Traditional knowledge as basis for phytochemical prospecting of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. aiming at conservation in the Brazilian semi-arid zone

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Research

Abstract

Background: The predatory exploitation of medicinal plants has been one of the factors with great impact on biodiversity, especially when the part used affects the survival of the plant. The bark of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. (Sapotaceae, common name quixabeira or jungleplum) is the part most frequently used in traditional medicine, and this study had as objective to verify if the leaves present the same metabolites as the the bark. Furthermore, based on the reported preparation mode, it was also analyzed whether there is a change in the quality of the metabolites extracted when different extraction methods are used (aqueous, cachaça or sugarcane liquor, as used in garrafadas, and ethanolic).

Methods: Quixabeia leaves and bark were collected, dried and ground, to prepare the raw extracts (aqueous, cachaça and ethanolic) separately. Phytochemical screening was performed to evaluate for the following chemical constituents: alkaloids, flavonols, flavones, flavononols, xanthones, triterpenes, tannins, saponins and steroids.

Results: The results obtained with both alcoholic extracts and with the aqueous extract showed similarity between the classes of medicinal interest compounds both in the leaves and in the bark. The results demonstrate that there are no losses of metabolites when the population uses cachaça or aqueous extract.

Conclusions: The obtained results open the possibility of replacing the use of the bark by the use of the leaves, reducing the impact of the extraction, promoting more time for the regeneration of the bark and favoring the conservation of the species, without loss of medicinal characteristics so appreciated by the local populations.

Key words: cachaça, aqueous extract, bark, traditional knowledge.

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Resumo
A exploração predatória das plantas medicinais vem sendo um dos fatores de grande impacto à biodiversidade, principalmente quando a parte usada afeta a sobrevivência da planta. Considerando que a casca de *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. é a parte mais utilizada por populações tradicionais, este estudo teve como objetivo verificar se as folhas apresentam os mesmos metabólitos que a casca. Além disso, tendo como base o modo de preparo relatado, também foi analisado se há alteração na qualidade dos metabólitos extraídos em função dos extratos utilizados (aquoso, cachaça, como usado na garrafada, e etanólico). Folhas e cascas da quixabeira foram coletadas, secas e trituradas, para preparação dos extratos brutos (aquoso, cachaça e etanólico) separadamente. A triagem fitoquímica foi realizada para a prospecção dos seguintes constituintes químicos: alcalóides, flavonóis, flavonas, flavononóis, xantonas, triterpenos, taninos, saponinas e esteroides. Os resultados obtidos com todos os três extratos apresentaram equidade entre as classes de compostos de interesse medicinal evidenciados tanto nas folhas quanto na casca. Os resultados demonstram que a população não há perda de dos metabólitos ao com todos os três extratos cachaça ou a água como solvente. Além disso, demonstram ter a possibilidade de substituição do uso das cascas pelo uso das folhas, diminuindo o impacto do extrativismo, promovendo mais tempo para a regeneração da casca e favorecendo a conservação da espécie.

Palavras chave: cachaça, extrato aquoso, casca, conhecimento tradicional.

Background
The preference for herbal medicines has increased over the years (Nalawade *et al.* 2003). It is estimated that 80% of the world's population use plants for curing basic diseases (Chia *et* al. 2017; Hamilton, 2004). In sub-Saharan Africa where people often have a low purchasing power, traditional popular medicines are the most used remedies both in rural and urban areas, as opposed to expensive pharmaceutical drugs (Angone *et al.* 2013).
However, the predatory exploitation of medicinal plants has been one of the factors of greatly impacting biodiversity (Albuquerque *et al.* 2011; Albuquerque & Andrade 2002a). Numerous cases of documented ecological imbalances are caused by the disorderly exploitation of non-timber products (leaves, wax, latex) (Agra *et al.* 2007; Albuquerque *et al.* 2011; Dantas *et al.* 2007; Lima *et al.* 2016; Lucena *et al.* 2007; Lucena *et al.* 2012; Lucena *et al.* 2015; Marques, 2008; Pedrosa *et al*. 2012), leading ultimately to biodiversity loss (Reddy *et al.* 2016).
Much of the impact on plant populations is in the form of removal of the resource. Species with a high value of medicinal use, especially in the Caatinga semi-arid region of Brazil, suffer from frequent extractive action, consequently generating problems for the conservation of the local flora (Cunha & Albuquerque, 2006; Lima *et al.* 2016;). This threat is considered even more serious because, for many woody species, the main part used is the bark (Ribeiro *et al.* 2017; Kings *et al.* 2017), which may affect the survival of the species, if carried out intensively.
Sideroxylon obtusifolium (Roem. & Schult.) T.D. Penn., a species of the Sapotaceae family, popularly known as Quixabeira, is distributed from the state of *Maranhão* in the North to *Rio Grande do Sul* in the South of Brazil. It is a deciduous or semi deciduous tree with dense crown, and a height of about 18m (Garrido *et al*. 2007; Matos, 2012). The species flowers between the months of October and November, its flowers are odoriferous (Delfino *et al.* 2005; Garrido *et al.* 2007; Marques *et al.* 2010;) and attract mainly bees and butterflies as pollinators (Gomes *et al.* 2010). Its fruiting occurs between the months of January and February, and the fruits are consumed by wild animals as part of their diet (Lorenzi, 1999), because they contain sweet pulp (Silva *et al.* 2012). The species is used for various purposes like construction, fencing, technology, in the production of hoe and scythe handles. The fruits are consumed raw by the population of rural communities and the leaves are used for fodder (Albuquerque & Andrade 2002; Albuquerque *et al.* 2012). However, quixabeira stands out for its medicinal use, and the bark is the most used part for the preparation of popular remedies used by the local populations (Albuquerque *et al.* 2011; Kings *et al.* 2017; Lima *et al.* 2016; Lucena *et al.* 2015; Pedrosa *et al.* 2012; Ribeiro *et al.* 2017). Due to the intensive extractive activity causing the uncontrolled removal of its bark (Albuquerque & Andrade 2002b; Albuquerque & Oliveira 2007; Almeida *et al.* 2002), quixabeira has become scarce in the Caatinga, because the collection techniques used are aggressive, often leading to plant death due to stress (Almeida *et al.* 2002; Albuquerque *et al.* 2007).
The main use of quixaba is as anti-inflammatory (Leite 2015). The preparation and administration may be carried out in a variety of ways, depending on the disease, either using water or cachaça (sugarcane liquor) as solvents, a process locally known as *'dressing'*, for which the bark of quixabeira is immersed in water from one day to the next, or '*garrafada',* where cachaça the bark is immersed in together with the barks of other plants (Lima *et al.*

2016; Lucena *et al.* 2015; Pedrosa *et al.* 2012). The bark after being soaked overnight is administered orally for the treatment of coughs and digestive problems (Agra *et al.* 2007; Lucena *et al.* 2015; Pedrosa *et al.* 2012;); it can be used as seat bath in case of genito-urinary problems, and also topically for injuries and eye washing (Agra *et al.* 2007; Monteiro *et al.* 2011; Pedrosa *et al.* 2012;). A tea of the bark can be prepared and given orally for genito-urinary diseases and pain treatment in general (Agra *et al.* 2007). The "garrafada" is also one of the recommended forms of use for inflammations in general (Marreiros *et al.* 2015). The use of the bark for the treatment of genito-urinary diseases is especially common.

From a phytochemical point of view, both the solvent used, and the part of the plant used, can influence the efficacy of the action of the phytotherapeutic compound. Several phytochemical studies performed with different parts of the plant, using organic solvents to extract secondary metabolites, demonstrated the existence of the medicinal action of Quixabeira (Figueiredo *et al.* 2015; Oliveira *et al.* 2012). The main secondary metabolite found were terpenoids (Montenegro, 2005). Besides these compounds, the presence of alkaloids, flavonoids, tannins and saponins was also described (Hussain *et al.* 2007), indicating the medicinal potential of the species. However, phytochemical studies using aqueous extraction, or maceration in cachaça are lacking, even though these are the main forms of preparation (Agra *et al.* 2007; Marques *et al.* 2010).

Because of the importance of Quixabeira tree bark for disease treatments and the impact that bark collection can cause (Albuquerque *et al.* 2011; Albuquerque & Andrade 2002), a viable alternative for the reduction of predatory extraction might be the extraction of the secondary metabolites from other parts of the plant that would have less impact on the species (Chen *et al.* 2016). This replacement of plant parts has already been observed in other species. For example, in the case of ginseng (*Panax ginseng* C.A. Mey., Araliaceae), the leaves exhibited pharmacological activities similar to those of their roots, thus providing a less impactant form of obtaining the medicinal drug (Wang *et al.* 2009). While studies have shown that quixabeira bark has anti-inflammatory, antinociceptive and antioxidant action for the treatment of pain and inflammation (Araújo-Neto *et al.* 2010; Leite *et al.* 2015), the leaves also demonstrated anti-inflammatory and antinociceptive action, besides the antifungal action (Aquino *et al.* 2016; Silva *et al.* 2017). However, leaves are not used by the local communities.

This study was based on the ethnobotanical information provided by traditional communities (Agra *et al.* 2007; Lucena *et al.* 2015; Monteiro *et al.* 2011; Pedrosa *et al.* 2012) and aims to verify the type of extraction (aqueous, cachaça as used in 'garrafadas', and ethanolic) of leaves and bark of *S. obtusifolium* yielded better performance than the crude extract. Phytochemical screening was performed to characterize the classes of secondary metabolites present in leaf and bark, with the intention of evaluating the possibility of replacing the main part of the plant by the traditional communities. Prioritizing the use of leaves is a way to favor the conservation of the species, reducing the extractive pressure caused by the removal of the bark, and promoting the plant's regeneration time.

Materials and Methods

Study Area

The municipality of Cabaceiras is characterized by a low rainfall (average of 300mm annually) with sparse rains that accumulate between the months of February and May (IBGE 2017). It has a pastoral tradition, with local communities practicing subsistence agriculture, with the planting of maize, beans and cassava in areas irrigated by drip irrigation or rainy periods (IBGE 2017).

The Tapera community is located in the vicinity of the Environmental Protection Area (Portuguese acronym for APA) of *Cariri Lajedo Pai Mateus*, about 20 km from the city of Cabaceiras, Paraíba (Figure 1). It consists of about 150 residents, who live in masonry houses and cement floors, with no schools, churches, and access to health care is carried out monthly by a general practitioner. Economically, they survive mainly from the incentives of the government (Program for low-income families - *Bolsa Familia* - and retirement) and concomitantly from subsistence agriculture, pastoral activity. Some residents still work at the *Pai Mateus* Hotel, located in the community surroundings and part of the Conservation Unit, where resource extraction is prohibited.

Plant collection

The bark and leaves of *S. obtusifolium* (Figure 2 and 3) were collected from eight individuals located about 500m, from the community's anthropic area from where residents prefer to remove botanical material for the preparation of their medicines. The stem bark (500g) was removed with a machete in vertical direction, removing superficial pieces of only a part of the stem, reproducing the process used by the local communities (Albuquerque *et al.* 2011). The leaves (500g) were removed one by one, with the lowest possible amount of branches cut to not damage the tree. The minimum amount required for the preparation of extracts for phytochemical screening was set as 100g of dry plant material (Matos 2009).

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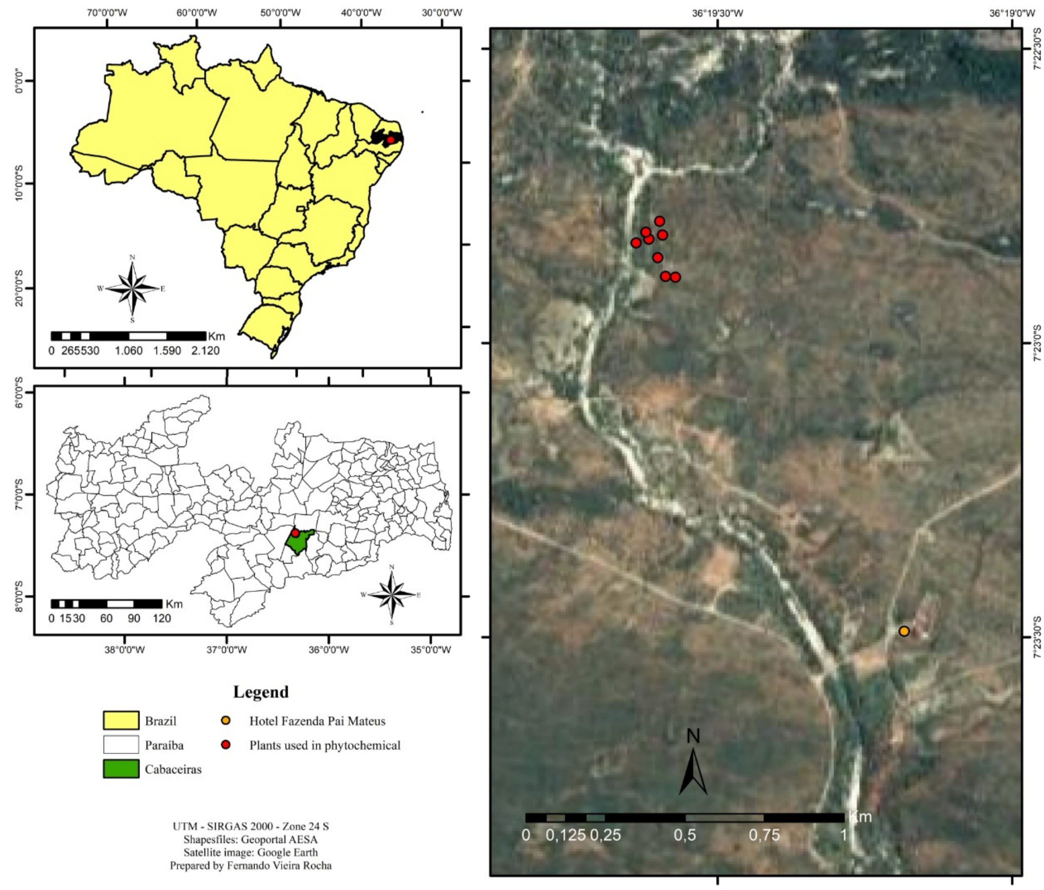

Figure 1. Geographic location of the study areas. Cabaceiras, Paraíba, Brazil.

Figure 2. *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. (Sapotaceae, common name Quixabeira). Cabaceiras, Paraíba, Brazil.

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Obtaining the crude extracts of the bark and leaves of *S. obtusifolium*

The botanical material (bark and leaf) of *S. obtusifolium* was oven dried with circulating air at 40 °C for 72 hours, and then mechanically ground separately.

The solvents used for the preparation of the extracts were chosen based on the literature, since the home remedy using Quixabeira bark is made mainly with water (*dressing*) or cachaça (*garrafada*) (Lucena *et al.* 2015; Pedrosa *et al* 2012). The ethanolic extract served as a basis for comparison to the other extracts, since it is usually used in phytochemical studies for its effectiveness in extracting the largest number of classes of metabolic compounds.

Three types of extracts were prepared: aqueous, with cachaça (39%) and ethanolic (98.5%), for each botanical part collected (leaves and bark), totaling six extracts. For each extract, 100g of the dry and crushed botanical material and 300ml of the solvent were used. The extracts were submitted to maceration for 3 consecutive days (Matos, 2009).

Afterwards the extractive solutions were concentrated in a rotary evaporator under reduced pressure at 40 °C coupled to a chiller in the case of the aqueous extract, obtaining the crude extracts of the bark and the leaves separately. The yield of each extract was calculated following the formula based on the methodology used by Rodrigues *et al.* (2011).

$$Re = (P\ extract / Weight\ partbot) \times 100$$

Where:

Re: yield of extract (%)

P extract: weight of the concentrated extract

Weight partbot: weight of the dry botanical material used in the preparation of the extract.

Phytochemical screening

Phytochemical screening was performed elucidate the following chemical constituents: alkaloids, flavonols, flavones, flavononols, xanthones, triterpenes, tannins, saponins and steroids, observing the change in color or presence of precipitate in the tested sample. The analyzes were adapted and based on the methodology proposed by Matos (2009), with the purpose of identifying the groups of secondary metabolites present in Quixabeira extracts through qualitative tests.

Nine numbered test tubes were separated for each prepared extract, totaling 27 tubes. From the tubes 1 to 8 an aliquot of the crude extract of 3 to 4 ml was withdrawn. All tubes were taken to the water bath to evaporate the contents of the tubes to half volume and to concentrate the samples.

Test for alkaloids

The contents of each test tube 1 were basified with 1 ml of 1% sodium hydroxide and the solvent evaporated in the water bath to total sample dryness. The resulting solid was dissolved with 6 ml distilled water and 6 ml chloroform ($CHCl_3$), filtered with a small cotton funnel into a separatory funnel where the chloroform portion was separated. To this 6 ml of 1% hydrochloric acid were added under stirring. The solution was allowed to settle until the solution became clear. The upper portion of the solution was distributed in tubes 2, 3, 4 and 5, with 1 ml in each tube. To tube 2, 5 drops of Bouchardat reagent were added, to tube 3, 5 drops of Mayer reagent were added, to tube 4, 5 drops of Dragendorff reagent were added and to tube 5, 5 drops of tungstic sodium acid reagent were added to test for alkaloids. In all cases, the presence or absence of precipitate was observed.

Test for steroids and triterpenes (Lieberman-Burchard)

In tube 6, 1-2 ml of chloroform was added and mixed with a glass rod until the complete homogenization of the residue of the tube. The solution was filtered through a glass funnel with cotton and sprinkled with anhydrous sodium sulphate (Na_2ONLY_4) to another dry test tube. 1 ml of acetic anhydride and three drops of concentrated sulfuric acid were added. The evaluation was performed with the observation of the color change of the sample. The change in staining of the sample to permanent green is indicative of free steroids and to permanent red is indicative of free pentacyclic triterpenoids.

Test for flavonols, flavones, flavononols and xanthones

To each tube 7 a tape of magnesium and 5 ml of hydrochloric acid (10%) were added. The end of the reaction indicated by the the total dissolution of the magnesium ribbon, and the color change of the sample were observed. The appearance or intensification of the pink coloration is indicative for the presence of flavonols, flavones, flavononols and free xanthones or their heterosides.

Test for phenols and tannins

To tube 8 of each sample three drops of an alcoholic solution of iron chloride III ($FeCl_3$) were added under stirring and the abundance of dark precipitation in the test tube was observed. The variable coloration between blue and red is indicative for phenols and the dark precipitate in the blue tint indicates the presence of pyrogallic tannins and in the green tonality indicates the presence of flobabenic tannins.

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Test for saponins

In tube 9, 10 ml of distilled water was added, and shaken strongly for three minutes, until frothing could be observed. Persistent and abundant foam for 5 minutes indicates the presence of saponins.

Results

Quixabeira leaves and barks: similar metabolites with therapeutic potential

Phytochemical screening revealed a greater diversity of metabolite classes when using cachaça and water as solvent (Table 1). In phytochemical screening, classes of secondary metabolites of the species and related to pharmacological activities (anti-inflammatory, cicatrizing, antioxidant) were identified (Leite *et al.* 2015; Araújo-Neto *et al.* 2010, Silva *et al.* 2017; Aquino *et al.* 2016.) (Table 2). The observed metabolites were similar in both solvents used and for both analyzed parts (leaves and barks), demonstrating that there was, in general, little variation between them. In the cachaça extract, both the bark and the leaves showed clear presence of triterpenes, flavonols, flavones, flavononols, xanthones, tannins and saponins. Studies carried out with other types of extracts on bark and leaves of *S. obtusifolium* presented the same substances extracted in the extract made with cachaça. In literature an ethanolic extract with Quixabeira leaves collected in the Restinga National Park of Jurubatiba-RJ in HPLC/PDA/MS analysis revealed as main constituents saponins and flavonoids (Oliveira *et al.* 2012). Araújo-Neto *et. al.* (2010) also recorded the presence of the same metabolites found in this study in the ethanolic extract of quixabeira bark from the state of Sergipe.

Table 1. Phytochemical screening of aqueous, cachaça (sugarcane liquor) and ethanolic extracts of bark and leaves of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. in the Tapera Community, Cabaceiras, in the Semi-arid Caatinga of Paraíba, Northeast of Brazil. - negative result, + weakly positive result; ++ moderately positive result; +++ strongly positive result.

TESTS	AQUEOUS EXTRACT		CACHAÇA EXTRACT		ETHANOLIC EXTRACT	
	BARK	LEAF	BARK	LEAF	BARK	LEAF
ALKALOIDS	-	-	-	-	-	-
STEROIDS	-	-	-	-	-	+++
TRITERPENES	+++	++	+++	+++	++	+
FLAVONALS	++	++	++	++	+	++
FLAVONS	++	++	++	++	+	++
FLAVANONOIS	++	++	++	++	+	++
XANTONAS	++	++	++	++	+	++
TANNINS	+++	+	+++	+++	+	+
PHENOLS	-	-	-	-	-	-
SAPONINS	+++	+++	+++	+++	++	+

Some metabolites found are characteristic of the Sapotaceae family. Metabolic classes similar to those found in *S. obtusifolium* were documented in the hexane extract of the leaves *Pouteria ramiflora* (Mart.) Radlk (Sapoaceae) (triterpenes, Rodrigues *et al.* 2017). From the aqueous and ethanolic extracts of the *Chrysophyllum pruniforme* Pierre ex Engl., an African plant widely used in Gabon and Congo, saponins, tannins and flavonoids were isolated (Angone *et al.* 2013).

In aqueous extracts of *S. obtusifolium* the presence of saponins, flavonols, flavones, flavononols and xanthones was evident both for the bark and the leaves. However, the concentratioon of triterpenes and the tannins in the bark was more pronounced than in the leaves. The presence of these metabolites in the aqueous extracts corroborates the information about quixabeira bark used by traditional communities (Lucena *et al.* 2015; Pedrosa *et al.* 2012), and generally the use of plants rich in tannins and flavonoids for the treatment of inflammations (Gurib-Fakim, 2006).

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In the ethanolic extracts of bark and leaves, all metabolites were less common when compared to the other two extracts (aqueous and cachaça). In the ethanolic leaf extract, flavonols, flavones, flavononols and xanthones were more present than in the bark extract. However, only the ethanolic extract of the leaves showed the presence of steroids. A similar result was observed in a study carried out with the species in the city of Canindé de São Francisco - SE (Araújo-Neto *et al.* 2010).

Table 2. Yield of the extracts of leaves and bark of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. in the Tapera Community, Cabaceiras, in the Semi-arid Caatinga of Paraíba, Northeast of Brazil.

	Aqueous extract	Cachaça extract 39%	Ethanolic extract 98%
Bark	8.4%	9.8%	2.8%
Leaves	6.6%	9.4%	8.3%

None of the extracts showed the presence of alkaloids and phenols. Meanwhile, Aquino *et al.* (2016) isolated N-methyl-(2S,4R)-trans-4-hydroxy-L-proline (NMP) from the leaves of *S. obtusifolium* using a methanolic extract.

Studies with quixaba leaves and with other members of the Sapotaceae family showed biological activities, demonstrating the presence of bioactive substances in their composition. An example are methanolic and ethyl acetate fractions of quixabeira stem bark collected in the municipality of Cabrobó-PE, which presented antioxidant and antimicrobial action for strains of *Staphylococcus aureus* Ruella *et al.* 2011). In Maurit in Ceará, the leaves presented antimicrobial and topical anti-inflammatory activity (Aquino *et al.* 2016). *Pouteria torta* (Mart.) Radlk, found in the Brazilian Cerrado, when macerated in hexane followed by ethanol extraction showed cytotoxic activity in the assay with *Artemia salina* (Crustacea) (Perfect *et al.* 2005). The methanolic extract of its leaves presented antimicrobial activity for some strains of bacteria (Alves *et al.* 2000). The extract of the leaves, the stem bark and the *Pouteria venosa* (Mart.) Baehni. presented positive preliminary results for larvicidal and antiradical action (Montenegro *et al.* 2006).

Importance of the solvent used in the extraction in the diversity of metabolites

The highest quantities of the crude extract were obtained from the extracts made with the cachaça, followed by the aqueous extracts of bark and leaves, respectively (Table 2). These results can be explained because cachaça is considered a mixture of solvents and, during the fermentation process, besides the presence of water and ethanol, there is formation of secondary alcohols, esters and methanol that become responsible for the improvement of the extraction, due to variations of polarity gradients (Cardoso, 2013), including water, a polar solvent. In general, more polar solvents extract polar molecules more easily, as well as more nonpolar solvents extract apolar molecules more easily (Martins *et al.* 2013). Extractions with moderately polar solvents (methanol and ethanol) have the capacity to extract both polar and nonpolar compounds (Yunes, 2014).

Therefore, based on the traditional knowledge of the communities, which use the bark of quixaba for medicinal purposes in the form of a water extract and alcoholic extract in a bottle (cachaça), we verified that both solvents are able to extract classes of secondary metabolites with therapeutic potential. The leaves, in aqueous extracts and in cachaça, obtained similar results to the bark. Quantitative studies with Quixabeira leaves are needed to demonstrate which bioactive substances are present within the metabolite classes found, in order to allow the use of the leaves for medicinal purposes by the traditional communities. The use of environmental education as a tool for the return of research to the community may allow information on Quixabeira use to reduce extractive pressure in the species and contribute to its conservation in the environment.

Declarations

List of abbreviations: Not applicable.

Ethics approval and consent to participate: The purpose of the study was explained to the community members interviewed, and they were asked to sign an informed consent form, as required by the Brazilian National Health Council through the Research Ethics Committee (Resolution 510/2016). The study is part of a larger project entitled Identification of Patterns of Use of Native Species in Caatinga Areas: An Ethnobiological and Conservationist Approach, which has already been approved by the Human Research Ethics Committee (CEP, for its initials in Portuguese) of the Lauro Wanderley Hospital, Federal University of Paraíba, registered under protocol CEP/HULW no. 297/11 (with cover sheet no. 420134).

Consent for publication: Not applicable.

Availability of data and materials: The data was not deposited in public repositories.

Competing interests: The authors do not have any competing interests.

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Authors' contributions: DAB carried out fieldwork, data analysis and drafted the manuscript. RFPL and DDC configured the research project, supervised the work and improved the manuscript. All authors read, reviewed and approved the final version of the manuscript

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