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Chemistry

In vitro antioxidant and anticholinesterase screening of five plant species collected from the Discovery Coast, Bahia, Brazil

Triagem in vitro antioxidante e anticolinesterásica de cinco plantas coletadas na Costa do Descobrimento, Bahia, Brasil

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ABSTRACT

Although most of the species *Dysphania ambrosioides* (L.) Mosyakin & Clemants, *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson, *Rosmarinus officinalis* L., *Schinus terebinthifolia* Raddi, and *Tragia volubilis* L. are not native to Brazil, they have been adapted and widely-used in folk medicine and spiritual rituals in the Discovery Coast region of Bahia. Within this context, considering the need for knowledge of the pharmacological properties of plants and awareness of the influence of environmental factors, this study was conducted. This study therefore investigated the phenolic composition, antioxidant, and anticholinesterase (Anti-AChE) properties of plant extracts collected from the area known as the Discovery Coast, in Porto Seguro, Bahia, Brazil. In addition, this study presented data on biological investigations described in the literature in order to contribute to scientific knowledge and gather evidence that validates the plants used in traditional medicine. Anti-AChE was strongly indicated in the extracts with higher antioxidant activity. Also, the results suggest that the higher phenolic content of the extracts may be responsible for their antioxidant and Anti-AChE activity. Although many results from the biological activities in the literature converge on some popular applications for the plants, further studies are still required to corroborate their therapeutic effectiveness as well as provide technical clarifications regarding their use, preparation, and purpose.

Keywords: Southern Bahia; Cholinesterase Activity; Atlantic Rainforest; Biological Research

RESUMO

A *Dysphania ambrosioides* (L.) Mosyakin & Clemants, *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson, *Rosmarinus officinalis L.*, *Schinus terebinthifolia* Raddi e *Tragia volubilis L.* embora a maioria das espécies não sejam nativas no Brasil, encontram-se adaptadas e muito usadas na medicina popular ou rituais espirituais na Costa do Descobrimento, Bahia, Brasil. Neste contexto, considerando a necessidade do conhecimento de propriedades farmacológicas de vegetais, perante a ciência da influência dos fatores ambientais este estudo foi realizado. Portanto, este trabalho investigou a composição fenólica, as atividades antioxidante e anticolinesterásica (Anti-AChE) dos extratos das plantas coletadas em Porto Seguro, Bahia, Brasil, território de identidade da Costa do Descobrimento. Além disso, o estudo apresentou dados sobre investigações biológicas descritas na literatura, a fim de contribuir para o conhecimento científico e reunir evidências para a validação das plantas na medicina tradicional. Desta maneira, Anti-AChE indicaram fortemente nos extratos com maior atividade antioxidante. Ainda, os resultados sugerem que o maior teor de fenólicos dos extratos podem ser os responsáveis por sua atividade antioxidante e Anti-AChE. Apesar dos resultados levantados das atividades biológicas convergirem para algumas indicações populares, ainda são necessários outros estudos para corroborar a efetividade terapêutica das plantas.

Palavras-chave: Sul da Bahia; Atividade Colinesterase; Mata Atlantica; Investigação Biológica

1 INTRODUCTION

Plants are responsible for the prevention and treatment of many diseases around the world and, in many cases, may be the only resources for low-income communities (Pinto et al., 2002). Also, because they can produce many substances, they have been the inspiration for the development of drugs that can be used in a variety of therapeutic applications (Calixto, 2000).

In view of the growing interest in this arsenal of natural bioactive compounds, Brazil occupies a privileged position due to the enormous diversity of plants found within its territory, estimated at approximately 43.020 species already known across its various biomes (Brasil, 2020). The country also harbors communities that have gathered immeasurable collections of knowledge about plants that has been passed down from generation to generation (Brasil, 2020). Understanding the biodiversity and cultural diversity of Brazil is therefore a promising source of investigation for new therapeutics.

The species *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Amaranthaceae), formerly known as *Chenopodium ambrosioides* L. (Senna, 2020), *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson (Verbenaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Schinus terebinthifolia* Raddi (Anacardiaceae), and *Tragia volubilis* L. (Euphorbiaceae) are popularly known as *mastruz*, *erva-cidreira*, *alecrim*, *aroeira*, and *urtiga*, respectively. Although most of them are not native to Southern Bahia Discovery Coast or even to Brazil, they have been adapted and are widely used in folk medicine and spiritual rituals.

However, despite their ubiquitous use, numerous reports in ethnopharmacological studies, and even some plants being included as therapeutic options in the Brazilian Public Health System by the National Policy for Medicinal Plants and Herbal Medicines (PNPMF) (Brasil, 2020), little is known about the therapeutic effectiveness of these plants. Similarly, studies on plants collected from regions such as the Discovery Coast are scarce, given that the chemical composition, responsible for the therapeutic effects, can be influenced by environmental factors inherent to location such as sun exposure, wind, and soil, among others (Gobbo-Neto & Lopes, 2007; Sampaio & Da Costa, 2018).

The investigation of substances capable of reducing oxidative stress caused by free radicals (FRs) (Alves et al., 2010, Aktumsek et al., 2013) that oxidize biomolecules and cause cellular damage is important, because these chemical compounds are often associated with chronic diseases, premature aging, and neurodegenerative disorders (Aktumsek et al., 2013; Ghribia et al., 2014; Haida & Kribii, 2020).

In light of the severe effects FRs can cause, many natural substances have been tested for their ability to minimize the symptoms of memory loss and reduced cognitive abilities that result from Alzheimer's disease (AD) and which reduce quality of life and life expectancy (Arantes et al., 2017). One of the most frequently employed strategies is to increase the synaptic availability of acetylcholine by inhibiting the enzyme acetylcholinesterase (AChE), the enzyme principally responsible for the hydrolysis of the neurotransmitter at the central cholinergic synapse (Arantes et al., 2017; Hlila et al., 2015).

Currently, many prescription drugs are effective inhibitors for mild and moderate cases, providing temporary symptomatic relief. However, they may have adverse effects, such as hepatotoxicity and gastrointestinal disturbance (Forlenza, 2005; Aktumsek et al., 2013; Hlila et al., 2015). In this context, natural substances are considered promising candidates to be efficient and safe inhibitors for the treatment of AD.

Given the above, this study investigated the phenolic composition, antioxidant, and anticholinesterase (Anti-AChE) activities of ethanolic extracts from the species *Dysphania ambrosioides*, *Lippia alba*, *Rosmarinus officinalis*, *Schinus terebinthifolia*, and *Tragia volubilis* collected in Porto Seguro, Bahia, Brazil from the territory known as the Discovery Coast. In addition, the study presents data on biological investigations described in the literature for these plants, in order to contribute to scientific knowledge and support future studies, as well as to gather evidence for the validation of commonly used plants in traditional medicine.

2 MATERIAL AND METHODS

2.1 Plant material

Plant materials were collected from the district of Arraial D'ajuda, located within the urban perimeter of the municipality of Porto Seguro, Bahia (16°27'4" S, 39°3'53" W). Following botanical identification, exsiccates were documented in the Professor Geraldo C.P. Pinto (GCPP) Herbarium at the Federal University of Southern Bahia, located at the Sosígenes Costa Campus in Porto Seguro, according to the APG IV classification system (CHASE et al., 2016). The species names were checked in the List of Species of the Flora of Brazil (Luz, Mitchell, & Pell, 2020) to ensure the use of the currently accepted names for the respective plant species.

2.2 Preparation of Extracts

The ethanolic extract was prepared with 200 g of leaves from *Tragia volubilis* (ETTV), *Schinus terebinthifolia* (ETST), *Rosmarinus officinalis* (ETRO), *Lippia alba* (ETLA), and *Dysphania ambrosioides* (ETDA) through cold maceration with ethanol (95%) three consecutive times over a 48-hour period, adapted from LIMA *et al*., (2020). In each preparation process, ethanol was removed using a rotary evaporator at 50°C under reduced pressure.

2.3 Total Phenolics (TP)

The phenolic content was determined using the Folin-Ciocalteu colorimetric method with the use of gallic acid as a standard (Neves et al., 2009; Mathew; Subramanian, 2014; Silva et al., 2022). Gallic acid solutions were prepared with concentration from 0.03 to 120 μg mL^{-1} in ethanol. In summary, 40 μL of each standard solution was added to 200 μL of Folin-Ciocalteu reagent plus 120 μL of 8.5% sodium carbonate (Na_2CO_3) and was then incubated at 50°C for one hour. The absorbance was measured at 760 nm. The calibration curve was constructed from the absorbance of the solution versus the concentration of gallic acid. The Detection Limit (DL) and Quantification Limit (QL) were obtained from equation (1):

$$DL = \frac{(3{,}3\ x\ s)}{S} \quad \text{and} \quad QL = \frac{10\ x\ s}{S} \qquad (1)$$

Where “s” represents the standard deviation from the blank reading and “S” represents the angular coefficient of the calibration curve of the standard.

The results are expressed as milligrams per gallic acid equivalent per gram of extract (mg GAE g^{-1}), based on the interpolation of the absorbance of the extracts in the linear regression equation for gallic acid.

2.4 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Antioxidant Test

The antioxidant activity was measured by the ability of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) to sequester free radicals. Serial dilutions of the extracts at concentrations between 5 and 30 μg mL^{-1} were prepared in the cavities of a 96-well microplate, after which 150 μL of the ethanolic DPPH solution (0.1 mMol L^{-1}) was added. Following incubation at room temperature for 30 minutes, the absorbance at 517 nm was measured. All experiments were performed in triplicate (Mathew, & Subramanian, 2014; Furlan et al., 2015; Silva et al., 2022).

The sequestration percentage (AA%) of the DPPH radical was calculated using equation (2):

$$AA\% = \frac{(|2| - |1|)}{|2|} x\ 100 \tag{2}$$

Where: Abs1 is the absorbance of the extracts with their respective blanks and Abs2 is the absorbance corresponding to the negative control (ethanol and DPPH).

Ethanol was used as the blank and quercetin as the positive control. The effective concentration (CE_{50}) was obtained from the straight-line equation of the plot of AA% against the sample concentration.

2.5 *In vitro* anticholinesterase (Anti-AChE) test

The spectrometric method developed by Ellman et al. (1961) and modified by Alves et al. (2013) was used to measure the anticholinesterase potential (Anti-AChE). This *in vitro* test consists of the hydrolysis of acetylcholine promoted by the enzyme acetylcholinesterase, monitored at 405 nm.

In this method, 40 μL of acetylthiocholine iodide (15 mM), 140 μL of 5,5’-dithiobis-[2- nitrobenzoic acid] (3 mM), 65 μL of phosphate buffer (pH 8) with 0.1% bovine serum albumin, and 40 μL of extracts at concentrations of 50, 100, 200, 350, and 500 μg mL^{-1} were placed in the cavities of a 96-well microplate and incubated at

37°C for 10 minutes. Following this, 40 µL of the enzyme solution (0.22 U mL^{-1}) was added and the absorbance at 405 nm was recorded after five minutes. The inhibition percentage (I%) was obtained by comparing the reaction rates of the extracts against the blank, using equation (3):

$$I(\%) = \frac{(AChE - AChI)}{AChE}\, x\, 100 \tag{3}$$

Where: AChI = activity obtained in the presence of the inhibitor; AChE = n the absence of the inhibitor.

The experiments were performed in triplicate and physostigmine (0.1 to 30 µg mL^{-1}) was used as reference.

2.6 Statistical analyses

All analyses were performed in triplicate. Results were reported with the mean standard deviation (SD). Calculation of IC_{50} values was done using GraphPad Prism version 4.00 for Windows (GraphPad Software Inc.).

3 RESULTS

Phenolic compounds are considered promoters in the maintenance of human health, mainly because they perform or are associated with a number of pharmacological actions (Aktumsek et al., 2013; Onofre et al., 2016; Kumar, & Goel, 2019). In light of this, the quantification of total phenolics (TP) is a fundamental tool to understand the health values of a plant. In this study, the TP and extraction yield for *Rosmarinus officinalis*, *Schinus terebinthifolia*, *Lippia alba*, *Dysphania ambrosioides*, and *Tragia volubilis* are shown in Table 1.

In the extracts, the highest TP values were found in *S. terebinthifolia* (ETST) and *R. officinalis* (ETRO) with levels of 330.32 ± 0.002 mg GAE g^{-1} and 179.23 ± 0.001 mg GAE g^{-1}, respectively. In other studies, with plants collected from other localities, Iwanaga et al. (2018) found a value of 312.50 ± 0.50 mg GAE g^{-1} and Uliana et al. (2016) a value of 221.63 ± 2.58 mg GAE g^{-1} for total phenols from the hydroethanolic extracts of *S. terebinthifolia* leaves. Tlili, Sarikurkcu (2020) found a value of 142.84 ± 1.13 mg GAE g^{-1} for the aqueous extract of *R. officinalis* leaves. Slight differences between the values obtained in this study and previous reports may be attributed to factors such as origin, climatic conditions, stage of ripening, soil, or other abiotic factors. In addition, the processing methods, solvents, and storage conditions, can also influence the final composition of the plant extracts (Gobbo-Neto et al., 2007; Sampaio et al., 2018; Tlili & Sarikurkcu, 2020).

Table 1 – Total phenolic content in ethanolic extracts of compounds of Rosmariuns officinalis (ETRO), Schinus terebinthifolia (ETST), Lippia alba (ETLA), Dysphania ambrosioides (ETDA), and Tragia volubilis (ETTV)

Extract	Yield	Phenolic content*		
	% (m/m)	mg GAE g^{-1}	Linear regression	R^2
ETRO	6.31	179.23 ± 0.001		
ETST	11.53	330.32 ± 0.002		
ETLA	9.02	95.24 ± 0.001	Y=0.0082x - 0.0036	0.9997
ETDA	7.53	166.14 ± 0.003		
ETTV	9.54	137.60 ± 0.003		

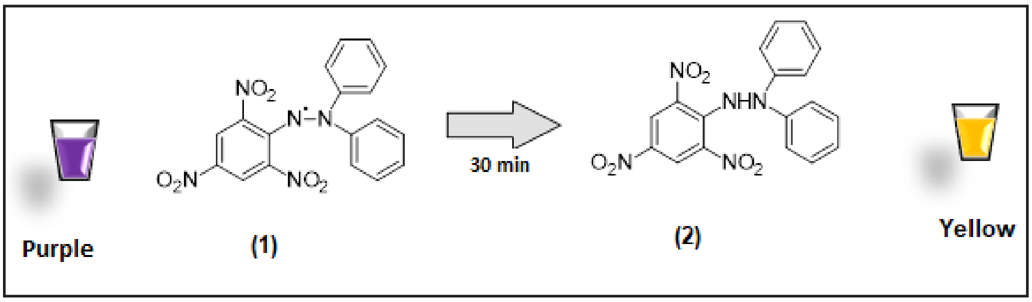
* LD = 1,36 μg mL^{-1} and LQ = 4,11 μg mL^{-1}

Source: Authors (2022)

Numerous studies point out the benefits of phenolic compounds to human health. For example, in decreasing the risk of heart disease (Aktumsek et al., 2013; Kumar & Goel, 2019), degenerative diseases such as cancer (Kumar et al., 2019; Soleas et al., 2002), diabetes (Palma-Duran et al., 2017), and inflammation (Kumar & Goel, 2019; Kumar et al., 2019). Similarly, they describe their important role in slowing aging and sequestering free radicals, acting as antioxidants.

Antioxidants are molecules capable of inhibiting FRs and delaying or reducing cellular damage. Due to their considerable chemical diversity, several *in vitro* tests have been described for evaluating antioxidants (Alves et al., 2010). However, the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) test has been widely used for natural extracts and substances. This method is based on monitoring the reduction of the picrylhydrazyl radical **(1)** (purple) to hydrazine **(2)** (yellow) by antioxidants (Aktumsek et al., 2013; Alves et al., 2010; Nimse, Palb, 2015) (Figure 2).

Figure 1 – Representation of the chemical structure of (1) Unreduced and (2) Reduced DPPH



Source: Authors (2022)

The antioxidant activity of the different extract concentrations and the standard quercetin, measured by the DPPH method, is shown in Table 2. All samples exhibited activity in a concentration-dependent manner, and the EC_{50} values showed the ability to sequester radicals (the lower the value, the higher the activity) (Table 3). ETST (EC_{50} 4.63) and ETRO (EC_{50} 4.27) had the highest DPPH reduction potentials (μg mL^{-1}) compared to the standard quercetin (EC_{50} 3.11). However, the other extracts, except for *T. volubilis* (ETTV), are also classified as intensely active antioxidants with EC_{50} < 50 μg mL, according to parameters described in Reynertson *et al*. (2005).

Table 2 – Concentration, sequestration (AA%), linear regression and linear coefficient (R^2) for the ethanolic extracts R. officinalis (ETRO), S. terebinthifolia (ETST), L. alba (ETLA), D. ambrosioides (ETDA), and T. volubilis (ETTV)

Extract	**Sequestration Percentage (AA%) ****					**Linear regression**	**R²**
	5*	**15***	**20***	**25***	**30***		
Querc	39.43 ± 0.002	53.25 ± 0.010	64.86 ± 0.002	71.91 ± 0.004	75.58 ± 0.004	Y=0.0145x + 0.3193	0.9996
ETST	24.62 ± 0.003	43.00 ± 0.002	52.01 ± 0.001	61.84 ± 0.006	73.01 ± 0.001	Y=0.0192x + 0.1446	0.9983
ETLA	24.44 ± 0.002	25.54 ± 0.002	25.56 ± 0.002	28.11 ± 0.001	30.24 ± 0.001	Y=0.0031x + 0.2084	0.9816
ETCA	21.58 ± 0.007	23.43 ± 0.002	23.43 ± 0.002	24.87 ± 0.004	26.99 ± 0.001	Y=0.0016x + 0.2074	0.9995
ETRO	25.31 ± 0.001	46.60 ± 0.008	57.66 ± 0.009	70.82 ± 0.001	73.44 ± 0.001	Y=0.0194x + 0.1687	0.9930
ETTV	21.53 ± 0.004	23.01 ± 0.002	23.23 ± 0.002	23.88 ± 0.001	24.78 ± 0.003	Y=0.0012x + 0.2094	0.9992

*Concentration in μg mL^{-1}

*** Each value is expressed as mean ± standard deviation (n=3)*

Source: Authors (2022)

A variety of plant extracts and their constituents that have antioxidant potential, have also shown Anti-AChE activity and may be useful for neurodegenerative disorders such as AD. The activity found in this study is summarized in Table 3.

All of the extracts, except ETTV, exhibited AChE inhibitory activity in a dose-dependent manner, although differences in their degrees of activity were observed. ETST (IC_{50} 46.76 μg mL^{-1}) and ETRO (IC_{50} 34.02 μg mL^{-1}) were the most effective at inhibition, with the IC_{50} value for the positive control, physostigmine, being 14.60 μg mL^{-1}. Indeed, the high acetylcholinesterase inhibitory activity of these extracts likely stems from the high concentration of phenolics and high antioxidant capacity shown in the earlier tests. Ramassamy (2006) highlights the fact that phenolic compounds have different intracellular targets, and constitute an efficient approach to reducing the incidence of AD. Tabet (2006) reports that antioxidants block the oxidation process that produces free radicals which contribute to the disease.

Table 3 – Antioxidant and anticholinesterase activity, respresented by the EC_{50} and IC_{50} values of the extracts R. officinalis (ETRO), S. terebinthifolia (ETST), L. alba (ETLA), D. ambrosioides (ETDA), and T. volubilis (ETTV). Quercetin and physostigmine were used as a reference.

Extract	**Part of Plant Used**	**Antioxidant Activity** EC_{50} (μg mL^{-1})	**Anti-AcHE Activity** IC_{50} (μg mL^{-1})
ETST		4.63	46.76
ETLA		23.52	178.57
ETRO	Leaves	4.27	34.02
ETDA		45.72	63.35
ETTV		60.54	Nd*
Physostigmine	-	-	14.60
Quercetin	-	3.11	-

* Not detected
Source: Authors (2022)

These results agree with data previously obtained by other authors. Sharma, Fagan and Schaefer (2020) found significant Anti-AChE activity (IC_{50} 8.57 ± 0.82 mg mL^{-1}) in the Soxhlet extract of organically grown *R. officinalis,* while Kamli et al. (2022) pointed out that ethyl acetate extract at a concentration of 250 μg mL^{-1} exhibited the highest significant inhibitory effect of 75%, comparable to the inhibitor galantamine, with 88% inhibition. In traditional medicine, *Rosmarinus officinalis* is used as an antispasmodic, renal colic, antirheumatic, diuretic, antiepileptic, expectorant, as well as in cases of diabetes, heart disease, and for relief of respiratory disorders. In addition, Heinrich (2006) records its use as a tonic to improve memory dysfunction. Therefore, the inhibitory effects found in the studies may explain in part the traditional use for improving memory and enhancing cognition, contributing to the validation of the plant in folk medicine.

A variety of popular uses are indicated for the plants of this study. Obviously, these do not, in general, present scientific validation that confirms their therapeutic actions. In order to gather evidence that supports their traditional uses, more information from the literature has been presented on the biological evaluation of the species (Table 4). This data highlights the scarcity of biological research information on *Tragia volubilis*, despite it being a plant popularly used as a diuretic (Agra, Freitas, & Barbosa-Filho, 2007).

Although many of the results converge with some popular indications, further studies are still required to reach wider conclusions, in addition to more technical guidance on conditions for the safe and effective use of the species.

Table 4 – Identification from numerous pharmacological investigations of plants

(To be continued)

Scientific name	Evaluation	Extract/Oil	Results	Ref.
Dysphania ambrosioides	Antitumor Antioxidant	Hydroalcoholic Extract	The isolated substance kaempferitrin exhibited antioxidant and antitumor activity (IC_{50} of 0.38 μM)	Li et al., 2022
	Antibacterial	Essential Oil	Broad spectrum activity (*Escherichia coli*, *Staphylococcus aureus,* and *Enterococcus faecalis*)	Kandsi et al., 2022
	Antidiabetic	Aqueous and methanolic	Antidiabetic potential in rats through significant reduction in blood glucose during fasting	Kasali et al., 2022

Table 4 – Identification from numerous pharmacological investigations of plants

(Continuation)

Scientific name	Evaluation	Extract/Oil	Results	Ref.
Dysphania ambrosioides	Scarring	Methanolic	Inhibition of rat paw edema and reduction of induced contortions. Antipyretic effect and induction of strong gastrointestinal propulsive movement	Olajide; Awe; Makinde, 1997
	Scarring	Methanolic	Inhibition of rat paw edema and reduction of induced contortions. Antipyretic effect and induction of strong gastrointestinal propulsive movement	Olajide; Awe; Makinde, 1997
	Anti-inflammatory Antinociceptive Scarring	Ethanolic	Validation of use for therapeutic treatment of wound healing processes in rats	Trivellatograssi et al., 2013
	Collagen-induced arthritis	Hydromethanolic	Reduced the percentage of neutrophils and macrophages and the number of bone marrow cells and increased the number of lymphocytes and cellularity of inguinal lymph nodes	Pereira et al., 2018
	Antioxidant Antimicrobial Antidiabetic	Hydromethanolic	Significant antioxidant, cytotoxic, antimicrobial, and antidiabetic potentials revealed	Zohra et al., 2019
	Hepatotoxicity and nephrotoxicity	EtOH Extract and fractions	Mercury-induced hepatoprotective and nephroprotective activities	Ogunleye et al., 2020

Table 4 – Identification from numerous pharmacological investigations of plants

(Continuation)

Scientific name	Evaluation	Extract/Oil	Results	Ref.
Lippia alba	Antithrombotic Antibacterial	Ethanolic	Promising *in vitro* anticoagulant activity for new antithrombotic agents	Leite et al., 2023
		Essential oil	Bacteriostatic and bactericidal action against Aeromonas isolates	Majolo et al., 2022
	Antifungal		Antifungal action with potential biotechnological application	Sales et al., 2022
	Antiviral against Zika virus		Antiviral effect in virucidal (IC_{50} 32,2 μg mL) and post-treatment (IC_{50} = 54,1 μg mL) activity trials	Sobrinho et al., 2021
	Trypanocidal Cardioprotective		Displayed a high trypanocidal therapeutic effect (similar to benznidazole) and positive cardioprotective effect in the course of chronic Chagas' cardiomyopathy, in rats	Ramírez et al., 2021
	Anti-ulcerogenic activity	Infusion	Oral treatment with the plant infusion did not cause gastric irritation. In addition, oral administration was shown to be effective in preventing indomethacin-induced gastric ulceration in rats	Pascual et al., 2001
Rosmarinus officinalis	Antibacterial	Essential oil	Active against Gram-positive bacteria Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermidis	Ghavam, 2022

Table 4 – Identification from numerous pharmacological investigations of plants

(Continuation)

Scientific name	Evaluation	Extract/Oil	Results	Ref.
Rosmarinus officinal	Anticancer Antioxidant	Essential oil	Showed significant cytotoxicity in colorectal cancer cells and a low antimicrobial effect	Dolghi et al.,2022
Rosmarinus officinalis	Anticancer Antioxidant	Ethanolic	Antiproliferative against human prostate cancer cell lines (LNCaP)	Bourhia et al., 2019
			The nano emulgel oil appeared to be a promising new form of dosage for pharmaceutical industries in the treatment of different cancer cell lines and microbial infections	Eid et al., 2022
		Hydroethanolic	Preliminary results suggest it as an alternative source of bioactive compounds to further examine its abilities against glioblastoma and rhabdomyosarcoma cancer cell lines	Kakouri et al., 2022
	Antifungal	Essential oil	The results of the diffusion test revealed a stronger antifungal effect from nystatin against all Candida strains analyzed	Murtiastutik et al., 2022
	Antidiabetic Anticholinergic Cognitive	Extract	Rosmarinic acid exhibited Kİ values of 9.67 ± 0.81 nM for acetylcholinesterase (AChE) and 33.56 ± 2.96 nM against α-glucosidase enzymes	Topal et al., 2022
		Aqueous Soxhlet	High AChE inhibition for the extract (8,57±0,82 mg mL^{-1}) compared to galantamine (6,33 μg mL^{-1})	Sharma; Fagan; Schaefer, 2020

Table 4 – Identification from numerous pharmacological investigations of plants

(Continuation)

Scientific name	Evaluation	Extract/Oil	Results	Ref.
Rosmarinus officinalis	Antibacterial	Methanolic/ Fractioned	Triterpenoid acids isolated from the fruit reduced *Staphylococcus aureus* virulence and dermonecrosis	Tang et al., 2020
Schinus terebinthifolia	Antiproliferative Cytotoxic Effects	Methanolic	Demonstrated that the leaf is harmful to thyroid cells after exposure to high concentrations for prolonged periods	Olinto et al., 2020
	Antinociceptive	Leaf lectin	Reduced hyperalgesia from sarcoma 180 in the paws of mice. The effect may be related to its antitumor action	Ramos et al., 2020
			The results provide new perspectives for the development of analgesic agents using leaf lectins	Marinho et al., 2023
	Antitumor Toxicity		Antitumor effect against sarcoma 180 without inducing hematological changes and genotoxic effects in mice. A degree of hepatic and renal toxicity was observed, suggesting the need for evaluation of future drug delivery strategies	Ramos et al., 2019
	Antiviral Activity	Hydroethanolic	The crude extract conferred protection against lesions caused by Herpes simplex virus type 1 *in vivo*	Nocchi et al., 2017

Source: Authors (2022)

4 CONCLUSIONS

The investigation of the therapeutic action of *Dysphania ambrosioides*, *Lippia alba*, *Rosmarinus officinalis*, *Schinus terebinthifolia,* and *Tragia volubilis* is an important contribution to the knowledge of plants that are commonly found along the Discovery Coast, especially because the region has a great diversity of flora and is surrounded by communities that traditionally use these plants in rituals and as medicine for the cure and prevention of diseases.

In vitro screening has been widely used as a preliminary approach to investigate the therapeutic action of plants. In this study, the Anti-AChE tests of the extracts indicated a significant causal relationship between antioxidant activity and phenolic content.

It is also important to consider the data collected from biological investigations in the literature in order to gather evidence to corroborate the therapeutic effectiveness of the plants. Although many results converge with many popularly indicated uses for the plants, further studies are still required, as well as technical clarifications on the use, preparation, and purpose of these plants.

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