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PHYTOCHEMICAL PROFILE AND ANTIOXISING ACTIVITY OF MEDICINAL PLANTS FROM CASTAINHO - PE

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Thaís V. da Rocha Ferro[1], Filipe de Santana Othmar[2], Daniel Medeiros Nunes[3], Marcio Michael Pontes[4], Natalie Emanuelle Ribeiro Rodrigues[5], Pedro Henrique Sette de Souza[6] and Alissandra Trajano Nunes[7].

ABSTRACT

The use of medicinal plants is deeply rooted in the history of traditional populations, representing an important therapeutic tool in vulnerable communities, such as quilombolas. These communities often depend on local natural resources for the treatment of various diseases, which justifies the search for scientifically validating the use of plant species with therapeutic potential. Therefore, this study aimed to investigate the phytochemical profile and antioxidant activity of three species with high use value in the quilombola community of

[1] Master's student in the Graduate Program in Health and Socio-Environmental Development
University of Pernambuco (UPE)
E-mail: thaisrferro@gmail.com
Orcid: https://orcid.org/0000-0001-5401-174X
Lattes: https://lattes.cnpq.br/5190908383395843
[2] Graduating in Medicine
University of Pernambuco (UPE)
E-mail: filipe.othmar@upe.br
Orcid: https://orcid.org/0009-0008-1159-9764
Lattes: http://lattes.cnpq.br/9197202929697127
[3] Graduating in Medicine
University of Pernambuco (UPE)
E-mail: daniel.medeirosnunes@upe.br
Orcid: https://orcid.org/0000-0003-0624-9459
Lattes: http://lattes.cnpq.br/1861837426303220
[4] Master's student in the Graduate Program in Animal Bioscience
Federal Rural University of Pernambuco (UFRPE)
E-mail: marcio.mpontes@ufrpe.br
Orcid: https://orcid.org/0000-0001-5912-9827
Lattes: http://lattes.cnpq.br/8131480125482359
[5] Dr. in Therapeutic Innovation
University of Pernambuco (UPE)
Email: natalie.rodrigues@upe.br
Orcid: https://orcid.org/0000-0003-2235-729X
Lattes: https://lattes.cnpq.br/4443122163296249
[6] Doctor of Dentistry
University of Pernambuco (UPE)
E-mail: pedro.souza@upe.br
Orcid: https://orcid.org/0000-0001-9119-8435
Lattes: https://lattes.cnpq.br/6730016892651974
[7] Dr. in Biotechnology
University of Pernambuco (UPE)
E-mail: alissandra.nunes@upe.br
Orcid: https://orcid.org/0000-0001-8830-3599
Lattes: http://lattes.cnpq.br/9481915719146847

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Castainho, Garanhuns-PE: Acanthospermum australe (Loefl.) Kuntze, Momordica charantia L. and Hyptis pectinata (L.) Poit. The leaves were collected, dried and submitted to infusion to obtain the extracts. Phytochemical screening revealed the presence of bioactive compounds, such as tannins, flavonoids, triterpenes, and saponins. The quantification of polyphenols and flavonoids indicated significant concentrations, especially Hyptis pectinata (503.86 µg/mg of polyphenols). The evaluation of the antioxidant activity by the DPPH method demonstrated that only Hyptis pectinata sequestered 50% of the DPPH radical, indicating its significant antioxidant potential. The fact that Acanthospermum australe and Momordica charantia did not present the same sequestration capacity may be related to the concentration of the extracts used in the assay. These findings suggest that the plants studied have compounds with therapeutic potential, reinforcing the importance of bioprospecting in traditional communities.

Keywords: Medicinal Plants, Traditional Communities, Antioxidant, Secondary Metabolites.

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INTRODUCTION

The use of plants as medicine is part of the evolutionary history of humanity, so the knowledge acquired as a survival strategy was passed on between generations, remaining until the present day in different populations. The cultural and biological heritage associated with traditional populations is recognized by the World Health Organization (2020) with regard to traditional medicine and its therapeutic practices for the treatment of diseases.

Traditional medical treatment practices in Brazil have been explored by researchers for decades, especially in view of the great diversity of traditional populations that inhabit the various biomes, favoring the wealth of knowledge associated with the use of plant resources (Brandelli, 2017; Lipporacci *et al*., 2017). Considering the rich biological diversity of the country, with about 20% of global diversity, science recognizes the great phytochemical potential for research in bioprospecting, as demonstrated in research in ethnobotany and ethnopharmacology in the search for active ingredients (Calixto, 2005).

These researches consist of a safe way to investigate species with medicinal properties for pharmacological application (Calixto, 2005; Zago, 2018). Thus, in view of the diversity of ethnicities and other populations existing in the territory, there is much to be known in the field of medicinal plants applied to bioprospecting. This need is even greater, given the growing scenario of suppression of native vegetation and changes in the way of life of traditional populations, who have been undergoing repression for centuries, resisting all types of pressure, such as indigenous populations and those of African origin, such as quilombolas**.**

About the quilombolas, a vast knowledge associated with the forms of treating diseases using medicinal plants is recognized (Da Silva, I; Da Silva, G., 2023). Some authors claim that this relationship still occurs due to the state of social vulnerability in such communities, which allege that there is a lack of medical care (Cardoso; Hawk; Nunes, 2019) or that the assistance is insufficient for local demands. However, the coexistence of biomedical and traditional treatment systems should be considered, as occurs in the community of Castainho (Garanhuns-PE), where there is a Basic Health Unit (UBS), within the quilombo, influenced by the form of treatment of diseases (Silvestre; Nunes, 2023), but maintaining the traditional forms.

In another study at the site, it was shown that the population preserves a vast knowledge about medicinal plants and cultivates most of the species in their backyards (Nunes *et al*., 2024), in addition to resorting to the remnant of the Atlantic Forest

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neighboring the community to collect native plants for therapeutic purposes (De La Cruz *et al.*, 2022), the authors comment on the possibility of knowledge erosion in this community, as younger people are not interested in learning and using traditional practices. The data obtained in Castainho show well-known species such as lemongrass, mint, boldo, some cited as multifunctional for having medicinal and food effects; others require more in-depth studies from a phytochemical point of view to recognize the pharmacological potential (De La Cruz *et al.*, 2022).

Of the various pharmacological effects of medicinal plants, one can mention their role in the scavenging of free radicals. These radicals are produced by organisms constantly, being atoms or molecules that contain one or more unpaired electrons in their last shell, so that they confer a reactive characteristic. The production of free radicals, when in excess, leads to oxidative stress, causing damage to the body, since it can result in the degradation of biological structures essential for cellular organic functioning and provide the development or worsening of diseases (Vellosa *et al.*, 2021). Antioxidants can act by delaying or preventing the oxidation of the substrate involved in oxidative processes, preventing the formation of free radicals and preventing the triggering of oxidative reactions. As sources of natural antioxidants, plants with their secondary metabolism stand out, which enables the formation of bioactive compounds (Santos, J. *et al.*, 2018).

Taking into account that the use of medicinal plants as a therapeutic basis is widely used in rural communities, but there are few studies that prove its pharmacological potential, the objective of this study was to investigate the phytochemical and antioxidant profile of three species with high value of use by the Castainho community.

METHODOLOGY

COLLECTION AND ANALYSIS OF ETHNOBOTANICAL DATA

The research was part of a broader project approved by the Ethics Committee (CAEE: 96751118.00000.5207), under which data from informants on the relationships of use with medicinal plants were obtained. This was divided into two moments: in the first, a general approach with the residents of the residences and in the second with the key informants, through semi-structured interviews directed to medicinal plants (Albuquerque *et al.*, 2014).

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CRITERIA FOR SELECTING SPECIES

The information acquired was categorized according to the indications for use and stored in the Excel software (Office 2013). For species selection, first the indications of plants with proven phytochemical studies were excluded. Then, applying the data filter, the species with the highest use value were selected, through the Use value formula (VU = ΣU/n, where, VU = Use Value, U = number of citations of the ethnospecies per informant, n = number of informants who mentioned the ethnospecies) of the species, the technique suggested by Phillips and Gentry (1993a, 1993b) and Phillips *et al*. (1994), modified by Rossato (1996), where the importance of a plant species is given by the number of uses it presents, which then indicates the local importance of the species in view of the agreement of the information about it cited in the interviews. From the species with the highest use value, *Acanthospermum australe (Loefl.) was selected. Kuntze, Momordica charantia* L. and *Hyptis pectinata* (L.) Poit., for the analysis of phytochemical constituents.

PRODUCTION OF EXTRACTS

The leaves were collected in the community of Castainho, located in Garanhuns-PE. The leaves were dried in a circulating air oven at a temperature of 45 ± 2 ºC and then ground. To obtain the extract, the plant raw material was infused (10:100, w/v) for 15 minutes using distilled water as the extractor solvent. Then, the extracts were filtered and concentrated in a freeze dryer (-80°C and 4.58 mmHg).

PHYTOCHEMICAL SCREENING OF EXTRACTS

To identify the main classes of chemical compounds, the methodologies proposed by Matos (1997) were used. For this, 1mg/mL of EAFCe was used for qualitative chemical tests. The presence of steroids was evaluated by the Liebermann-Buchard reaction, while the presence of tannins was evaluated by the precipitation of iron salts. Flavonoids were investigated by Shinoda and Taubouk reactions, while saponins were analyzed by foam persistence after shaking the extract. In addition, the evaluation of the presence of phenols, flavones, flavonols, xanthones, catechins, anthocyanins, anthocyanidins, terpenoids and flavanones were tested with the use of specific chemical reagents. The interpretation was made based on the visual characteristics. The detection of the alkaloid chemical group was performed by thin layer chromatography (CCD) using specific eluents and development systems according to Cechinel and Yunes (1998).

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QUANTIFICATION OF SPECIALIZED METABOLITES

The determination of the total polyphenol content was carried out by the method of Chandra and Mejía (2004). In 1 mL of the aqueous solution of the extract, 1 mL of the Folin-Ciocalteau reagent 1N was added, and this mixture remained at rest for 2 minutes. Then, 2 mL of an aqueous solution of 20% (w/v) Na2CO3 was added, and the mixture remained at rest for another 10 min. Then, the absorbance was read at 757 nm in a spectrophotometer (Shimadzu® UV mini –1240) against a blank composed of distilled water, Folin-Ciocalteau reagent and 20% solution of Na2CO3. The analytical curve was obtained from a standard solution of 100 µg/mL of gallic acid. The concentration of polyphenols was expressed in micrograms per milligrams of gallic acid equivalent extract. The determination of the total flavonoid content followed the method of Meda *et al*. (2005). To 5 mL of each solution (in methanol) of the extract, the same volume of a solution (in methanol) of AlCl3a 2% (w/v) was added. The mixture remained at rest for 10 min before the absorbance reading at 415 nm against a white composed of the AlCl3 solution. The calibration curve was obtained from a standard solution at 100µg/mL of quercetin in methanol. The concentration of flavonoids was expressed in micrograms per milligram of quercetin equivalent extract.

EVALUATION OF ANTIOXIDANT ACTIVITY BY THE DPPH METHOD

To carry out this trial, the methodology used was the one described by Furlan *et al*., 2015. Initially, 1 mg of the substance was solubilized in methanol P.A. to a concentration of 0.1 mg/mL. Next, 10 µL of the sample in methanol was added to the 96-well plate, and then 140 µL of the DPPH solution was added to the wells. Subsequently, the reading was performed in a spectrophotometer at a wavelength of 517 nm, every 5 minutes, for one hour, establishing the following times: T0, T5, T10, T15, T20, T25, T30, T35, T40, T45, T50, T55, T60. The calculation of the percentage of antioxidant activity (% AAO) follows the following equation: % AAO = Abs DPPH – Abs sample/ Abs DPPH x 1000.

RESULTS

Phytochemical screening revealed the presence of hydrolyzable tannins, flavanols, triterpenes and saponins in M . *charantia extract.* In the extract of *A. australe, the presence of phenols, hydrolyzable tannins, flavonoids, friterpenoids and steroids was observed; while*

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in the extract of Hyptis pectinata, the presence of phenols, condensed tannins, flavanols, steroids, saponins and antrons was observed (Table 1).

Table 1 - Prospection of chemical constituents of extracts of medicinal plants selected for analysis of metabolite compounds.

Chemical Constituents	*Hyptis pectinata* (L.) Poit.	*Momordica charantia* L.	*Acanthospermum australe (Loefl.)* Kuntze
Phenols	+	-	+
Hydrolyzable Tannins	-	+	+
Condensed Tannins	++	-	-
Anthocyanins	-	-	-
Chalconas	-	-	-
Leucoantocianidinas	-	-	-
Catechins	-	-	-
Flavanols	+	+	+
Steroids	+	-	+
Triterpenóides	-	++	+
Saponins	+	++	-
Alkaloids	-	-	-
Anthraquinone	-	-	-
Antronas	+	-	-
Coumarins	-	-	-

-: Absent coloration; +: Weak coloration; ++: Intense coloration. Source: The authors.

Still on metabolites, the study quantified total polyphenols and flavonoids (Table 2). The results showed that the extract of *H. pectinata, M. charantia* and *A. australe have polyphenol concentrations of 503.86 ± 10.27, 201.95 ± 22.51 and 230.76 ± 23.10 µg/mg gallic acid equivalent, respectively. For flavonoids, the results obtained were: 13.96 ± 8.11, 16.38 ± 5.66 and 14.12 ± 8.38 µg/mg quercetin equivalent), respectively. The presence of the mentioned compounds, in the reported concentrations, is a factor that may explain the biological effects of the plants studied.*

Table 2- Quantification of Total Flavonoids and Polyphenols

Extracts	Flavonoids Totu		Total Polyphenols	
	µg/mg	SD	µg/mg	SD
Hyptis pectinata (L.) Poit.	13,96	± 8,11	503,86	± 10,27
Acanthospermum australe (Loefl.) Kuntze	14,12	± 8,38	230,76	± 23,10
Momordica charantia L.	16,38	± 5,66	201,95	± 22,51

Total Flavonoids are given in Quercetin Equivalents (in 1 mg of the extract, one has X µg of Quercetin Equivalent Flavonoids). Total Polyphenols are given in Gallic Acid Equivalents (in 1 mg of the extract, one has µg of Gallic Acid Equivalent Polyphenols). Source: The authors.

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Table 3 presents the results of the percentage of antioxidant activity by the DPPH method. It was possible to observe that only the extract of *H. pectinata* was able to sequester 50% of the DPPH radical.

Table 3: Evaluation of antioxidant capacity by the DPPH method

Extracts	T0	T5	T10	T15	T20	T25	T30	T60
Hyptis pectinata (L.) Poit.	18,32%	24,17%	27,68%	30,60%	32,94%	36,06%	38,79%	58,09%
Acanthospermum australe (Loefl.) Kuntze	0,00%	3,51%	6,04%	8,38%	10,72%	12,67%	15,20%	35,48%
Momordica charantia L.	0,00%	0,39%	2,14%	3,31%	4,68%	5,65%	6,43%	14,23%

Source: The authors

DISCUSSION

Studies of the constituents present in botanical extracts, extracts obtained through extraction processes already widely known by science and the population, are necessary to support the beginning of scientific investigation, especially in relation to secondary metabolites that are generally responsible for biological actions linked to extracts (Simões *et al*., 2010)

Mada *et al.* (2013) showed that the extract of M. *charantia* leaves revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids. In a study conducted by Bonella *et al.* (2011), the authors demonstrated the presence of alkaloids, flavonoids, glycosides, phenols, tannins and steroids in the extract of *A. australe*. Also, Amusan *et al.* (2007) also demonstrated the presence of phenolic compounds in the species. Vasconcelos *et al.*, (2020), when studying the phytochemical profile of the ethanolic extract of *Hyptis pectinata* leaves, evidenced the presence of classes of chemical constituents tannins, alkaloids, and terpenes. Therefore, it is possible to infer that these results corroborate the findings of our study.

Secondary compounds are known to be biologically active and thus aid in the treatment of diseases. The antimicrobial activity of phenols, flavonoids, and tannins is already well reported in the literature (Bouyahya *et al*., 2022). A study by Kamran *et al*. (2022) highlighted the anti-cancer potential of terpenes. The authors reported that these secondary metabolites were able to lead to suppression of the early stage of tumorigenesis by inducing cell cycle arrest, through inhibition of cancer cell differentiation and activation of apoptosis. In addition to terpenes, flavonoids and alkaloids have been widely studied for their potential action against human cancer cells (Mada *et al*., 2013; Forni *et al.,* 2021).

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Among the secondary metabolites, saponins and their derivatives stand out, which are versatile glycosides capable of performing relevant functions in the pharmaceutical, food and agricultural industries. Its applications in the pharmaceutical sector demonstrate few cytotoxic adverse effects, in addition to a diverse therapeutic potential, such as antioxidant, antihypertensive, antimicrobial, hypolipidemic, and hypoglycemic activity (Sharma *et al.*, 2023).

Fruits, grains and leaves are rich in flavonoids, a substance that is part of the polyphenol family. These compounds are known for their various phytochemical effects, such as: antioxidant, anti-inflammatory, antitumor, antifungal capacity, among others (Ekalu; Habila, 2020). However, it is important to emphasize that negative results do not mean the total absence of a certain metabolite, considering that its quantity in the sample, when less than semimicro, is usually not detected during the qualitative analysis of the extract. In addition, it is known that other factors can influence the extraction of phytoconstituents, such as the extractive method and the type of extracting solvent used (Santos, T., 2018; Oliveira *et al.*, 2016)

The identification analyses of secondary compounds through preliminary phytochemistry can contribute to the identification of secondary metabolisms of plants with the possibility of further investigations as a source of new medicinal agents. The presence of these secondary metabolites in the species studied has justified the claim of the Castainho community for the use of these plants for the treatment of various diseases (Costa *et al.*, 2017).

In recent years, there has been a growing interest in the use of plant species for the production of herbal products, with the antioxidant property being one of the most evaluated biological activities in these plant materials (Londoño-Londoño, 2013). The evaluation of the antioxidant activity of phytochemical compounds present in plant extracts can be carried out through several methods, among which the DPPH assay stands out (Číž, 2010).

The assay based on the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a relatively simple and fast method that measures the antioxidant elimination capacity against the stable free radical DPPH (Yamauchi *et al.* 2024). This method aims to calculate the IC50 parameter, which represents the so-called "efficient concentration", that is, the concentration of the extract necessary for a 50% reduction in DPPH activity to occur (Molyneux *et al.*, 2004).

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Among the secondary metabolites present in plants, the ones that showed the most antioxidant activity are phenols, due to the presence of an aromatic ring that facilitates the stabilization and redistribution of unpaired electrons, allowing the donation of hydrogen atoms and electrons of the hydroxyl groups (Cömert; Gökmen, 2017). Thus, the antioxidant potential of *H. pectinata* extract can be explained by the large amount of phenolic compounds and flavonoids quantified in the samples.

Corroborating the results of the research, Vasconcelos *et al*. (2020) showed that the amount of ethanolic extract from *H. pectinata* leaves needed to decrease the initial concentration of DPPH by 50% is 133.32 ± 1.59 µg/mL, showing that this extract showed DPPH free radical scavenging activity. Paixão *et al*. (2013) stated in their study that the aqueous extract of *Hyptis pectinata* leaves has moderate antioxidant action against the DPPH free radical. In another study, Serafini *et al.* (2012) attributed the antioxidant activity of the essential oil of *H. pectinata leaves* to the presence of sesquiterpenes. In the literature, it is possible to observe that plants of the same genus as *H. pectinata* also have free radical scavenging capacity (Xu *et al*., 2013; Ghaffari *et al.*, 2014).

Our study revealed that the extracts of *M. charantia and A. australe* did not show a good antioxidant capacity. However, Wu and Ng (2008) observed that both the aqueous extract and the ethanolic extract of *M. charantia* were able to eliminate the DPPH radical at the concentration of 129.94 mg/ml and 156.78 mg/ml, respectively. Also, Oragwa, Efiom and Okwute (2013) showed in their study that at the concentration of 1 mg/ml, the radical scavenging activities of *M. charantia* extract were comparable to those of vitamin C used as a control, but at lower concentrations, the scavenging activity decreases. Regarding the antioxande activity of the species *A. australe, Moglad et al*. (2024) showed that the ethanolic extract of *A. hispidum,* a plant of the same family, exhibited potent activity in the elimination of the DPPH radical at a concentration of 0.5 mg/ml. Thus, we can infer that the absence of antioxidant activity of the extracts *M. charantia* and *A. australe in our study is probably related to the concentration tested, which was 0.1 mg/mL for both.*

CONCLUSION

Phytochemical screening of *Acanthospermum australe* (Loefl.) species Kuntze, *Momordica charantia* L. and *Hyptis pectinata* (L.) Poit. confirmed the presence of bioactive compounds, such as flavonoids, tannins and triterpenes, reinforcing the traditional use of these plants by the community studied. The results obtained in the quantification of

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polyphenols and flavonoids were particularly relevant for *Hyptis pectinata*, which presented a high concentration of polyphenols (503.86 µg/mg), suggesting a strong antioxidant potential, corroborated by the ability of this species to sequester 50% of the DPPH radical in the antioxidant assay. This finding indicates that *Hyptis pectinata* may be a promising source of natural antioxidants, having a prominence among the species studied.

The low antioxidant activity observed for *Acanthospermum australe* and *Momordica charantia* may be related to methodological variables, such as the concentration of the extracts used in the assays, since these plants also presented bioactive compounds that, in other studies, have already demonstrated antioxidant activity. This aspect deserves to be investigated in future studies, which could adjust concentrations or explore other extraction methods.

In general, the results reinforce the relevance of investigating medicinal plants used by traditional communities, not only to scientifically validate their use, but also to explore new therapeutic alternatives. In this way, the continuity of studies with these species can contribute to the development of herbal products, in addition to preserving ancestral ethnobotanical knowledge. Bioprospecting plants such as these not only enhances local biodiversity, but also offers new opportunities in the field of health research and pharmaceutical innovation.

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