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Original Article

Traditional knowledge and uses of medicinal plants by the inhabitants of the islands of the São Francisco river, Brazil and preliminary analysis of *Rhaphiodon echinus* (Lamiaceae)

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(With 2 figures)

Abstract

This study aimed to carry out an ethnobotanical survey of medicinal plants used by inhabitants of the Rodeadouro Island, Jatoba Island and Massangano Island, located in The Submedium São Francisco River Valley. Also phytochemicals and preliminary pharmacological tests were performed to species most cited by the community. Ethnobotanical data were collected through observation visits and semi-structured interviews with 12 key informants. We calculated the relative importance (RI), the percentage of agreement related to the main uses (cAMU) and use value (UV). The aerial parts of *Rhaphiodon echinus* (Ness & Mart.) Schauer were used to obtain the lyophilizate (LYO-Re), crude ethanol extract (CEE-Re) and their hexanic (HEX-Re), chloroform (CLO-Re) and ethyl acetate (EA-Re) fractions. The microdilution technique was used for determining Minimum inhibitory concentration (MIC) for selected microorganisms. Already the spasmolytic effect was evaluated in isolated uterus fragments of Wistar rats, pre contracted with KCl 60 mM. We found 34 species cited, belonging to 22 families. The most plants were grown by locals. There were 51 different diseases, but the main indication was infectious and parasitic diseases. The species *R. echinus* was the most reported and it was indicated for urinary tract infection and dysmenorrhea. The screening revealed a higher prevalence of flavonoids, tannins, lignans and saponins in LYO-Re and AE-Re. Already terpene compounds were more present in HEX-Re and CLO-Re. The RE-Re fraction stood out with strong effect against *E. coli* and *S. aureus* while CEE-Re has moderate effect against gram-negative bacteria. The evaluation of the spasmolytic activity showed that LYO-Re, CEE-Re and HEX-Re fractions have similar activity, with partial effect and concentration-dependent response. This work brought about knowledge and use of medicinal plants by the riparian of the São Francisco River. It also revealed the importance of other methodologies for scientific evidence for the popular use of *R. echinus*.

Keywords: ethnobotany, traditional medicine, ethnopharmacology, traditional knowledge.

Conhecimento tradicional e usos de plantas medicinais por habitantes das ilhas do rio São Francisco, Brasil e análise preliminar de *Rhaphiodon echinus* (Lamiaceae)

Resumo

Objetivou-se realizar um levantamento etnobotânico de plantas medicinais utilizadas nas ilhas do Massangano, Jatobá II e Rodeadouro, localizadas entre Petrolina-PE e Juazeiro-BA, no submédio do rio São Francisco e posterior investigação fitoquímica e farmacológica da espécie mais citada, conforme a indicação da comunidade. Realizaram-se visitas de observação nas ilhas e os dados etnobotânicos foram coletados por entrevistas semiestruturadas com 12 informantes-chave. Calculou-se a Importância Relativa (IR), a Porcentagem Corrigida de Concordância quanto ao Uso Principal (CUPc) e o Valor de Uso (VU). Utilizaram-se as partes aéreas da espécie mais citada para obtenção do extrato etanólico bruto (EEB-Re), suas frações hexânica (HEX-Re), clorofórmica (CLO-Re) e acetato de etila (AE-Re); o produto liofilizado (LIO-Re) e o óleo essencial (OE-Re). A técnica de microdiluição foi usada para determinar a Concentração Inibitória Mínima (CIM) para microorganismos selecionados. A atividade espasmolítica foi avaliada em fragmentos isolados de útero de ratas Wistar pré-contraídos com KCl 60 mM. Foram relatadas 34 espécies, contidas em 22 famílias

diferentes. A maioria das plantas era cultivada pelos moradores. Registraram-se 51 enfermidades diferentes, mas a principal indicação foi doenças parasitárias e infecciosas. A espécie *Rhaphiodon echinus* (Ness & Mart.) Schauer foi a mais citada, com principal indicação para tratar infecção do trato urinário e dismenorreia. O *screening* fitoquímico revelou prevalência de flavonoides, taninos, lignanas e saponinas em LIO-Re e AE-Re e compostos terpênicos em HEX-Re e CLO-Re. Os testes antibacterianos mostraram que AE-Re é mais atuante contra *E. coli e S. aureus* do que para *P. aeruginosa*. O EEB-Re tem efeito parcial contra bactérias gram-negativas e OE-Re possui moderada resposta para todos microorganismos testados. LIO-Re, EEB-Re e HEX-Re possuem atividade espasmolítica dependente da concentração, sem diferença significativa e sem relaxamento total. Este trabalho trouxe conhecimento sobre o uso de plantas medicinais pelos ribeirinhos do rio São Francisco e revela a importância de estudos mais aprofundados para a comprovação científica do uso popular da *R. echinus.*

Palavras-chave: etnobotânica, medicina tradicional, etnofarmacologia, conhecimento tradicional.

1. Introduction

The search for reliefs and cures for diseases by using plants is possibly one of the first forms of human use of natural products (Zuanazzi and Mayorga, 2010). In Brazil, the use of herbs for medicinal purposes is a common practice, strengthened by the cultural diversity that came from colonization by European and African populations and indigenous traditional knowledge (Oliveira et al, 2011). Currently, estimates of the World Health Organization (WHO) stated that about 80% of the population uses the principles of traditional medicine in primary health care (Oliveira et al., 2010). This scenario, coupled with limitations of current pharmacotherapies, made pharmaceutical industries consider nature's resources as an important source of raw material for new drugs (Rates, 2001).

The Submedium São Francisco River Valley has an extension of about 700 kilometers. There are many fluvial islands located between the cities of Petrolina (Pernambuco) and Juazeiro (Bahia), in the northeastern region of Brazil. In these islands, the plants are subjected to the climate of the Caatinga, but have abundant water availability, constituting mixed vegetation (Godinho and Godinho, 2003). The association of the richness of plant species with poor access to health care caused the population of these islands to consolidate the use of resources of the region to treat and cure their ailments (Aquino, 2012). Considering the importance of the rescue of traditional knowledge of medicinal plants and of research of bioactive substances, this study aimed to carry out an ethnobotanical survey of medicinal plants used in communities of the Submedium São Francisco River Valley. Phytochemicals and preliminary pharmacological tests also were performed to species most cited by the community. This research is the first such work with the population of the islands. It contributes to enhance the knowledge of this community about the diversity of local vegetation.

2. Material and Methods

2.1. Study area

The study was conducted on three islands: Massangano Island, Jatoba Island and Rodeadouro Island, located in the Submedium São Francisco River Valley, between the cities of Petrolina, Pernambuco, and Juazeiro, Bahia. There are no official reports on the settlement process of these islands. Some records say that the island of Massangano began to be inhabited in 1830 and its name comes from a farmer who lived there and who owned much of the local land (Aquino, 2012). According to Article 20 of the Federal Constitution, paragraph IV, the river islands of São Francisco river are the property of the Union (Brasil, 1988), but most were inhabited by families who have no record of statutory individual ownership of the land. Thus only the houses are defined as individual plots, with the remainder of the territory regarded as an area for community use, regulated by custom and internally shared norms (Campos, 2002).

The soil is sandy and a range of less than 10 m wide at the edges of the islands is occupied by plants of riparian forests. Despite their importance for ecological and cultural reasons, these islands are not considered Permanent Preservation Areas (PPA) which represent fragile habitats under legal protection in the country (Brasil, 2012; Fabricante et al., 2015).

The visits were carried out during the months of December /2014 and January/2015. Twelve (12) key informants were selected, permanent residents, older than 18 years, with knowledge and skill with medicinal plants. In the sampling technique used, known as "snowball", a specialist recommends other with similar competence, repeating the process from new included (Andrade et al., 2006).

2.2. Ethnobotanical data

The ethnobotanical information of the use of plants was obtained through interviews based on semi-structured protocols with five triggering questions. The data on education, occupation and residence time on the islands were recorded to outline the profile of respondents. The people interviewed were asked to enumerate medicinal plants found locally and used frequently (popular name, preparation method and therapeutic indications). It was also asked about the reason for the use of medicinal plants and how this knowledge was obtained.

The plants cited were organized by common name, botanical family and species, statement, used parts and usage (Silva and Albuquerque, 2004). The species were identified by the comparison between individuals, studies on medicinal plants of the *caatinga* biome and the consultation of information sites of the The Plant List (2015) and

The Missouri Botanical Garden (2016). The term status was used to refer to plant classification as spontaneous or cultivated in the answers to questions about how the informants could medicinal plants used. It was considered that wild species are those that occur naturally in the bush, without being cultivated, including in this category the native and invasive plants (Albuquerque et al., 2007).

The therapeutic indications were grouped based on the classification of diseases proposed by the World Health Organization (WHO, 2007) and the diseases or conditions that could not be included in this classification were grouped in category "general symptoms and signs".

In order to compare the species of medicinal plants to the importance of its use, it was calculated the relative importance (RI), based on the proposal of Bennett and Prance (2000) and according Albuquerque et al. (2007). The value of the RI has maximum 2.00 for a species. Plants with higher RI have more medicinal properties and act in various body systems (Albuquerque et al., 2006). Additionally, two other methodologies had used, named agreement on the main use (AMU) and use value (UV). For the UV calculation, it followed the methodology of Silva and Albuquerque, 2004. The comparison between the ethnobotanical indicators was made considering that each of the indexes evaluate different aspects of the relationship between man and plants. The RI and UV make reference to multiplicity of use of medicinal plants (Albuquerque et al., 2006). On the other hand cAMU evaluates how the therapeutic indication is consensus in the group of experts assessed (Amorozo and Gely, 1998).

2.3. Preliminary phytochemical and pharmacological analysis of Rhaphiodon echinus (Nees & Mart.) Schauer (Lamiaceae)

The *Rhaphiodon echinus* samples collected (09°27'36.10"S, 040°34'46.50" W) were prepared as exsiccate (I.D.S.L. Pio 1, nº. 22526) and deposited in the "Vale do São Francisco Herbarium" (HVASF). The HVASF was responsible for making taxonomic classification.

Dried and powdered *Rhaphiodon echinus* samples were extracted with ethanol PA 95% by maceration (3×72h), yielding crude ethanol extract (CEE-Re) and after complete extraction the solvent was removed by distillation in rotary evaporator.. The crude ethanol extract was fractioned using hexane (HEX-Re), chloroform (CLO-Re) and ethyl acetate (EA-Re). Fractionates and the extract were stored in refrigerator for the following studies. The lyophilized (LYO-Re) was obtained by decoction following the orientations of the investigated community. The aqueous solution was filtered, lyophilized and stored in a dark bottle at 4 °C. The lyophilized powder was reconstituted freshly before use.

2.3.1. Phytochemical screening

Preliminary identification of phytochemical of *R. echinus* was performed by screening by analytical thin layer chromatography using the method described by Wagner and Bladt (1996). It made even the differential search for alkaloids by using Mayer's reactive (iodine + potassium mercurato) as revealing.

2.3.2. Pharmacological tests

The determination of antimicrobial activity used the microorganisms: *Pseudomonas aeruginosa* ATCC 8027; *Pseudomonas aeruginosa* ATCC 23243; *Staphylococcus aureus* ATCC 25619; *Staphylococcus aureus* ATCC 25925; *Escherichia coli* 2536; *Escherichia coli* 105. The microorganisms were obtained from Tropical Culture Collection (CCT), American Type Culture Collection (ATCC) and clinical origin. The Minimal inhibitory concentration (MIC) determination was carried out by microdilution technique determined by Viljoen et al. (2003). A starting concentration of 100 mg/ml chloramphenicol (Merck) was used as a positive bacterial control and dimethylsulfoxide (DMSO) as negative control. MIC values were at least determined in duplicate.

The determination of spasmolytic activity was with virgin female Wistar rats (150-250 g) treated for 18 to 24 hours before the experiment with diethylstilbestrol (Merck) at a dose of 1.0 mg/kg subcutaneously to induce the estrus. The rats were euthanized by cervical dislocation and exsanguination. The uterus was removed, cleaned in a nutrient solution and each piece was individually suspended in glass tanks of a bath system for isolated organs EFF-321model (Insight® Instruments, Brazil). After stabilization, an initial isometric contraction was induced in each piece of the uterus using potassium chloride (KCl; Dynamic) 60 mM. Then the solutions of the extracts were added to the tanks individually and cumulatively in increasing concentrations (1-729µg/ml) to obtain the results. Cremophor EL 3% was used as negative control. Isometric contractions were recorded in computer through the WINDAQ / DATAQ DI200 software. The spasmolytic activity was assessed by relaxation, i.e. reversal of the contraction obtained by addition of KCl. Numerical data were expressed as mean ± standard error of the mean ($x \pm$ s.e.m.). The sets of test data were statistically compared with the control group, by analysis of variance (ANOVA) and Tukey test. All statistical analyzes were performed using Prism 5 software for Windows (GraphPad Software Inc., San Diego, CA, USA), and the differences were considered significant when p-value ≤ 0.05. The data were plotted in concentration-response curves adjusted by nonlinear regression. The results for each tested extract were expressed in EC_{50} value (concentration able to cause a 50% maximal effect) and E_{max} (maximal effect obtained in percentage).

This study was approved by the Research Ethics Committee and Ethics Committee on the Use of Animals of the Federal University of São Francisco Valley, with protocols nº 0006/240914 CEDEP / UNIVASF and 0006/120215 CEUA-UNIVASF, The free and informed consent was obtained by reading, full understanding and signature of the document of Informed Consent that contained adaptation to cultural and linguistic peculiarities of those involved.

3. Results and Discussion

3.1. Knowledge and use of medicinal plants

The respondents had an average age of 64.83 ± 16.95 years with a female majority (83%). Most had little or no schooling, and lived on the islands for many years (median years 44.58 years). These communities have a diverse ethnic composition including indigenous descendants, sons of miners, boatmen and farmers and cowboys of the northeastern Brazilian region. However, these people retain their identity, based on "being of the island" that is strengthened every day with in cultural rituals and commemorations (Aquino, 2012).

The use of plants in health care is a daily activity on the islands. This knowledge was transmitted orally from generation to generation. The oral transmission of traditional knowledge is well elucidated in other ethnodirected studies (Carneiro et al., 2010; Firmo et al., 2012; Griz et al., 2017) (Table 1).

The determinants for the use of medicinal plants were defined as confidence in the potential of natural resources; preference front industrialized pharmaceuticals and ease of access. These reasons have been identified in several studies (Alvim et al., 2006; Carreira and Alvim, 2008; Rodrigues and Carvalho, 2001; Stalcup, 2000). However it is possible that plants are widely used also because of their lower prices when compared to synthetic drugs or because plants present a more viable alternative to the difficulties of access to medical care (Stalcup, 2000).

The Table 1 also shows that the most common forms of use were tea, bath and syrup. These forms of preparation were well reported in other studies of the Brazilian Northeast (Moreira et al., 2002; Freitas et al., 2012). Few listed plants were found simultaneously in the region and this was explained by the environmental changes (for example, small animal breeding that led to reduction of the original vegetation) forcing the residents to cultivate plants in peridomestic regions. This confirms the proposition Amorozo (2002) which says that the anthropic actions arising from changes in patterns of use of local natural resources can reduce the availability of plants with status of native and spontaneous.

Table 1. Data on knowledge and use of medicinal plants of residents (n = 12) of Massangano Island, Rodeadouro Island and Jatoba Island (PE/BA), 2015.

Inf*	Source of knowledge about medicinal plants	Reason for use of medicinal plants	Main forms of use of medicinal plants	Status of the most used medicinal plants
01	Family of indigenous descents**	Belief and because they easily found on the island.	Teas; baths; infusion	Cultivates at home
02	Parents	Feels better than when using industrialized drugs	Teas; baths	Cultivates at home
03	Mother	Preference for natural products	Teas; syrup; juices	Cultivates at home and neighborhood
04	Older family members	Preference for natural products	Teas; syrup*	Cultivates at home and spontaneus
05	Grandmother	Preference for natural products	Teas; syrup; baths	Cultivates at home and spontaneus
06	Parents	Confidence in the potential of plants	Teas; baths	Cultivates at home and spontaneus
07	Family	Feels better than when using industrialized drugs	Teas; baths	Cultivates at home
08	Mother	Believes more in plants than in industrialized drugs	Teas; juices; "in natura consumption"	Cultivates at home and spontaneus
09	Family	Confidence in the potential of plants	Teas	Cultivates at neighborhood and spontaneus
10	Parents	Why live in places with abundant medicinal plants ; ease of access	Teas; baths	Cultivates at home
11	Mother	Feels better than when using industrialized drugs	Teas; syrup	Cultivates at home and spontaneus
12	Older family members	Best alternative care	Teas; baths	Cultivates at home

*Informant's identification number; **Several family members were quoted as uncles, grandparents and great-grandparents.
Source: Authors.

Although there are no specific studies on the subject, it is clear that the status of medicinal plants used in traditional communities is a topic that has been discussed by ethnobotany researchers (Albuquerque and Andrade, 2002a; Amorozo, 2002; Batista Silva et al., 2015; Carneiro et al., 2010). It is possible that the status change of used medicinal plants is a reflection of the characteristics of each community, or of their care practices, the relationship with nature, or of the exchange of information between ethnically distinct peoples.

In the medicinal plants free-listing, the respondents identified 34 different species, belonging to 22 families and 51 different indications (Table 2). The most common family was Lamiaceae who were also often cited in studies of Moreira et al. (2002) and Souza et al. (2002). In addition, Lamiaceae and Asteraceae were often cited in the collection of medicinal plants of the *caatinga*, made by Albuquerque et al. (2007). They are also among the most representative in ethnobotanical study conducted in other Brazilian biome, the Atlantic Forest (Gazzaneo et al., 2005).

The leaves were further part of the plant used, with 73.5% of citations. However found other studies of the *caatinga* with different results (Albuquerque and Andrade, 2002a, b; Almeida et al., 2006). Albuquerque and Andrade (2002b) emphasize that in the *caatinga* biome, usually the stem or its bark is collected for medicinal use due to continuous supply of these resources (since they are minimally affected by water irregularity). So it appears that seasonality significantly influences the relationship between people and plants in the region (Table 2).

The highest number of reported medicinal species had indications in infectious and parasitic diseases, such as intestinal infections, diarrhea and worms (17.14%), followed by the genito-urinary problems (14.28%) and skin problems and subcutaneous fabric (11.43%) (Figure 1). A great reference these medical problems is justified by its high prevalence in the region, but also because they belong to the scope of primary health care. Thus, these diseases can be easily and effectively treated with medicinal plants (Almeida et al., 2006). Unlike what was seen, others studies of the *caatinga* biome more reference indications related to the respiratory system (Albuquerque and Andrade, 2002a, b; Freitas et al., 2012; Roque et al, 2010). It is important to emphasize the subjectivity that characterizes the identification of these medical problems, changing from community to community. This difference confirms the idea that each social group has its own diagnostic system, classification (Moreira et al., 2002) and treatment of diseases, influenced by their beliefs and worldviews.

Table 2. Plant resources used by residents (n = 12) of Massangano Island, Rodeadouro Island and Jatoba Island (PE/BA), 2015.

N	COMMON NAME	SPECIES	INDICATION	PART USED	METHOD OF USE	RI
			1. Lamiaceae Family*			
01	Agua-de-alevante	*Mentha gentilis* L.	Hypertension	Leaves	Infusion	0.8
02	Erva-cidreira	*Melissa officinalis* L.	Intestinal colic	Leaves	Tea	0.8
03	Hortelã, Hortelã-miúda	*Mentha x villosa* Huds.	Flu; intestinal colic	Leaves	Tea	2.0
04	Betônica	*Rhaphiodon echinus* (Nees & Mart.) Schauer	Flu; intestinal colic	Leaves	Tea, bath	2.0
05	Vick, Hortelã-Vick,	Mentha arvensis L.	Flu	Leaves	Tea	0.8
			2. Anacardiaceae Family			
06	Aroeira	*Myracrodruon urundeuva* Allemao	Urinary tract infection and dermatological	Stalk	Infusion	1.7
07	Cajueiro	*Anacardium occidentale* L.	Diabetes mellitus; healing	Stalk	Infusion	17
08	Mangueira	*Mangifera indica* L.	Whooping cough; respiratory infection	Leaves	Tea	0.8
			3. Fabaceae Family			
09	Jatobá	*Hymenaea courbaril* L.	Anemia	Stalk	Infusion	0.8
10	Pau-ferro	*Caesalpinia férrea* Mart.	Anemia	Stalk	Molasses	0.8
11	Jurema-preta	Mimosa tenuiflora (Willd) Poir.	Skin lesions	Stalk	Bath	0.8

*Classification based on site information The Plant List (2015). Source: Authors.

Table 2. Continued...

N	COMMON NAME	SPECIES	INDICATION	PART USED	METHOD OF USE	RI
			4. Malvaceae Family			
12	Malva-santa, Malva-branca	*Sida cordifolia* L.	inflammatory processes; healing; diarrhea	Leaves	Tea	2.0
13	Quiabo	*Abelmoschus esculentus* (L.) Moench	inflammatory processes; healing; diarrhea	Leaves	Tea	0.8
14	Colônia	*Alpinia speciosa* (Blume) D. Dietr.	Hypertension	Leaves	Tea	0.8
15	Gengibre	*Zingiber officinale* Roscoe	Joint pain, flu	Roots	Tea, syrup	0.8
			6. Asteraceae Family			
16	Macela	*Egletes viscosa* (L.) Less.	Intestinal colic	Leaves	Tea	0.8
17	Picão-preto	*Bidens* sp.	Whooping cough; respiratory infection	Leaves	Tea	1.7
			7. Rutaceae Family			
18	Arruda	*Ruta graveolens* L.	Urinary tract infection; colic, digestive	Leaves	Tea, juice	1.5
19	Limão	*Citrus limonum* Russo	Worm	Fruit	Juice	0.8
			8. Xanthorrhoeaceae Family			
20	Babosa	*Aloe vera* (L.) Burm. f.	Worm	Leaves	Juice	0.8
			9. Amaryllidaceae Family			
21	Cebola	*Allium cepa* L.	Diabetes mellitus	Leaves	Tea	0.8
			10. Aristolochiaceae Family			
22	Jarrinha	*Aristolochia* sp.	Flu	Roots	Syrup	0.8
			11. Myristicaceae Family			
23	Noz moscada	*Myristica fragrans* Houtt.	Articular pain	Seed	Tea	0.8
			12. Rhamnaceae Family			
24	Juazeiro, Juá	*Ziziphus joazeiro* Mart.	Flu, tooth decay	Stalk	Powder (topic)	1.7
			13. Lauraceae Family			
25	Abacate, Abacateiro	*Persea americana* Mill.	Renal pain	Leaves	Tea	0.8
			14. Moraceae Family			
26	Amoreira Amora-negra	*Morus nigra* L.	Weight Loss	Leaves	Tea	0.8
27	Capim-santo	*Cymbopogon citrates* (DC.) Stapf	Hypertension	Leaves	Tea	0.8
			16. Combretaceae Family			
28	Castanhola	*Terminalia catappa* L.	Renal pain	Leaves	Tea	0.8
			17. Annonaceae Family			
29	Graviola	*Annona muricata* L.	Renal pain	Leaves	Tea	0.8

*Classification based on site information The Plant List (2015). Source: Authors.

Table 2. Continued...

N	COMMON NAME	SPECIES	INDICATION	PART USED	METHOD OF USE	RI
			18. Amaranthaceae Family			
30	Mastruz	*Chenopodium ambrosioides* L.	Flu	Leaves	Tea; juice	1.2
			19. Cucurbitaceae Family			
31	Melão-de-são-caetano	*Momordica charantia* L.	Pain (inflammatory processes)	Fruit and leaves	Tea	0.8
			20. Rubiaceae Family			
32	None	*Morinda citrifolia* L.	Weight Loss; inflammatory processes; Skin lesions (healing)	Fruit and leaves	Juice	2.0
			21. Phytolaccaceae Family			
33	Pipi, Tipim	*Petiveria alliacea* L.	Fever; dental cavities	Leaves and roots	Bath, tea, powder	0.8
			22. Myrtaceae Family			
34	Pitanga	*Eugenia uniflora* L.	Fever	Leaves	Tea	1.2

*Classification based on site information The Plant List (2015). Source: Authors.

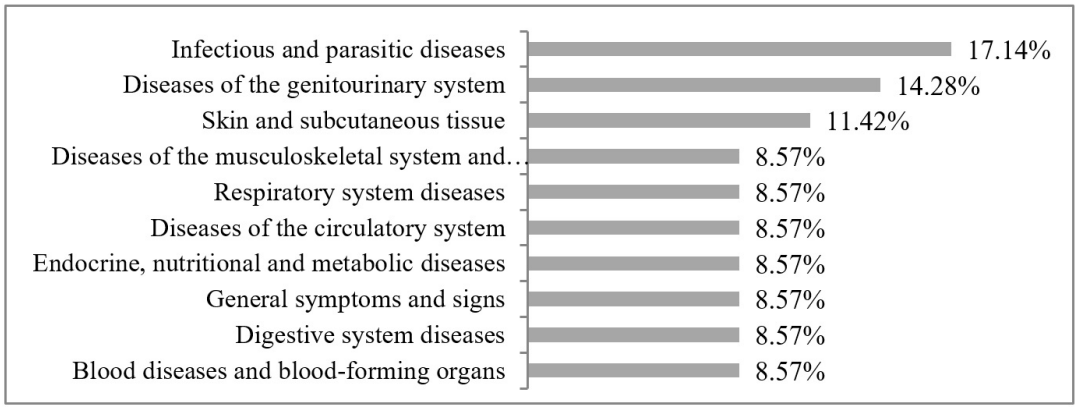

Figure 1. Percentage of medicinal plants cited by residents of Massangano Island, Rodeadouro Island and Jatoba Island (PE/BA), distributed by chapters of ICD 10, 2015. Source: Authors.

3.2. More important medicinal plants

According to the RI shown in Table 2, the most important plants for this community were: *Rhaphiodon echinus*, *Malva parviflora*, *Morinda citrifolia, Mentha x villosa* (RI = 2.0), *Myracrodruon urundeuva*, *Anacardium occidentale*, *Ziziphus joazeiro e Bidens* sp. (RI= 1.7). Some studies in areas of *caatinga* (Albuquerque and Andrade, 2002a, b; Albuquerque et al., 2007; Freitas et al., 2012) also indicate the use of these species in folk medicine, except for *Morinda citrifolia*. However, RI values for the most important plants in this study are very different from the amounts presented in an analytical study of 21 scientific publications (1986-2006), directed by Albuquerque et al. (2007). As an example, there is the set of IR values *Raphiodon echinus* and *Sida cordifolia* (IR = 2.0) very discrepant from those found in Albuquerque et al. (2007) (IR = 0.2 and 0.5, respectively). This significant difference stems from the different individual perceptions of natural resources in a given environment. The meaning of utility for medicinal plants changes between cultures and even among people of the same community. This concept becomes even greater when extrapolated understanding the utility beyond medicinal purposes (Albuquerque and Andrade, 2002b).

There was no information to *Morinda citrifolia*, possibly because this Polynesian origin plant only reached the US market in the 1990s (Potterat and Hamburger, 2007) and in Brazil only in the 2000s. Therefore the national scientific research for this species yet it is quite recent.

Thus the joint analysis of these three ethnobotanical indicators (RI, cAMU and UV) shows that the importance of a medicinal plant for a community is defined by its versatility and the consistency of information obtained by respondents. The comparison of indicators revealed *Rhaphiodon echinus* (called popularly "Betônica" or "Flor-de-urubu") as the most important medicinal plant to the community studied (Table 3). This means that this plant has importance in health care that people, both the versatility of therapeutic indications described in IR and VU, but also by the degree of consensus on its main use, among the experts interviewed (defined by cAMU). That is why preliminary tests were conducted to identify the phytochemical composition and pharmacological activity of extracts of *R. echinus*.

3.3. Chemical constituents of Rhaphiodon echinus

The Betônica (*Rhaphiodon echinus*) belongs to Lamiaceae family, subfamily Ocimoideae, subtribe Hyptidineae (Forzza et al., 2010). It is a creeping plant and its main characteristics are the presence of fruitful cup with spinescent lacínios and prostrate habit. *R. echinus* is considered invasive, typical of the caatinga biome, with wide distribution in Brazil, located in the states of Bahia, Pernambuco, Paraíba, Ceará and Minas Gerais (Dias and Kiill, 2007). This plant commonly occurs in areas of irrigated fruit in the São Francisco Valley, where it forms dense populations.

In the interviews, the residents said the main indication of *R. echinus* is urinary tract infections and dysmenorrhea. This information also appears in the study of Albuquerque et al. (2007). Another study reports that *R. echinus* is used to treat cough and dental inflammation (Menezes et al., 1998). The aqueous extract of this plant has anti-inflammatory and analgesic activity (Torres et al., 2009) and its essential oil has antimicrobial effect against *E. coli* and *K. pneumoniae* (Souza and Rodrigues, 2012).

In the screening phytochemical HEX-Re, CLO-Re and AE-Re fractions and the LYO-Re showed a higher prevalence of flavonoids and tannins, lignans and saponin. It is described in the scientific literature that flavonoids, saponins and terpenes are constituents found in many Lamiaceae family species as *Orthosiphon Stamineus* Benth, *Mentha spicata* L., *Coleus amboinicus* Lour, *Ocimum basilicum* L., *Bolivian Satureja* (Benth) Briq. L. and *Ocimum gratissimum*. The concentration of these compounds may vary depending on the solvent of each phase. But these metabolites are always present and can be a mark of this family (Andrade et al., 2010; Fiuza et al., 2010; Galvez et al., 1993).

All fractions showed the presence of flavonoids. Those compounds are important because of its various activities on the biological system, in particular, on the cardiovascular system and antioxidant action (Andrade et al., 2010; Scur et al., 2016). In the digestive system, some flavonoids have been reported with antispasmodic potential (Galvez et al., 1993). Moreover the species Hyptis - most studied genus of subtribe Hyptidineae - are recognized for having medicinal properties with antioxidant potential, attributing part of its therapeutic properties to phenolic compounds detected (Povh et al., 2012).

The HEX-Re and CLO-Re showed the mainly presence of terpenes and steroids components.

This result is supported by the study Menezes and Kaplan (2006) have identified a mixture of pentacyclic triterpenes in crude acetone extract of *R. echinus*. In addition, some work on the Hyptis gender report high concentration of terpene compounds, considered the main components of

Table 3. Comparison ethnobotanical indicators for the plants most often cited by residents of Massangano Island, Rodeadouro Island and Jatoba Island (PE/BA), 2015.

COMMOM NAME	FAMILY SPECIES	RI*	VU**	cAMU (%)***
Betônica	Lamiaceae *Rhaphiodon echinus* (Nees & Mart.) Schauer	2.0	1.7	83.3
Malva-santa	Malvaceae *Sida cordifolia* L.	2.0	1.0	16.7
None	Rubiaceae *Morinda citrifolia* L	2.0	1.5	16.7
Hortelã	Lamiaceae *Mentha x villosa* Huds.	2.0	2.0	33.3
Cajueiro	Anacardiaceae *Anacardium occidentale L*	1.7	1.0	33.3
Aroeira	Anacardiaceae *Myracrodruon urundeuva Allemao*	1.7	1.3	50.0
Juazeiro	Rhamnaceae *Ziziphus joazeiro* Mart.	1.7	1.0	16.7
Picão-preto	Asteraceae *Bidens* sp.	1.7	2.0	16.7

*RI: Relative Importance; **UV: Use Value; ***cAMU: Agreement on the Main Use. Source: Authors.

the essential oils of these species (Basílio et al., 2007; Falcão et al., 2003; Moreno et al, 2005; Torres et al, 2009). Terpenes have great importance in the new drugs research area, since they have different biological activities, such as expectorant, anesthetic, carminative, anthelmintic, antispasmodic and antiseptic (Astudillo-Vázquez et al., 2009). The therapeutic potential of terpenes can explain the popular use of *R. echinus* observed.

The alkaloids detection tests suggested the presence of these metabolites in the composition *R. echinus*. We did not find other studies confirming this result. However, the scientific literature suggests the existence of different metabolism in some Lamiaceae representatives, as the Hyptis genus characterized by remarkable variability. A study of Falcão et al. (2003) reported the isolation of a compound alkaloid, (R) -5-hydroxypyrrolidine-2-one, from *Hyptis verticillata* extract.

3.3.1. Antimicrobial activity of *R. echinus*

Because of the increasing development of drug resistance to human pathogens, medicinal plants have been extensively studied as alternative agents for the prevention and treatment of infections (Höfling et al., 2010). According to the classification by Sartoratto et al. (2004), Re -EA showed strong antimicrobial potential against *E. coli* and *S. aureus* and a moderate effect on *P. aeruginosa*. Furthermore the CEE-Re showed moderate effect against gram-negative bacteria (Table 4).

The results of phytochemical screening indicate that EA-Re and LYO-Re fractions contain high concentration of phenolic compounds. The antimicrobial activity of AE -Re might be attributed to the presence of the foresaid secondary metabolites. However the LYO-Re fraction did not show antimicrobial activity, which can be explained by the variation of the concentration of metabolites in each fraction (Andreo and Jorge, 2006).

3.3.2. Spasmolytic activity *R. echinus*

The spasmolytic substances have wide application in pathophysiological processes, such as hypertension, cardiac arrhythmias, angina pectoris, diarrhea, intestinal spasms, asthma and menstrual colic (Souza et al., 2013). Then the use of these substances in the uterine muscle may represent an important therapeutic alternative in combating problems like dysmenorrhea or diseases in pregnancy (Young, 2007). Therefore the research conducted in this study revealed that CEE-Re, LYO-Re e HEX-Re relaxed utero fragment rats, pre-contracted with KCl 60mM with concentration-dependent response.

The three derivatives *R. echinus* showed similar activity without statistical difference (Figure 2). None of them could completely relax the uterine muscle, although the HEX-Re has reached about 80% of the relaxation (Figure 2A). There was no statistically difference in the mean values of EC_{50} (the extracts were equipotents) (Figure 2B). As for the E_{max} (relative efficacy parameter) the HEX-Re fraction was more effective compared to other two products tested (Figure 2C).

No records were found in the literature on the spasmolytic properties of the species *R. echinus*. For the Lamiaceae family, there were few studies which showed activity of the same species on uterine muscle, but with spasmogenic or uterotonic activity, that is, with opposing goals for this study (Ladeji et al., 2005; Chen et al., 2000; Gruber and O'Brien, 2011; Kamatenesi-Mugisha and Oryem-Origa, 2007). However, other studies have indicated the spasmolytic property of essential oils of plants, such as the study Centeno (2010), for the essential oil of *Rosmarinus officinalis* L. and Shih et al. (2009) which assessed one flavonoid from *Scutellaria baicalensis* Georgi in reducing contractions in the uterus of female rats.

The absence of previous studies with *R. echinus* should be considered a limitation to this study, given the

Table 4. Antimicrobial activity of extracts and fractions of the aerial parts of *R. echinus*, Petrolina (PE), 2015.

SAMPLE	BACTERIAL STRAINS					
	S. aureus **ATCC 25619**	***S. aureus*** **ATCC 25925**	***P. aeruginosa*** **ATCC 8027**	***P. aeruginosa*** **ATCC 25243**	***E. coli*** **ATCC 2536**	***E. coli*** **ATCC 105**
	MIC (mg/mL)	**MIC (mg/mL)**	**MIC (mg/mL)**	**MIC (mg/mL)**	**MIC (mg/mL)**	**MIC (mg/mL)**
LYO-Re	-	-	-	-	-	-
CEE-Re	-	-	**1.024**	**1.024**	**1.024**	**1.024**
HEX-Re	-	-	-	-	-	-
CLO-Re	-	-	-	-	-	-
AE-Re	**0.512**	**1.024**	**1.024**	**1.024**	**0.512**	**0.512**
Neg. Con.*	-	-	-	-	-	-
Pos. Con.**	+	+	+	+	+	+

LYO-Re: lyophilized (aqueous extract); CEE-Re: crude ethanol extract; HEX-Re: hexane fraction; CLO-Re: chloroform fraction; AE-Re: Ethyl Acetate Fraction; *Negative control: DMSO; **Positivo control: chloramphenicol, 100 µg/mL. *Note*: Antimicrobial potential classification (Sartoratto et al., 2004): (1) Strong antimicrobial power = 0.05 ≤ MIC ≤ 0.5 mg/mL; (2) moderate antimicrobial power = 0.6 ≤ MIC ≤ 1.5 mg/mL e; (3) weak antimicrobial power = MIC ≥1.6mg/mL. Source: Authors.

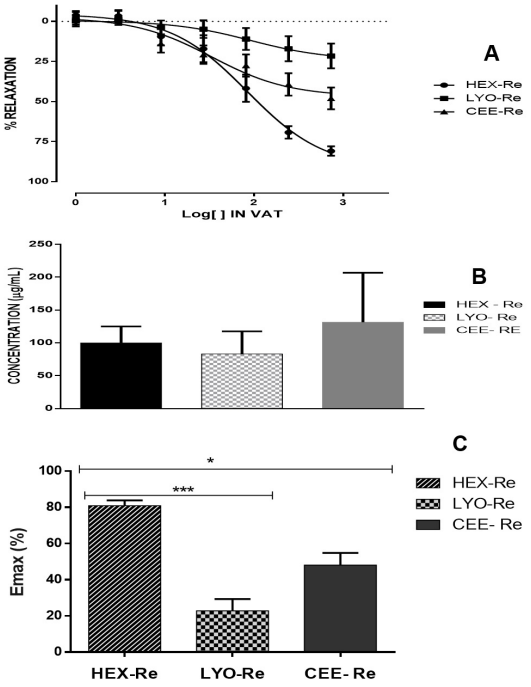

Figure 2. Effect of spasmolytic derived from *R. echinus* on the tonic contractions induced with 60 mM KCl in rat isolated uterus Petrolina (PE), 2015. (A) concentration-response curves; (B) EC_{50} values of the tested compounds; (C) E_{max} values for the tested compounds. Data are presented as mean ± S.E.M. (N = 5). *Represents Emax values were statistically different (ANOVA, $p < 0.05$); ***Represents Emax values were statistically different (ANOVA, $p < 0.01$). LYO-Re: lyophilized (aqueous extract); CEE-Re: crude ethanol extract; HEX-Re: hexane fraction. Source: Authors.

difficulty of drawing comparisons. However it reinforces the relevance of this research to the knowledge of the Lamiaceae family and the plant considered so important by the riparian river São Francisco.

4. Conclusions

In this study was reported the use of 34 different medicinal plants. The most common form of use was tea and the reason for the use of medicinal plants was the confidence in the potential of natural resources. The main indications for the use of medicinal plants were different conditions from those carried out in other studies conducted in the caatinga biome. This difference can have a relationship with local care systems, specific to each community.

The comparison of ethnobotanical indicators revealed the importance of *R. echinus* between the riparians of São Francisco River, justifying the phytochemical and pharmacological research conducted then.

The ethnopharmacological data were results of unpublished reports of evaluation of action in two experimental models in vitro. However the weak antimicrobial activity and spasmolytic of the LYO-Re (preparation similar to the form of use reported by experts) inserts doubts about the popular indication for use of *R. echinus*. Therefore, the scientific evidence for the traditional use of this medicinal plant requires further confirmations based on other experimental protocols.

This study provides an advance to approximate the popular knowledge with the research developed within the University. Thus, the prospect of this work is to collaborate with academic and scientific activities committed to valorization of the identity of the residents of the islands of the São Francisco river, while contributing to the investigation of natural resources with therapeutic potential from the regional flora.

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