Albuquerque *et al*., 2022). Researchers are equipped with a number of selection methods based on well-established criteria, such as ethology, chemosystematics and an ethno-directed approach (Crawford & Giannasi, 1982; Albuquerque & Hanazaki, 2006; Albuquerque *et al*., 2014; Costa, 2017).

The ethnobotanical approach has pointed to the importance of cultural criteria in selecting species, taking into consideration their efficacy in treating diseases and also the importance assigned to the plants by the local community (Nascimento *et al*., 2000; Pedrollo *et al*., 2016). This is an increasingly common method for studying species, particularly because it involves selection tools, which use the traditional local knowledge to anticipate promising species for new pharmaceuticals (Araújo *et al*., 2008; Silva *et al*., 2013). It is also important not to overlook the large amount of experimental data gathered in these communities, since it can provide evidence-based treatment discoveries (Gheler-Costa & Comi, 2022).

However, the ethno-directed studies are full of issues to consider: superficial or flawed data collection and misguided interpretations, such as erroneous association of local diseases with those recognized by the traditional medical establishment. Another difficulty may be presented by small sample sizes that make it impossible to infer on large populations (Silva *et al*., 2021). Therefore, it is important to consider the difficulty of interpreting responses in ethnobotanical interviews and establishing the species to be studied from a biological point of view (Albuquerque *et al*., 2022). A misleading case in the selection of species, for example, considers different activities attributed to a plant: a plant reported to have anti-inflammatory properties may also, in fact, be an antimicrobial agent. As the inflammation is the first response of the organism to tissue damage (caused by a physical or microbial injury), there will be a decrease in the inflammation caused by an infection when the pathogen is eliminated (Coelho-Castelo *et al*., 2009; Cabral, 2014).

The present study thus aims to evaluate the antimicrobial properties of three groups of plants selected using the ethno-directed method: GI - plants reported as having antimicrobial properties; GII – plants reported as having anti-inflammatory properties and GIII – plants reported as having both antimicrobial and anti-inflammatory properties, according to one rural community.

MATERIALS AND METHODS

Species selection

The species were selected from a database formed by the survey of traditional knowledge, carried out by the application of the main techniques of ethnopharmacological data collection, such as free-list and semi-structured interviews, described in Araujo *et al*. (2008). This database, previously developed by the Laboratory of Ecology and Evolution of Socio-Ecological Systems (LEA-UFPE), has already been used in several studies for anti-inflammatory and antimicrobial purposes (Alencar *et al*., 2009; Silva *et al*., 2011; Ferreira Junior *et al*., 2011; Albuquerque *et al*., 2012; Siqueira *et al*., 2012; Melo *et al*., 2017).

Considering this database, the plants were grouped as follows. First, the GI group – plants reported as having only antimicrobial properties. This group is recommended, for example, for the treatment of tuberculosis, bronchitis, boils, cold sores, pneumonia and general infections. Second, the GII group – plants reported as having only anti-inflammatory properties. Finally, the GIII group – plants reported as having both antimicrobial and anti-inflammatory properties, covering both of the above sets of descriptors. Species were excluded if there were less than three reports per person, to ensure minimal consensus among informants (Araújo *et al*., 2008). These criteria produced a total of 22 species, which are listed in Table No. 1. The species with the larger number of reports were selected.

Collection of species was carried out in spiny deciduous vegetation (Caatinga), in the Carão community, in the Municipality of Altinho/PE (08°35’13.5”S x 36°05'34.6”W) in January 2014. A minimum of three samples of each were collected and these were mixed together in order to minimize idiosyncratic effects (Araújo *et al*., 2008).

Table No. 1
Selection of plants by the ethnodirected method in the Carão community, Altinho-PE reported as antimicrobials and/or anti-inflammatories

	Used plants	Family	Popular name	Used part	Voucher
GI	*Dysphania ambrosioides* (L.) Mosyakin & Clemants	Amaranthaceae	mastruz	leaf	IPA 81001
	Chloroleucon extortum Barneby & J.W. Grimes	Fabaceae	jurema branca	bark	UFP 53492
	Citrus x aurantium L.	Rutaceae	laranjeira	leaf	IPA 89972
	Cleome spinosa Jacq.	Cleomaceae	mussambê	flower	UFP 46366
	Lippia origanoides Kunth	Verbenaceae	alecrim de caboclo	leaf	IPA 91588
	Plectranthus amboinicus (Lour.) Spreng.	Lamiaceae	hortelã folha grande	leaf	IPA 91000
	Ziziphus joazeiro Mart.	Rhamnaceae	juazeiro	bark	UFP 46186
GII	*Boerhavia diffusa* L.	Nyctaginaceae	pega pinto	root	IPA 91066
	Libidibia ferrea (Mart.) L.P. Queiroz	Caesalpinaceae	jucá	bark	UFP 46664
	Cedrela odorata L.	Meliaceae	cedro	bark	UFP 54186
	Cereus jamacaru DC.	Cactaceae	mandacaru	root	UFP 58750
	Crateva tapia L.	Capparaceae	trapiá	bark	UFP 46188
	Schinopsis brasiliensis Engl.	Anacardiaceae	baraúna	bark	IPA 91044
	Spondias tuberosa Arruda	Anacardiaceae	umbu	bark	UFP 54171
GIII	*Amburana cearensis* (Allemão) A.C. Sm.	Fabaceae	imburana açu	bark	PEUFR 50486
	Anacardium occidentale L.	Anacardiaceae	caju roxo	bark	HST 20410
	Anadenanthera colubrina (Vell.) Brenan	Fabaceae	angico	bark	UFP 46181
	Erythrina velutina Willd.	Fabaceae	mulungu	bark	UFP 46180
	Maytenus rigida Mart.	Celastraceae	bom nome	bark	UFP 46182
	Mimosa tenuiflora (Willd.) Poir.	Fabaceae	jurema preta	bark	IPA 91039
	Myracrodruon urundeuva Allemão	Anacardiaceae	aroeira	bark	IPA 91068
	Handroanthus impetiginosus (Mart. Ex DC.) Mattos	Bignoniaceae	Pau d`arco roxo	Bark	UFP 17536

GI- Plants reported as antimicrobials, **GII-** Plants reported as anti-inflammatories, **GIII-** Plants reported as antimicrobials and anti-inflammatories

Preparation and Chemical Characterization of Extracts

The samples were dried, grounded in a vertical Wiley-type knife mill and cut into standard 20 mesh granules. The samples then underwent extraction by maceration for 48 hours at a proportion of 1:10 (m/v) in 80% ethanol. The extraction liquid was renewed twice, giving a total of three macerations. The extracts were, then, put together, filtered and evaporated under reduced pressure at a temperature of 40 ± 2ºC until they became completely dried.

Qualitative analysis of extracts (10 mg/mL) was carried out using thin-layer chromatography with eluent systems and specific revealers (Wagner & Bladt, 1996), with the capacity to identify the main secondary metabolism groups.

Determination of the total phenolic content (TPC) and total tannin content (TTC)

The TPC of the extracts was determined by the Folin-Ciocalteu method and the residual phenolic content was determined by the method of precipitation of casein followed by Folin-Ciocalteu, where the TTC is the difference between the levels of total and residual phenols (Amorim *et al*., 2008).

The TPC was calculated from 0.2 mL of

diluted extract (1 mg/mL, w/v), 0.5 mL of aqueous solution of Folin-Ciocalteu (10%, v/v), 1 mL of aqueous solution of sodium carbonate (7.5%, w/v) and 8.3 mL of distilled water. The solution was placed in the dark for 30 minutes and the absorbance was measured at 760 nm. In order to calculate the residual phenolic content, 6 mL of diluted extract (1 mg/mL, w/v) and 12 mL of distilled water were agitated for 3 hours with 1 g of casein, then filtered and adjusted to a final volume of 25 mL with distilled water. The residual phenolic content was determined with 1 mL of the filtrate by the Folin-Ciocalteu method. The TTC was calculated as the difference between the content of total phenols and residual phenols. TPC and TTC were expressed as one milligram of tannic acid per each gram of sample (mg TAE/g). The samples were evaluated with three replicates.

Determination of total flavonoid content (TFC)

The TFC of the extracts was estimated by a colorimetric method based on the formation of a flavonoid-aluminum complex (Peixoto & Sobrinho *et al*., 2008). The TFC was calculated using 0.2 mL of diluted extract (1 mg/mL, w/v), 0.12 mL of glacial acetic acid, 2 mL of pyridine in methanol (20%, v/v), 0.5 mL of aluminum chloride in methanol (5%, w/v) and 7.18 mL of distilled water. The solution was placed in the dark for 30 minutes and the absorbance was measured at 420 nm. The results were expressed as one milligram of rutin per each gram of sample (mg RE/g). Threereplicated samples were evaluated.

Antimicrobial Properties

Test Microorganisms

Antimicrobial assays were carried out for nine micro-organisms from the Federal University of Pernambuco's Antibiotics Department Microorganism Collection. The organisms were the Gram- positive bacteria *Staphylococcus aureus* (UFPEDA 01), *Bacillus subtilis* (UFPEDA 16), *Enterococcus faecalis* (UFPEDA 138), and *Micrococcus luteus* (UFPEDA 06); the Gram-negative bacteria *Escherichia coli* (UFPEDA 224), *Pseudomonas aeruginosa* (UFPEDA 39) and *Serratia marcescens* (UFPEDA 398); the acid-alcohol resistant bacillus *Mycobacterium smegmatis* (UFPEDA 71); and the yeast *Candida albicans* (UFPEDA 1007).

Disc Diffusion Test

Antimicrobial activity was confirmed *in vitro* using the disc diffusion method (Bauer *et al*., 1966). The concentration of extract was 200.000 µg/mL in dimethyl sulfoxide (DMSO) and the 6 mm diameter paper discs were soaked in 10 µL of solution corresponding to 2.000 µg of raw extract per disc. The suspensions were standardized at 0.5 on the McFarland scale (Barry, 1986; Koneman *et al*., 1997), corresponding to a concentration of approximately 10^8 UFC/mL.

Results with haloes of less than 9 mm indicated inactivity, 9-12 mm partial activity, 13-18 mm activity and greater than 18 high activity (Alves *et al*., 2000).

The antibiotic gentamicin and the antifungal ketoconazole were used in the tests as standard drugs, at concentrations of 10 µg/disc and 30 µg/disc, respectively.

Minimum inhibitory concentration - MIC

The MIC was calculated using extracts which exhibited disc test haloes ≥ 15 mm, using 96- well plates evaluated according to the criteria adopted by the Clinical and Laboratory Standards Institute - CLSI (2015). A 0.5 MacFarland scaled suspension was used for *S. aureus, B. subtilis* and *M. luteus* in the tests. Each well received 180 µL of the Müeller Hinton agar medium, 10 µL of the inoculum and 20 µL of the extract, to obtain final concentrations of 3,9-2.000 µg/well. A 100 µL of Müeller Hinton agar (sterility control) and 100 µL of Müeller Hinton agar controls were combined to 10 µL of a standardized microbial suspension (positive control). The plate was incubated for 24 hours and a revealing coloring (resazurin) was used to accurately verify the turbidity in the well.

Plant extracts showed good antimicrobial activity if MIC < 100 µg/mL, moderate activity for 100 < MIC < 500 µg/mL, low activity for 500 < MIC < 1.000 µg/mL and inactive for MIC > 1.000 µg/mL (Tanaka *et al*., 2005).

Statistical Analysis

The degree of similarity of the phytochemical profile of the 22 plants studied was investigated. In a paired combinatorial analysis, the extracts were considered similar when a given compound showed presence (+) or absence (-) in both species. The similarity was expressed as a percentage, relating the number of common metabolites to the total (13 groups of secondary metabolites) and qualitatively tested (Araujo *et al*., 2015). Similar plants were considered only for a similarity percentage equal to or greater than 75%.

The Shapiro-Wilk test confirmed the normality of the data obtained by quantification of content. The data were expressed as a mean ± standard deviation and analyzed using ANOVA followed by the Tukey test. Pearson's test of correlation could only be used to compare the total phenol compound content and tannins of GIII with the respective means for the haloes obtained in the disc tests for Gram-positive microorganisms. The BioEstat 5.3 software package was used for statistical analysis.

RESULTS

Chemical Characterization of Extracts

The thin-layer chromatography (TLC) tests revealed the presence of alkaloids, anthrone and anthranol, anthraquinones, anthracene derivatives, naphthoquinones, phenolic compounds (simple phenols, flavonoids, tannins and coumarins), saponins, terpenoids, steroids and xanthines. All metabolite groups were identified in at least one of the species evaluated and phenol compounds, anthracene derivatives and saponins were identified in all samples, with the exception of *B. diffusa* and *C. tapia*. The greatest diversity of compounds were found in the *A. cearensis* and *E. velutina* samples, where only anthracene derivatives and xanthines (Table No. 2) were not found.

D. ambrosioides was phytochemically a 100% similar to *C. aurantium*, as were *S. brasiliensis* x *A. colubrina*, *A. cearensis* x *E. velutina* and *A. occidentale* x *E. velutina*. *C. jamacaru* was the most similar specie, identified with other five species. The similarity percentages of the studied groups showed that the GIII plants had 7 of 28 allowed combinations, while GI and GII had 4 and 1 similar combinations out of 21, respectively.

Phenolic compounds were identified in all the species under investigation. This is not a surprise, since this secondary metabolite group is found in a plethora of fruits, vegetables, nuts, seeds, stems and flowers, and also tea, wine, propolis and honey. A further investigation for these compounds can be made by using the High-Performance Thin Layer Chromatography (HPTLC) method. For instance, Figure 1 shows the GI and GIII phenolic compound plates.

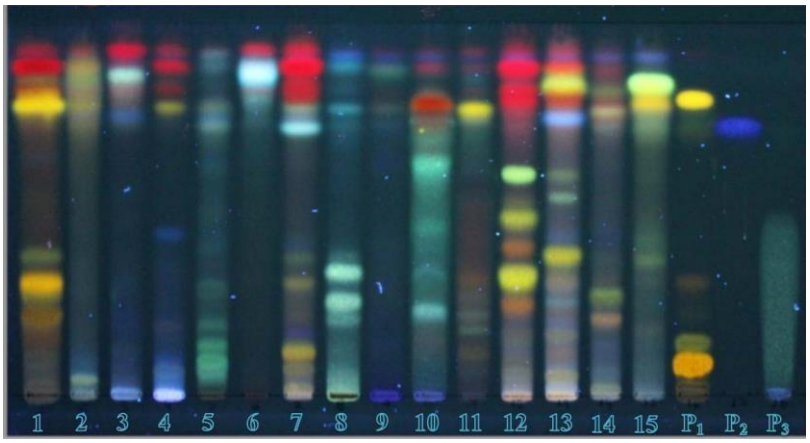

Figure No. 1

Chromatogram for phenolic compounds performed in HPTLC, in which eluent is Ethyl acetate:formic acid : water (in 90:5:5 proportion) and NEU as reagent. The samples are the following for each group: GI = 1 – *Lippia sp.*; 3 – *Z. joazeiro*; 4 – C. extortum; 7 – *C. aurantium*; 12 – *D. ambrosioides*; 13 – *C. spinosa.*; 14 – *P. amboinicus*; GIII = 2 – *E. velutina*; 5 – *M. tenuiflora*; 6 – *M. rigida*; 8 – *H. impetiginosus*; 9 – *A. occidentale*;10 – *M. urundeuva*; 11 – *A. colubrina*; 15 – *A. cearensis*. Also, P1 – rutin; P2 – gallic acid; P3 – ellagic acid

Table No. 2
Phytochemical characterization of extracts of plants selected by the ethnodirected method in the Carão community, Altinho-PE reported as antimicrobials and anti-inflammatories

	Species/Metabolic Group	Alkaloids	Antrona and Anthranol	Anthraquinone	Phenolic compounds	Coumarins	Anthracene derivatives	Flavonoids	Tannins	Mono, sesqui and diterpenes	Naphthoquinones	Saponins	Triterpenes and steroids	Xanthines
GI	*Dysphania ambrosioides*	+	+	+	+	+	+	+	-	+	-	+	+	-
	Chloroleucon extortum	-	-	-	+	+	+	-	-	-	-	+	-	-
	Citrus x aurantium	+	+	+	+	+	+	+	-	+	-	+	+	-
	Cleome spinosa	+	+	-	+	+	+	+	-	-	-	+	+	-
	Lippia origanoides	+	-	+	+	+	+	+	+	+	+	+	+	+
	Plectranthus amboinicus	-	+	-	+	+	+	-	-	+	-	+	+	-
	Ziziphus joazeiro	-	-	-	+	+	+	+	-	+	-	+	+	-
GII	*Boerhavia diffusa*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cedrela odorata	-	-	-	+	+	+	+	+	-	+	+	+	+
	Cereus jamacaru	+	+	-	+	+	+	+	-	+	+	+	+	-
	Crateva tapia	+	-	-	-	-	-	-	-	+	-	-	+	-
	Libidibia ferrea	-	+	+	+	+	+	+	+	-	-	+	+	+
	Schinopsis brasiliensis	-	-	-	+	-	+	-	+	-	-	+	-	+
	Spondias tuberosa	+	+	-	+	+	+	+	+	-	-	+	+	+
GIII	*Amburana cearensis*	+	+	-	+	+	+	+	+	+	+	+	+	-
	Anacardium occidentale	+	-	-	+	-	+	-	+	-	+	+	-	+
	Anadenanthera colubrina	-	-	-	+	-	+	-	+	-	-	+	-	+
	Erythrina velutina	+	+	-	+	+	+	+	+	+	+	+	+	-
	Maytenus rígida	-	+	-	+	-	+	+	+	+	+	+	+	-
	Mimosa tenuiflora	+	-	-	+	-	+	-	+	-	+	+	-	+
	Myracrodruon urundeuva	-	-	-	+	-	+	+	+	+	+	+	+	+
	Handroanthus impetiginosus	-	-	-	+	+	+	-	+	+	-	+	+	+

GI- Plants reported as antimicrobials, **GII-** Plants reported as anti-inflammatories, **GIII-** Plants reported as antimicrobials and anti-inflammatories; **(-)** = absence; **(+)** = presence

Determination of Phenol Content

The total phenol, tannin and flavonoid content of species of plant reported as active against infections caused by fungi and bacteria and also inflammations are presented in Table No. 3.

Overall, a wide variation was found between levels of phenol compounds in the analyzed extracts. For the total phenols, the results varied from 27,6 ± 2,6 to 497,1 ± 12,1 mg/g EAT, with the highest level found in the extract of *Myracrodruon urundeuva* bark, although no statistically significant difference was found for another five species (*A. occidentale*, *A. colubrina*, *M. tenuiflora*, *C. odorata* and *S. brasiliensis*) and no total phenols were identified in extracts of *D. ambrosioides*, *Z. joazeiro*, *B. diffusa*, *C. jamacaru* and *C. tapia*.

The extract of bark of *A. occidentale* had the highest mean tannin content (460,8 ± 15,9 mg/g EAT), but the results for another three species provided no statistically significant results (*M. urundeuva*, *C. odorata* and *S. tuberosa*). On the contrary, the extracts of *D. ambrosioides, C. extortum, C. aurantium, P. amboinicus, Z. joazeiro, B. diffusa, C. jamacaru* and *C. tapia* contained no tannins.

The extracts of *C. extortum, P. amboinicus, A. occidentale, A. colubrina, M. tenuiflora, H. impetiginosus, B. diffusa, C. tapia, S. brasiliensis and S. tuberosa* contained no flavonoids, while the extract of bark of *E. velutina* presented the highest levels of this metabolite (257,4 ± 11,9 mg/g ER). This variation between samples was expected as the extracts came from different plant species/organs and also due to different phenol compounds observed.

The group of plants reported as having both antimicrobial and anti-inflammatory properties had higher levels of phenol compounds and tannins, with 376,0 and 305,3 mg/g EAT, respectively, as the mean for the samples in this group. In the case of flavonoids, GI had higher mean levels (74,41 mg/g ER), mainly because it contained more herbaceous species and leaf extracts, although *E. velutina*, from GIII, had the highest levels of this class of metabolite.

Table No. 3
Concentration of total phenols, tannins and flavonoids obtained from hydro-alcoholic extracts of plants selected using the ethnodirected method in the Carão community, Altinho-PE, reported as antimicrobials and/or anti-inflammatories

Species/Phenolic Compounds		Total Phenolic Content ± SD	Total Tannins Content ± SD	Total Flavonoids Content ± SD
GI	*Dysphania ambrosioides*	N/D	N/D	46,15±2,75 i
	Chloroleucon extortum	67,87±6,03 j	N/D	N/D
	Citrus x aurantium	115,89±5,75 h	N/D	101,20±4,04 h
	Cleome spinosa	81,84±1,81dj	14,80±1,22 h	122,84±3,88 g
	Lippia origanoides	326,17±4,99 g	204,33±6,89 g	196,37±18,20 f
	Plectranthus amboinicus	27,64±2,64 i	N/D	N/D
	Ziziphus joazeiro	N/D	N/D	54,33±4,66 i
	Mean GI	88,49	31,30	74,41
GII	*Boerhavia diffusa*	N/D	N/D	N/D
	Cedrela odorata	477,78±28,24 be	447,53±28,69 c	31,80±2,37 c
	Cereus jamacaru	N/D	N/D	69,11±6,05 e
	Crateva tapia	N/D	N/D	N/D
	Libidibia ferrea	370,33±35,28 c	370,33±35,28 b	13,41±1,30
	Schinopsis brasiliensis	493,88±13,23 b	367,12±21,35 b	N/D
	Spondias tuberosa	452,56±29,33 e	452,10±7,23 c	N/D
	Mean GII	256,36	233,87	16,33
GIII	*Amburana cearensis*	216,63±17,92 f	186,31±17,79 g	150,77±11,10 d
	Anacardium occidentale	488,43±6,58 b	460,85±15,90 c	N/D
	Anadenanthera colubrina	492,09±3,57 b	331,24±3,52 d	N/D
	Erythrina velutina	186,28±12,51 a	147,88±13,83 a	257,14±11,95 a
	Maytenus rigida	382,43±8,33 c	296,20±9,37 e	42,20±1,87 ci

	Mimosa tenuiflora	478,46±11,62 be	379,50±17,28 b	N/D
	Myracrodruon urundeuva	497,07±12,13 b	441,66±30,52 c	13,33±1,26 b
	Handroanthus impetiginosus	107,22±10,46 dh	79,78±6,28 f	N/D
	Mean GIII	376,00	305,30	44,67

Plants reported as antimicrobials, **GII-** Plants reported as anti-inflammatories, **GIII-** Plants reported as antimicrobials and anti-inflammatories; Means followed by the same letter in the column do not differ significantly from one another ($p<0.05$)**; Content =** mg/g tannic acid or rutin per extract; **DP =** Standard Deviation; **N/D =** not detected

Minimum Inhibitory Concentration - MIC

According to the parameters developed by Tanaka *et al*. (2005), all the extracts selected, the *L. origanoides* (250 µg/mL) excepted, were inactive in relation to *B. subtilis*. The results for *S. aureus* showed moderate activity for the following MICs: *L. origanoides* (500 µg/mL), *C. odorata* (500 µg/mL), *L. ferrea* (250 µg/mL), *S. brasiliensis* (500 µg/mL), *A. occidentale* (250 µg/mL), *A. colubrina* (500 µg/mL), *M. rigida* (500 µg/mL), *M. tenuiflora* (500 µg/mL) and *M. urundeuva* (250 µg/mL).

Within the stipulated parameters, the *M. luteus* micro-organism was found to be the most sensitive to the extracts, with moderate activity for the samples of *L. origanoides* (125 µg/mL), *A. colubrina* (250 µg/mL) and *M. rigida* (250 µg/mL) and good activity for extracts of *M. urundeuva*, *M. tenuiflora*, *C. odorata* and *S. brasiliensis* (MIC 62,5 µg/mL) and extracts of *L. ferrea* and *S. tuberosa* (MIC 31,25 µg/mL) (Table No. 5).

DISCUSSION

It can be seen that there is a need of carefully interpretation of ethnobotanical results in relation to the selection of plants with antimicrobial activity, since the group of reported antimicrobial plants obtained less results for antimicrobial activity than the plants reported as having promising anti-inflammatory properties or those reported as possessing both properties.

Phenol compounds such as tannins, flavonoids, coumarins and terpenoids are responsible for antibacterial action in a plethora of studies in the literature. Phenols are the most abundant secondary metabolites in plants and have been described by various authors as the components responsible for the antimicrobial activity of some species (Daglia, 2012; Silva *et al.*, 2010). Accordingly, the present study found a positive correlation between the phenol content of plants in GIII and antimicrobial activity against some strains investigated by the disc test.

As tannins are able to precipitate proteins, they enhance antimicrobial and antifungal activity in the extracts in which they are the most abundant metabolite (Santos & Mello, 2004; Monteiro *et al*., 2005). *L. origanoides*, *A. colubrina*, *M. urundeuva*, *M. rigida*, *A. occidentale* and

M. tenuiflora were thus selected to determine the MIC, as these six species had the highest levels of tannins. A positive correlation between tannin content and antimicrobial activity against strains (investigated using the disc test) was also found for plants in GIII. This result is corroborated by data in the literature that indicate that such activity is characteristic of this group of secondary metabolites (Scalbert, 1991; Santos & Mello, 2002; Almeida *et al*., 2005; Obiang-Obounou & Ryu, 2013).

Using the calculation developed by Araújo *et al*. (2015), to determine the percentage of chemical similarity between bark and leaves of *Anacardium humile, Brosimum gaudichaudii* and *Tabebuia avellanedae*, this study found a larger number of phytochemically similar species in GIII. This group also featured the broadest spectrum of activity, as all species showed antimicrobial activity against at least one of the Gram-positive bacteria tested.

Bennett & Prance (2000), evaluate the versatility of species in communities using the relative importance (RI) index: the larger the number of indications it receives for different body systems, the larger the RI index. The literature reports high RI values for three plants in GIII (*M. urundeuva*, *A. occidentale* and *A. colubrina)* (Silva & Albuquerque, 2005; Monteiro *et al*., 2011; Souza *et al*., 2016), thereby corroborating their inclusion in the group of plants useful for treating both inflammations and diseases caused by fungi or bacteria.

Table No. 4
Mean antimicrobial inhibition haloes (in mm) for hydro-alcoholic extracts of plants selected by the ethnodirected method in the Carão community, Altinho-PE, reported as antimicrobials and anti-inflammatories

	Species/Micro-organisms	Hydroalcoholic Extracts (2.000 µg/disc) (mean of haloes in mm)								
		Gram-positives bacteria				Gram-negatives bacteria			BAAR	Y
		Sa	Bs	Ef	Ml	Ec	Pa	Sm	Ms	Ca
GI	*Dysphania ambrosioides*	-	-	-	-	-	-	-	-	-
	Chloroleucon extortum	-	-	-	-	-	-	-	-	-
	Citrus x aurantium	-	-	-	-	-	-	-	-	-
	Cleome spinosa	-	-	-	-	-	-	-	-	-
	Lippia origanoides	21,0	23,0	-	25,7	-	-	-	17,0	-
	Plectranthus amboinicus	-	-	-	-	-	-	-	-	-
	Ziziphus joazeiro	-	-	-	13,3	-	-	-	14,7	-
GII	*Boerhavia diffusa*	-	-	-	-	-	-	-	-	-
	Cedrela odorata	15,7	-	-	16,7	-	-	-	-	-
	Cereus jamacaru	-	-	-	-	-	-	-	-	-
	Crateva tapia	-	-	-	-	-	-	-	-	-
	Libidibia ferrea	19,3	14,7	14,7	16,3	-	-	-	20,3	-
	Schinopsis brasiliensis	18,0	16,7	16,7	15,0	-	-	-	16,7	
	Spondias tuberosa	13,3	13,0	13,0	14,0	-	-	-	12,0	-
GIII	*Amburana cearensis*	14,3	11,3	14,7	12,3	-	-	-	12,0	-
	Anacardium occidentale	17,7	12,3	16,0	17,0	-	-	-	16,0	-
	Anadenanthera colubrina	18,0	16,7	15,0	23,3	-	-	-	23,3	-
	Erythrina velutina	-	-	-	12,3	-	-	-	-	-
	Maytenus rígida	17,7	10,7	13,7	17,3	-	-	-	18,0	-
	Mimosa tenuiflora	19,3	12,3	17,7	14,0	-	-	-	15,0	-
	Myracrodruon urundeuva	19,3	13,7	17,3	17,3	-	-	-	17,0	-
	Handroanthus impetiginosus	-	14,7	14,7	-	-	-	-	17,0	-
Standard	Gentamicin (10 µg/disc)	22,7	27,7	14,7	30,7	22,7	21,3	15,0	33,3	-
	Ketoconazole (30 µg/disc)	-	-	-	-	-	-	-	-	39,7

GI- Plants reported as antimicrobials, **GII-** Plants reported as anti-inflammatories, **GIII-** Plants reported as antimicrobials and anti-inflammatories; **BAAR –** Acid-Alcohol Resistant Bacillus, **Y -** Yeast; **Sa -** *Staphylococcus aureus*, **Bs –** *Bacillus subtilis*, **Ef -** *Enterococcus faecalis*, **Ml -** *Micrococcus luteus*, **Ec -** *Escherichia coli*, **Pa -** *Pseudomonas aeruginosa*, **Sm -** *Serratia marcescens*, **Ms -** *Mycobacterium smegmatis*, **Ca -** *Candida albicans*; **(-)** – No activity

Table No. 5
Minimum inhibitory and bactericidal concentrations (µg/mL) of hydro-alcoholic extracts of plants used to cure infections caused by fungi and bacteria showing haloes >15mm on the disc test

Species/Micro-organisms		Gram-Positives Bacteria (mean of concentrations in µg/mL)		
		Sa	**Bs**	**Ml**
GI	*Lippia origanoides*	500±0,0	250±0,0	125±0,0
GII	*Cedrela odorata*	500±0,0	>2000	62,5±0,0
	Libidibia ferrea	250±0,0	2000	31,25±0,0
	Schinopsis brasiliensis	500±0,0	>2000	62,5±0,0
GIII	*Anacardium occidentale*	250±0,0	>2000	62,5±0,0
	Anadenanthera colubrina	500±0,0	1000±0,0	250±0,0
	Maytenus rígida	500±0,0	>2000	250±0,0
	Mimosa tenuiflora	500±0,0	>2000	62,5±0,0
	Myracrodruon urundeuva	250±0,0	>2000	62,5±0,0

GI- Plants reported as antimicrobials, **GII-** Plants reported as anti-inflammatories, **GIII-** Plants reported as antimicrobials and anti-inflammatories; **Sa-** *Staphylococcus aureus* (UFPEDA 01), **Bs –** *Bacillus subtilis* (UFPEDA 16), **Ml -** *Micrococcus luteus* (UFPEDA 06) **(-)** – No activity

Among the Gram-positive microorganisms used, *Staphylococcus aureus* is the main cause of various diseases pointed in the ethno-pharmacological research that preceded this study, including boils(Razera *et al*., 2009; Arnaiz-Garcia *et al*., 2015), cold sores (Park *et al*., 2011; Rocha *et al*., 2015) and bronchitis (Santos *et al*., 2017). The results of the present study provide further evidence of the antimicrobial properties of the plants analyzed against Gram-positive microorganisms, including *S. aureus*, according to the disc diffusion test.

The MIC is used in the literature as a standard method for quantifying the susceptibility of organisms to possible antimicrobials and also helps to refine the results of other susceptibility tests, such as the disc diffusion test (Andrews, 2002). Thus, the MICs of the present study corroborate the results of the disc diffusion test, with the best results obtained for plants in GIII and GII. This is not the case for those in GI, which were indicated by the local community for treatment of diseases caused by fungi and bacteria only.

Although some studies in the literature have compared existing methods of plant selection and found ethno-directed approaches to be more effective (Silva *et al*., 2013), others have tried to evaluate the antimicrobial activity of plants reported as treatments for infections (Vieira *et al*., 2014; Chander *et al*., 2016) which were found to be not fully active. Not all plants, therefore, are in fact active and this suggests some placebo effect or some combination of species that the informant failed to mention during data collection or even a misinterpretation of a given researcher regarding the traditional receipt for the treatment.

Our results may point to a misinterpretation of the data, showing that plants commonly indicated as anti-inflammatories may, in fact, work as an antimicrobial agent rather than acting on the cascade of inflammatory mediators (Cabral, 2014).

It is known that inflammation plays an important role in defending the body from harmful agents by diluting, destroying or neutralizing them. Various kinds of molecules and cells are essential for inflammatory processes, including plasma proteins, macrophages, monocytes and polymorphonuclear leukocytes. In the case of infection-related inflammatory processes, these molecules circulate in the bloodstream and are brought to the infection site by inflammatory reactions in order to eliminate the intruder micro-organisms (Kumar *et al*., 2008). When the pathogen has been eliminated, either by the action of the immune system itself or by the administration of antibiotics, the inflammation subsides. Common symptoms of inflammation, such as edema, heat, flushing and pain, are then relieved and it is possible that, after the use of an antimicrobial plant, the local people believe that the effect has been anti-inflammatory.

Starting from the assumption that interpretation of ethnopharmacological studies is complex, Ferreira-Junior *et al*. (2016), agree that inflammation may be confused with other medical

processes, since they found a variation in plants used to treat inflammation according to the type of inflammation perceived by the informant. Thus, difficulties regarding interpretation of the data collected from local populations are important, since the perception of diseases may affect evaluation of the responses obtained and, consequently, affect the selection of medicinal plants (Herndon *et al*., 2009; Reyes-Garcia, 2010).

CONCLUSION

The present research thus suggests that there are, in fact, limitations regarding the ethno- directed approach to the selection of antimicrobial plants. Plants that are "genuinely" anti- inflammatory were found to be more effective as antimicrobials than "genuine" antimicrobials. This may suggest three mutually compatible scenarios: (i) people cannot easily distinguish different kinds of inflammation; (ii) researchers do not do enough to collect information on the local medical system; and (iii) plants may go through a process of evolution in popular knowledge and be used initially as anti-inflammatories, since inflammation has more visible symptoms, and only later perceived to be antimicrobials.

It is suggested that researchers bioprospecting antimicrobial plants using an ethno-directed approach may also include in their list of study candidates those plants reported by the population to be anti-inflammatories, so as not to overlook a potential antimicrobial species. However, studies using this very model are necessary to evaluate whether those species reported as anti-inflammatories, but in fact acting as antibiotic agents, do also have anti-inflammatory properties, as suggested by the hypotheses raised in the present paper.

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