Phytochemical screening of *Cenostigma microphyllum* (Mart ex G. Don) E. Gagnon & G. P. Lewis (Fabaceae)

Edward Teixeira Albergaria[1, *], A. F. M. Oliveira[1]

[1] Laboratório de Ecologia Aplicada e Fitoquímica, Departamento de Botânica, Universidade Federal de Pernambuco, Av. Prof. Moraes do Rego s/n, Cidade Universitária, Recife, Pernambuco, CEP 50670-901, Brazil

[*] Correspondence: edward.teixeira.albergaria@gmail.com

Introduction

Seasonally Dry Tropical Forest and Woodlands (SDTFW) is a global biome of 2,700,000 km² characterized by low annual rainfall (< 1800 mm/year), high seasonality (periods of 5–6 months characterized by less than 100 mm of rainfall) and deciduous, not fire-tolerant vegetation (Pennington et al., 2009; Queiroz et al., 2017). The Caatinga, as integral part of this biome, is the largest dry forest nucleus, with a total area of 849,516 km² and corresponding to 31% of the SDTFW biome in the Neotropical region and it is entirely located in the Brazilian Northeastern region (Pennington et al., 2009; Queiroz et al., 2017).

The SDTFW Caatinga, besides facing multiple anthropic pressures, is also one of the regions most vulnerable to the global climate crisis (Collevatti et al. 2013; Ripple et al. 2019) owing to low rainfall and soil nutrient depreciation (Borchert 1994; Menezes et al. 2012; Buzzard et al. 2015). These two factors that are limiting to the growth and development of plant life, may accelerate the desertification process and affect all biodiversity and ecosystem services (IPCC 2019; Santos et al. 2014; Vieira et al. 2015). However, despite of it, SDTF Caatinga is estimated to be between 5 and 8 thousand plant species, which about 318 of them are endemic, turning it in one of the most privileged as a resource with bioprospective potential that has fundamental importance in pharmacological studies of its flora (Novais et al., 2003; Giulietti et al., 2006).

Furthermore, plants that grow up under scarce climatic conditions may have higher concentrations of natural products compared to individuals of the same species that grow in habitats with wide availability of water (Selmar, 2008). There have been reports about the positive influence of water stress on the concentration of various secondary metabolites, including cyanogenic glycosides,

and catechins (Selmar and Kleinwächter, 2013).

Among the several species present in the *Caatinga*, *Cenostigma microphyllum* (Mart ex. G. Don) E. Gagnon & G. P. Lewis (*Fabaceae*) is an endemic, native and highly abundant species in the SDTFW Caatinga (Fernandes et al., 2019 ; Gagnon et al., 2016), showing considerable importance to the local population, being used for medicinal purposes (in the treatment of digestive diseases, infections and inflammations, due to its antibiofilm and antiprotozoal properties) (Agra et al., 2008; Silva et al., 2020,2014), beyond that it is used as timber and fuel (Gomes et al., 2019). Thus, it is an interesting species to study regarding phytochemical aspect, given local relevance and low availability of information on it in the literature.

Therefore, the objective of this work was to perform the phytochemical screening of hexanic (nonpolar) and hydroalcoholic (polar) extracts, both of leaves and stem barks of the *Cenostigma microphyllum* to verify the presence of secondary plant metabolites with potential use in human health.

Materials and methods

Study site

The study was conduced at the Catimbau National Park, located in the state of Pernambuc—Brazil (8°24'00" and 8°36'35" S; 37°00'30" and 37°01'40" W). The Park is located 281 km from the state capital Recife and covers part of the municipalities of Buíque, Ibimirim and Tupanatinga. Its area comprises approximately 62,300 ha, with a semi-arid climate (Köppen-Geiger Bsh classification), deciduous vegetation typical of hyperxerophytic Caatinga and predominantly lithosols. It presents an average annual temperature of 23 °C and a high variation in its average annual precipitation (480-1,100 mm/year) (Rito et al., 2017) (Fig. 1).

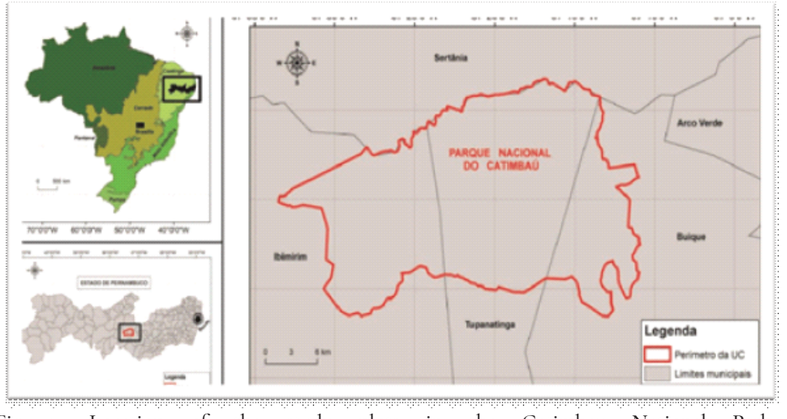

Fig 1. Location of the study plots in the Catimbau National Park, PE—Brazil

Experimental design

The plant material collected consisted of 500 grams of leaves and stem bark of the Cenostigma microphyllum obtained in January 2019 from adult individuals approximately three meters high, which the stem barks samples were removed at one meter over the ground level, while leaves were always mature, without signs of predation and obtained from shaded site on all trees. Then, samples were stored in plastic bags and dehydrated at room temperature (26 ° C) in order to better preserve the phytochemical properties of the material, being subsequently sprayed and sieved before the phytochemical screening process.

Sample collection was authorized by the national Biodiversity Authorization and Information System (SISBIO), registered under number 64367–1. Collection and herbori-zation followed the guidelines as suggested by Mori et al. (1989) with identification performed by Dr. Rita de Cássia Araújo Pereira. Voucher samples were deposited in Dárdano de Andrade-Lima Herbarium, at Instituto Agronômico de Pernambuco (IPA—PE- Brazil) under no. 91695.

Phytochemical screening

Hexanic and hydroalcoholic extracts of the leaves and stem barks were prepared by double maceration technique. In a 50 ml Erlenmeyer flask about ten grams of the pulverized plant material were imerged into 100 ml of hexane PA, keeping under constant agitation for one hour. Then, the crude hexanic extract was filtered and taken to a hot water bath at 110 °C. Remaining material was re-suspended in hydroalcoholic solvent (70 ml ethyl alcohol PA plus 30 ml distilled water) and followed to the same procedure as descripted previously to obtain the crude hydroalcoholic extract. Subsequently, aliquots of both extracts were removed for phytochemical screening.

Hexanic extracts were tested for presence or absence of saponins, alkaloids, coumarins, volatile oils/terpenoids and simple phenols, while hydroalcoholic extracts were tested for presence of flavonoids (glycosides and aglycone) using the Thin-layer chromatography (TLC) technique, which they were compared to well-known standards as reference model (Tab. 1). Analyzes were carried out applying aliquots of these extracts under silica gel 60 F254 chromatographic plates with aluminum support (20 × 20 cm) (Merck) using capillary tubes (15µL). Then, such plates were eluted in different mobile phases and stains, as suggested by conventional methods (Wagner and Bladt, 1996) and visualized under UV light chamber.

Tab. 1. Secondary metabolite classes, mobile phases, stains and standards used in the phytochemical screening of *Cenostigma microphyllum* (Mart. Ex G. Don) E. Gagnon & G.P. Lewis (*Fabaceae*)

Class	Mobile phase	Stain	Standard
Hexanic extract			
Alkaloids	Precipitation reaction	Dragendorff + HCl 10%	Scopolamine butylbromide (Buscopan© tablets) and *Catharanthus roseus* (*Asteraceae*)
Phenylpropanoids/ Coumarins	Toluene: ethyl ether (1:1 v/v)	Ethanolic KOH 10%	Chloroformic extract of Justicia pectoralis and 1,2-benzopyrone
Saponins	-	Foam test	Aqueous extract of stem bark of *Zizipus joazeiro* (*Rhamnaceae*)
Volatile oils/ terpenoids	Toluene: ethyl acetate(93:7 v/v)	Anisaldehyde-sulphuric acid + heating	Ethanolic extract of *Mesosphaerum suaveolens* (*Lamiaceae*) and *Justicia gendarussa* (*Acanthaceae*)
Simple phenols	Toluene:chloroform: ethanol (40:60:15 v/v)	NP/PEG (NEU)	-
Hydroalcoholic extract			
Flavonoids (glycosides)	Ethyl acetate: formic acid: glacial acetic acid: distilled water (100:11:11:26 v/v)	NP/PEG (NEU)	Quercetin
Flavonoids (aglycones)	Toluene:ethyl ether (1:1 v/v)	NP/PEG (NEU)	Quercetin

Results and discussion

The phytochemical profile of the leaf and stem bark extracts of *C. microphyllum* detected strong presence of phenols, however the presence of flavonoids (glycosides and aglycone) was also observed. On the other hand, the presence of alkaloids, phenylpropanoids/coumarins, saponins and volatile oils / terpenoid was not detected (Table 2).

Tab. 2. Metabolites present in the hexanic and hydroalcoholic extracts of *Cenostigma microphyllum* (Mart. Ex G. Don) E. Gagnon & G.P. Lewis (Fabaceae), using the Thin- layer Chromatography (TLC) method

Class	Stem bark	leaves
Hexanic extract		
Alkaloids	-	-
Phenylpropanoids/ Coumarins	-	-
Saponins	-	-
Volatile oils/terpenoids	-	-
Simple phenols	++	++
Hydroalcoholic extract		
Flavonoids (glycosides)	+	+
Flavonoids (aglycones)	+	+

Subtitle: (-) Absent; (+) Present and (++) Strongly present

Many phenolic compounds, terpenoids and phytosterols have been isolated from several species of the *Cenostigma genus* (Zanin et al., 2012). The results observed in *C. microphyllum* are similar to those obtained in the leaves or stem barks of the species Cenostigma pyramidale (Tul.) E. Gagnon & G.P. Lewis, which it is an other widely used medicinal species that it has already possible to isolate phenolic acids (such as gallic acid and derivatives of cinnamic acid) and polyphenols, such as catechins, lignans, flavonoids and derivatives (aglycone, chalcones, catechins, flavonones, flavones and flavonols) (Bahia et al., 2010; Bahia

et al., 2005; Mendes et al., 2000; Monteiro et al., 2005; Saraiva et al., 2012; Silva et al., 2011).

Studies also shows *Cenostigma pyramidale* has metabolites such as condensed tannins, phenylpropanoids, lupeol (a triterpene) and phytosterols (such as β–sitosterol and stigmasterol) (Bahia et al., 2010; Bahia et al., 2005; Mendes et al., 2000; Saraiva et al., 2012; Siqueira et al., 2012), which they were not detected or not tested in the present study. On the other hand, Silva et al. (2020,2014) confirmed the presence of hydrolyzable tannins, terpenoids, phytosterols and amines in the fruits of *Cenostigma microphyllum*. Bueno et al. (2016) observed the presence of hydrolyzable tannins in the stem barks of the Cenostigma pluviosum (DC.) E. Gagnon & G. P. Lewis species, it may also be an indication of the presence of hydrolyzable tannins in the stem bark of this study plant species.

Conclusion

Due to the phytochemical similarities in the composition between *Cenostigma pyramidale* and *Cenostigma pluviosum*, the species *Cenostigma microphyllum* may work as a promising alternative in the treatment of several diseases of traditional populations in the Brazilian semi-arid region, requiring further studies regarding its biological activities, cytotoxicity, mutagenicity and hepatotoxicity.

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