Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2018.9.18

Research Article
In vitro Antioxidant and Anticholinesterase Activities of Colombian Plants as Potential Neuroprotective Agents

[1]Erika A. Plazas, [2]Monica C. Ávila, [2]Willman A. Delgado, [2]Oscar J. Patiño and [2]Luis E. Cuca

[1]Departamento de Quimica, Universidad Nacional de Colombia, Bogotá, Cr 30 N°45-03 Colombia
[2]Laboratorio de Productos Naturales Vegetales, Departamento de Quimica, Universidad Nacional de Colombia, Sede Bogotá, Colombia

Abstract

Background and Objective: Neurodegenerative diseases are complex and heterogeneous group of multifactorial and progressive disorders, which constitute a worldwide health problem. Plants are a potential source of multi-target neuroprotective agents for the treatment of multifaceted diseases such as neurological disorders. In this study, *in vitro* antioxidant and anticholinesterase screening of 81 ethanolic extracts of 43 Colombian species (Lauraceae, Piperaceae, Rutaceae and Myristicaceae) was performed in order to determine their neuroprotective potential. **Materials and Methods:** The preliminary activity was tested using TLC-bioautography assay with 2,2-Diphenyl-1-picrylhydrazyl (DPPH·), β-carotene and acetylcholinesterase (AChE). The inhibitory effect (IC_{50}) of the most active extracts in each assay was quantified by a microplate colorimetric test. **Results:** From the TLC-bioautography assay, almost 36 extracts showed radical scavenging capacity and antioxidant activity by β-carotene bleaching mainly of Lauraceae, Myristicaceae and Piperaceae. In addition, 13 extracts exhibited anticholinesterase activity and the most potential extracts were characterized by the presence of active alkaloids. Extracts of Lauraceae and Rutaceae were more active against enzymatic inhibition than DPPH assay. Five extracts of *Zanthoxylum* (Rutaceae) and *Ocotea* (Lauraceae) presented strong inhibition of AChE with IC_{50} lower than 50 μg mL^{-1}. However, most of these were inactive against butyrylcholinesterase. **Conclusion:** The most active extracts showed the presence of isoquinoline alkaloids, this characteristic may be related with the strong AChE inhibition.

Key words: DPPH· scavenging, anticholinesterase, Colombian plants, TLC-bioautography screening, isoquinoline alkaloids

Citation: Erika A. Plazas, Monica C. Ávila, Willman A. Delgado, Oscar J. Patiño and Luis E. Cuca, 2018. *In vitro* antioxidant and anticholinesterase activities of Colombian plants as potential neuroprotective agents. Res. J. Med. Plants, 12: 9-18.

Corresponding Author: Erika A. Plazas, Departamento de Quimica, Universidad Nacional de Colombia, Bogotá, Cr 30 N°45-03 Colombia Tel: +57-1-3165000

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neurodegenerative diseases (ND) comprise a diverse group of late-onset central nervous system (CNS) disorders, which affect more than 30 million people worldwide. The most common ND includes Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD)[1]. AD and PD are the most prevalent and high-cost disorders and constitute a rising public health problem due to high incidence and the low effectiveness of current treatments[2].

The neurodegenerative disorders were characterized by the progressive and irreversible loss of neuronal cell populations from specific regions of the brain, with distinct morphological and pathophysiological features[3]. They have complex and multifactorial pathology; although, the specific mechanisms of neuronal death in ND were unknown currently, huge evidence suggested that many genetic and environmental factors play an important role in the pathophysiology[4]. In the last decades neuroscience has achieved significant advances in the understanding of the neurodegenerative process and basic biochemical pathways have been recognized in different pathogenic cascades[5,6]. The most common factors involve oxidative stress, metal dyshomeostasis, mitochondrial dysfunction, misfolding and dysfunctional trafficking of proteins[7,8].

The reactive nitrogen and oxygen species (RNS and ROS) produced in the respiratory chain play important functions in the physiology of the CNS, mainly as biological messengers. Nevertheless, different exogenous factors such as high cholesterol diets, pollution and metal overexposure increase the imbalance between generation and elimination of RNS and ROS, triggering an oxidative response in nervous system. The brain is particularly susceptible to damage by oxidative stress due to their dependence on oxygen consumption and deficit of endogenous antioxidant systems[9]. The overproduction of nitric oxide (NO·) and RNS promote inflammatory process elicit mitochondrial, activated microglia, cytotoxicity and apoptosis; which finally lead to massive neuronal death. Thus, the oxidative stress is a key biochemical cascade associated whit neurodegeneration[10,11]. Therefore, the search of antioxidants is a promising strategy to the development of neuroprotective agents as prevention or clinical treatment[12].

Due to the multifactorial nature of ND, the pharmacotherapy based on a single target has been insufficient, lead to ineffective or symptomatic drugs[13]. For these reason, the medicinal chemistry had proposed different pharmacological approaches to counteract these problems, as well as to improve the efficiency of current treatments. The use of combinations of drugs with different therapeutic mechanisms showed synergistic effects, however, might be disadvantageous due to increase the toxicity by biotransformation. Thus, emerge the multi-target directed ligands or MTDLs approach focused on development to a single agent capable to modulate different targets involved in the cascade of pathological events leading to a given disease[14,15]. The most extensive group of MTDLs agents explored in the last years comprises mainly cholinesterase inhibitors with some additional properties that increase the pharmacological profile[16]. The inhibition of cholinesterase is an important target strategy not only for AD, also for senile dementia, ataxia and PD. Actually, cholinesterase inhibitors were the only approved drugs for treating of AD as Galantamine and Rivastigmine, however, they only treat cognitive symptoms[17]. Natural products, one of the most promising sources of privileged scaffolds with multimodal characteristics for the treatment of neurodegenerative diseases[18,19]. Alkaloids and polyphenols are the secondary metabolites with greater neuroprotective potential against different biochemical pathways of ND diseases[20-22].

The present study achieved an *in vitro* screening antioxidant and anticholinesterase activities of Colombian Lauraceae, Piperaceae, Rutaceae and Myristicaceae species, in order to select plant extracts for the subsequent isolation of secondary metabolites with neuroprotective potential.

MATERIALS AND METHODS

Plant material: Eighteen Lauraceae, fifteen Piperaceae, four Myristicaceae and five Rutaceae species were collected between August, 2015 and June, 2016, in different regions from Colombia. The plant material was identified by the Herbario Nacional Colombiano de la Universidad Nacional de Colombia, where voucher specimens have been deposited (Table 1).

Preparation of the extracts: Plant material of each species was separated into different parts (leaves, stems, flowers and fruits when it was possible) and was dried at room temperature. The powdered air-dried plant material was extracted by maceration with ethanol at room temperature. Resulting extracts were concentrated under vacuum at 40°C to obtain dry extracts.

Chemicals: Acetylcholinesterase from electric eel (E.C.3.1.1.7, Type VI-S), butyrylcholinesterase from horse serum (E.C.3.1.1.8), butyrylthiocholine iodide (BTCI), Fast Blue B salt,

Table 1: Extracts of Colombian plants tested using TLC-bioautography assay with DPPH, β-carotene and acetylcholinesterase

				Bioautography		
Family	Species	Place of collection	Used part	DPPH	Carotene	AChE
Lauraceae	*Cinnamomum triplinerve*	Santa Barbara (Santander)	Leaves	+	-	-
			Bark	+	+	-
			Wood	+	+	-
	Nectandra sp.	Santa Barbara	Leaves	-	-	-
			Bark	-	-	-
	Endlicheria oreocola	Santa Barbara	Leaves	-	-	+
	Cinnamomum cinnamomifolium	Santa Barbara	Leaves	-	-	-
			Bark	+	+	-
	Endlicheria paniculata	Santa Barbara	Bark	-	-	-
	Cinnamomum triplinerve	Nocaima (Cundinamarca)	Leaves	-	-	-
	Nectandra membranacea	Santa Barbara	Bark	-	-	-
			Flowers	+	+	-
	Nectandra reticulata	Granada (Meta)	Leaves	-	-	-
	Ocotea macrophylla	La vega (Cundinamarca)	Wood	+	+	-
			Bark	+	+	-
	Rhodostemonodaphne laxa	Acacias	Leaves	+	+	-
	Nectandra reticulata	Puerto López (Meta)	Leaves	-	-	-
	Persea perseiphylla	Puerto López	Leaves	-	-	-
	Ocotea longifolia	Leticia (Amazonas)	Leaves	+	+	-
			Bark	+	+	-
	Beilschmiedia costaricensis	La vega	Leaves	+	+	-
	Aniba robusta	La vega	Leaves	-	-	-
			Bark	-	-	-
	Nectandra lineata	Nocaima	Leaves	-	-	-
	Ocotea discolor	Santa Barbara	Leaves	+	+	+
			Wood	+	+	+
			Bark	+	+	+
Myristicaceae	*Virola carinata*	Puerto Lopez (Meta)	Leaves	-	-	-
	Virola sebifera	Puerto Lopez	Seeds	+	+	-
			Wood	+	+	-
	Virola carinata	Granada (Meta)	Leaves	+	-	-
			Bark	-	-	-
			Fruits	-	-	-
			Peels	-	-	-
	Virola sp.	Leticia	Leaves	+	+	-
			Wood	-	-	-
			Bark	+	-	-
	Compsoneura sp.	Leticia	Leaves	+	+	-
			Bark	+	-	-
Piperaceae	*Piper imperiale*	Santa Barbara	Leaves	-	-	-
			Wood	-	-	-
	Piper pesaresanum	Santa Barbara	Leaves	+	+	-
			Wood	-	-	-
	Piper artanthe	Fusagasuga	Aerial part	+	+	-
			Inflorescences	-	-	-
	Piper reticulatum	Arauca	Leaves	-	-	-
	Piper cumanense	Quipile (Cundinamarca)	Leaves	-	-	-
			Wood	-	-	-
			Inflorescences	-	-	-
	Piper bogotense	Granada (Cundinamarca)	Leaves	-	-	-
			Inflorescences	-	-	-
	Piper sp.	Acacias (Meta)	Leaves	+	+	-
	Piper asperiusculum	San Mateo (Boyacá)	Leaves	+	+	-
			Inflorescences	+	+	-
	Piper pertomentellum		Leaves	-	-	-
			Inflorescences	-	-	-
	Piper bogotense	Uvita (Boyacá)	Leaves	+	+	+
			Wood	+	+	+

Table 1: Continue

Family	Species	Place of collection	Used part	Bioautography		
				DPPH	Carotene	AChE
	Piper nubigenum	Cascajal (Boyacá)	Leaves	-	-	-
			Wood	-	-	-
	Piper marginatum	Tibacuy (Cundinamarca)	Aerial part	-	-	-
	Piper holtonii	Nocaima (Cundinamarca)	Leaves	+	+	-
			Wood	-	-	-
	Piper elbancoanum	Granada (Cundinamarca)	Leaves	-	-	-
	Piper arboreum	Arbelaez (Cundinamarca)	Leaves	-	-	-
			Wood	-	-	-
	Piper peltatum	Pandi (Cundinamarca)	Leaves	-	-	-
	Piper amalago	Tibacuy	Leaves	-	-	-
Rutaceae	*Zanthoxylum rhoifolium*	Guayabal de siquima	Leaves	-	-	-
			Wood	-	-	-
			Bark	+	+	+
			Fruits	+	-	-
	Zanthoxylum monophyllum	San bernardo (Cundinamarca)	Leaves	-	-	+
			Bark	+	-	+
	Zanthoxylum rhoifolium	Santander	Leaves	+	+	+
			Fruits	-	-	-
	Zanthoxylum rigidum	Icononzo (Tolima)	Leaves	+	+	-
	Zanthoxylum quinduensis	Alban (Cundinamarca)	Leaves	+	-	-
			Bark	+	+	+
	Zanthoxylum fagara	Icononzo	Bark	-	-	-

+: Active (Presence of spots with intensities comparable to positive controls), -: Non-active

1-Naphthyl acetate, dimethylsulfoxide (DMSO), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), β-carotene and bovine serum albumin (BSA) were purchased from Sigma-USA. Acetylthiocholine iodide (ATCI) and dithiobis-nitrobenzoic acid (DTNB) were obtained from Alfa Aesar. Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$), disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$), sodium bicarbonate ($NaHCO_3$) and silica gel plates (silica gel 60 F254) were obtained from Merck (Darmstadt, Germany).

TLC bioautography: The preliminary activity of 81 extracts was evaluated by TLC-bioautography assay using silica gel as stationary phase and different mobile phases. The antioxidant potential was assessed by DPPH• scavenging and β-carotene bleaching test; the acetylcholinesterase inhibitory was carried out by Fast Blue assay. The extracts were solubilized in methanol at a final concentration of 10 mg mL^{-1} and 10 µL of these were spotted at the TLC plates. The plates were developed with two different mobile phases (hexane-ethyl acetate 8:2 and chloroform-methanol 9:1) to ensure that most compounds were separated and analyzed. Then, the developed plates were dried and sprayed with the reagents for each assay.

Antioxidant activity: A methanolic solution of the 0.2% DPPH radical was used, past 5 min after the plates were examined and the active compounds (free radical scavengers) appeared as yellow to white spots against to the purple plate. Additionally, two plates were sprayed with a β-carotene solution (0.05% in chloroform) and left at UV light (365 nm) until degradation of the reagent. Active compounds (β-carotene protectors) remained as yellow-orange spots on a white background. As a positive control, a stock solution of quercetin (1 mg mL^{-1}) was used[23,24].

Acetylcholinesterase inhibition: This was performed as by Komsta *et al.*[25]. with a few modifications. The plates were sprayed with enzyme solution (4 U mL^{-1}), thoroughly dried and incubated at 36°C in a humid chamber for 30 min.

Subsequently the plates were sprayed with α-naphthyl acetate solution (2.5 mg mL^{-1}) and re-incubated under the described conditions. Finally, the Fast Blue B solution (2.5 mg mL^{-1}) was sprayed; and the characteristic purple coloration was developed. Potential AChE inhibitors were appeared as clear zones on a purple-colored background. Berberine and galantamine (1 mg mL^{-1}) were used as positive controls in each plate.

The results of the AChE-TLC were compared with separate TLC plates with the same mobile phases spraying with Dragendorff reagent.

Microplate assays: The active extracts in TLC assays were tested in microdilution to determine the inhibitory concentration (IC_{50}) according to following methodologies:

DPPH· scavenging: A 0.1 mM solution of DPPH radical in methanol was used. From a stock solution (500 pmm) of each extract serial dilutions were made. DPPH solution of 195 µL was added to 5 µL of samples in methanol at different concentrations. The absorbance was measured at 517 nm at 0, 10 and 30 min. Positive controls were quercetin and ascorbic acid (100-1 ppm). Each measurement was made at least in triplicate. The inhibitory concentration (IC_{50}) was calculated and expressed as VCEAC (mg L^{-1}) in comparison with ascorbic acid[26].

Anticholinesterase activity: The enzymatic activity of positive TLC-extracts was measured using an adaptation of the colorimetric Ellman's method[27] against AChE and BChE. The enzyme solution was prepared at 0.2 U mL^{-1} in phosphate buffer solution (0.1 M, pH 8 with 0.1% BSA). As substrate, ATCI or BTCI (15 mM) was used and Ellman *et al.*[27] reactive (DTNB 3 mM) in phosphate buffer solution[28,29]. The extracts were solubilized in DMSO (10% in phosphate buffer) at final concentration of 500 µg mL^{-1} and serial dilutions were made. In a 96 well plate, 30 µL of samples, 125 µL of DTNB and 30 µL of enzyme were mixed, the plate was incubated at 36°C for 10 min and the absorbance was measured at 405 nm 3 times every 5 min. Then 30 µL of substrate was added and then absorbance was measured at 1 min intervals for 30 min. Assays were performed in triplicate and compared to the negative control. Berberine and galantamine (1 and 100 µg mL^{-1}) were used as positive controls.

RESULTS AND DISCUSSION

TLC bioautography: According to the results of preliminary screening by TLC-bioautography (Table 1), among 36 extracts of 22 species showed compounds with DPPH radical scavenging capability, being the extracts with more antiradical metabolites mainly of the Lauraceae, Myristicaceae and Piperaceae families. Antiradical scavenging compounds were found to be predominant with medium to high polarities and were eluted with chloroform-methanol (Fig. 1). However, a few extracts of Piperaceae specifically, *Piper pesaresanum*, *Piper artanthe* and *Piper bogotense* exhibit nonpolar compounds with high radical scavenging capacity. The 36 extracts with radical scavenging capacity were also tested for antioxidant activity by β-carotene bleaching; in this test, the extracts presented similar results to those of the DPPH assay. Most of the extracts showed active spots, mostly in medium and low R_f regions (Fig. 1). The antiradical active nonpolar spots of *P. pesaresanum*, *P. artanthe* and *P. bogotense* were also active in the protection of β-carotene retained a strong orange color. From *P. bogotense* and *P. pesaresanum* different phenolic metabolites have been isolated, predominantly, benzoic acid derivatives, prenylated quinones, flavonoids and amides. In previous studies, the antioxidant activity of Piperaceae extracts has been related with the presence of flavonoids and derivatives of benzoic acid, consequently, this kind of compounds could be related with the antiradical activity observed in the results of this study[30,31].

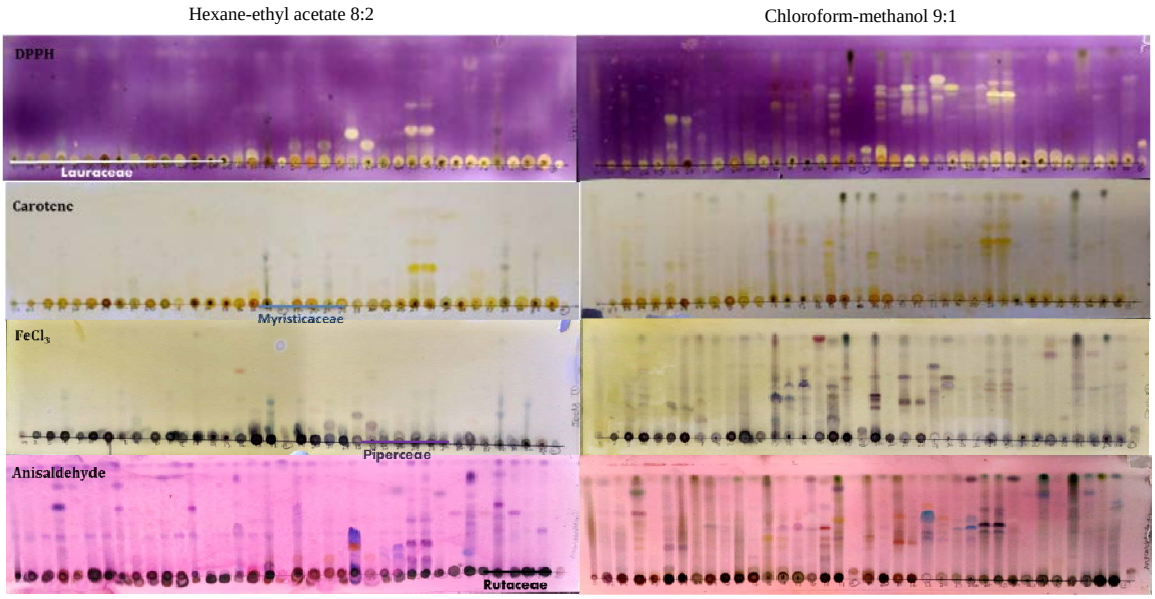

Fig. 1: TLC-Bioautography of the most active antioxidant extracts

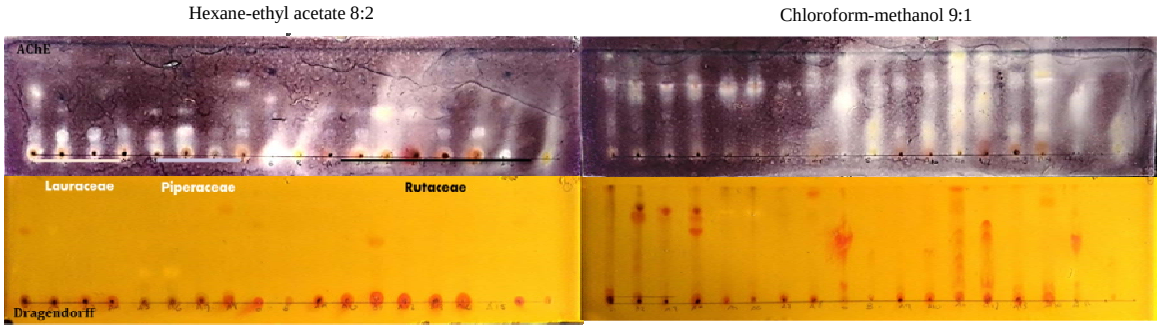

Fig. 2: TLC-Bioautography of the most active anticholinesterase extracts

Also, was noted that all *Ocotea discolor*, *Virola sebifera* and *P. bogotense* exhibited very strong active polar compounds in both bioassays.

To detect the presence of phenolic antioxidant compounds the active extracts were analysed in TLC with the same mobile phases spraying with ferric chloride (2%) and anisaldehyde sulphuric acid (Fig. 1). In these results medium and high polarity active compounds (antiradical/antioxidant) were appeared as green, blue or purple spots by reaction with $FeCl_3$; indicating the possible presence of free phenolic hydroxyls. On the other hand, with anisaldehyde reagent a variety of colours was observed, showing the presence of different metabolite scaffolds in the study extracts. Compounds that showed positive reaction with $FeCl_3$ appeared as red, blue or purple spots with anisaldehyde, which confirmed the presence of phenolic type antioxidants in the most active extracts[32]. The highest antioxidant/radical scavenger potential by TLC was detected in the extracts of *O. discolor*, *V. sebifera*, *Virola* sp., *Compsoneura* sp., *P. pesaresanum*, *P. artanthe* and *P. bogotense*.

In the AChE inhibitory assay, 13 extracts of 7 species showed one or more inhibition zones on the purple background (Fig. 2). In the hexane mobile phase fewer and weakly active spots were observed. While, in chloroform mobile phase the strongest AChE inhibitory potential was detected. Some extracts of Rutaceae and Lauraceae showed the highest number of active spots, placed in the middle and high polarity. Particularly the most potent extracts as *O. discolor*, *Z. monophyllum*, *Z. rhoifolium* and *Z. quinduensis* exhibited white spots whose intensities comparable with the positive controls (berberine and galantamine) located at the end of the plate (Fig. 2).

Considering that *Zanthoxylum* and *Ocotea* species are characterized by the presence of alkaloids[33,34] and this kind of metabolites were categorised by being cholinesterase inhibitors[35] a TLC analysis spraying with Dragendorff was achieved to detect the possible presence (Fig. 2). The most active extracts (*O. discolor*, *Z. monophyllum* and *Z. quinduensis*) showed AChE inhibitory alkaloids; both genus have in common occurrence of isoquinoline alkaloids such as aporphine, protoberberine or benzophenanthridine. Some isoquinoline alkaloids have a neuroprotective potential against different targets[36].

Microplate assays: DPPH· scavenging assay is the most extensively used test for screening antioxidant activity in extracts, due to, easiness and low cost. In this assay, the antioxidant compounds reduced the radical to pale yellow hydrazine, which involved the measurement of decrease in absorbance proportional to concentration of free radical scavenger added to DPPH reagent solution[37]. The IC_{50} amount of antioxidant necessary to decrease by 50% the initial DPPH· concentration, thus, lower IC_{50}, indicated high antiradical scavenging efficiency. Therefore, according to the results (Table 2), the most active extracts were *C. cinnamomifolium, N. membranaceae* and *O. longifolia* which presented IC_{50} lower than 50 μg mL^{-1}, all belonging to Lauraceae family. Some species of Lauraceae have shown antioxidant potential by DPPH* scavenging with variable inhibitory concentrations[38,39]; leaves and stems extracts of species of *Ocotea* genus like *O. minor* and *O. ceanothifolia* exhibited highly activity in DPPH assay[40]. In the present screening leaves extract of *O. longifolia* present the lowest IC_{50} (22.46 μg mL^{-1}), this was the first report of antioxidant activity for the specie. In previous research of the authors, from the bark of *O. longifolia* were isolated eight secondary metabolites mainly terpenes and the chemical composition of leaves essential oil was studied; however, the radical scavenging capacity was not determined[41]. The leaves of *O. longifolia* were more active than bark, thus, could be supposed that active compounds in the leaves may be

Table 2: Antioxidant activity of selected extracts by DPPH

Plants	Parts	IC_{50} (μg mL^{-1})
C. triplinerve	Leaves	66.41±1.32
	Bark	71.43±2.34
C. cinnamomifolium	Bark	50.04±0.98
N. membranaceae	Flowers	40.91±1.01
O. macrophylla	Wood	90.05±2.80
	Bark	92.46±1.98
R. laxa	Leaves	64.72±2.22
O. longifolia	Leaves	22.46±0.88
	Bark	72.38±1.32
B. costaricensis	Leaves	108.09±2.44
	Bark	90.91±1.98
O. discolor	Leaves	190.11±5.02
	Wood	104.63±4.22
	Bark	125.45±1.12
V. sebifera	Leaves	117.22±6,30
	Bark	84.02±2.12
V. carinata	Leaves	70.01±1.40
Virola sp.	Leaves	59.61±1.12
	Bark	91.53±2.80
Compsoneura sp.	Leaves	91.18±3.12
	Bark	65.42±1.01
P. pesaresanum	Leaves	136.40±1.12
P. artanthe	Aerial part	146.42±2.56
P. bogotense (Uvita)	Leaves	74.42±1.20
	Wood	78.16±1.70
P. holtonii	Leaves	153.02±1.05
Z. rhoifolium	Bark	193.11±6.26
	fruits	115.20±5.35
Z. monophyllum	Bark	102.01±1.34
Z. rigidum	Leaves	98.61±0.98
Z. quinduensis	Leaves	104.82±2.44
Positive controls	Quercetin	4.16±0.042
	Ascorbic Ac.	1.60±0.012

IC_{50} values represent the mean±SEM of three parallel measurements ($p<0.05$)

different from those previously isolated in the bark. According to the profiles in TLC-bioautography (Fig. 1), both organs differ mainly in the most polar compounds (origin).

On the other hand, relating the bioautography and quantification results, the extracts with the highest potential in TLC (*O. discolor*, *V. sebifera*, *Virola* sp., *Compsoneura* sp., *P. pesaresanum* and *P. artanthe*) did not necessarily show lower $IC_{50,}$ which may be due to antagonistic effects in the total extracts.

Since the main limiting of the of DPPH· is the steric accessibility with this test it is possible to determine mostly small compounds with radical scavenging capacity, hence its advisable to use another assay if it is necessary to determine the presence of large antioxidant compounds. For the purpose of this screening research, the results obtained were satisfactory with this test.

Data in Table 3 showed the inhibitory activity against cholinesterases was determine by Elman's colorimetric test, the inhibition percentage was determined for each concentration and IC_{50} was calculated by log Probit analysis. Five extracts of three species showed strong inhibition of AChE (higher than 80%) at 250 μg mL^{-1} and IC_{50} lower than 50 μg mL^{-1}. However, all extracts were inactive against BChE.

The active species against AChE (*O. discolor*, *Z. quinduensis* and *Z. monophyllum*) exhibited AChE inhibitory alkaloids in TLC-bioautography. Extract of bark to Z. *monophyllum* was the most powerful with an inhibitory concentration (10.2 μg mL^{-1}) comparable with the positive

Table 3: Acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) inhibitory activity of selected extracts

		IC_{50} (μg mL^{-1a})	
Plants	Parts	AChE	BChE
E. oreocola	Leaves	148.2 (120.5-194.1)	656.7 (542.8-678.8)
O. discolor	Leaves	35.1 (22.3-44.3)	188.9 (102.6-216.3)
	Wood	35.1 (17.2-39.9)	198.9 (120.8-264.6)
	Bark	34.5 (26.4-40.1)	170.4 (118.9-200.8)
V. carinata	Leaves	70.4 (63.3-94.5)	646.5 (632.2-733.4)
	Bark	158.6 (120.4-170.1)	569.4 (518.5-606.6)
	Fruits	112.7 (89.9-135.8)	756.3 (659.5-880.4)
P. bogotense (Uvita)	Leaves	70.4 (48.9-102.1)	324.2 (303.1-356.2)
	Wood	114.3 (105.5-138.0)	686.2 (589.0-732.1)
P. holtonii	Leaves	374.5 (338.5-416.2)	787.1 (702.2-884.1)
	Wood	96.7 (70.2-122.5)	376.4 (239.7-414.7)
Z. fagara	Bark	116.8 (100.9-158.5)	569.8 (454.1-707.3)
Z. rhoifolium	Leaves	107.4 (100.1-120.3)	838.4 (902.3-756.6)
	Bark	103.7 (89.9-118.7)	198.7 (182.8-255.6)
Z. monophyllum	Bark	10.2 (4.4-33.1)	194.4 (179.9-209.9)
Z. quinduensis	Leaves	123.0 (112.7-140.0)	697.8 (564.2-722.0)
	Bark	31.6 (12.3-46.5)	92.2 (78.4-106.1)
Positive controls	Berberine	1.9 (1.0-5.2)	35.0 (28.2-41.6)
	Galantamine	4.1 (1.4-7.5)	48.9 (36.2-61.8)

[a]IC_{50} values determined by log-probit analysis 95% confidence interval in parentheses

controls. In previous chemical studies of *Z. monophyllum* the presence of alkaloids, lignans, terpenes and coumarins has been reported[42,43]. From the bark of this specie were previously isolated protoberberine alkaloids as berberine and jathrorrhizine[44] and recently they have been reported as AChE inhibitors[45] which could explain the strong inhibitory activity of the extract.

From the *Z. quinduensis* in our research group were isolated some benzophenanthridine alkaloids[44], including chelerythrine, which in recent studies showed inhibitory activity against AChE and BChE[45].

Finally, from wood of *Ocotea discolor* were isolated 4 aporphine alkaloids: ocoxilonine, ocoteine, dicentrine and 1,2-Methylenedioxy-3,10,11-trimethoxyaporphine[46], which didn't reported previously for anticholinesterase activity. Therefore, to continue with the present research, currently being determined the inhibitory activity of these alkaloids and molecular docking studies were also performed to explore the detailed interaction with AChE.

The Genus *Zanthoxylum* and *Ocotea* are characterized by the occurrence of isoquinoline alkaloids and this feature seems to be related to AChE inhibitory activity. From this perspective, they can be considered promising for target isolation of potential multitarget therapeutic agents for the treatment of neurodegenerative diseases.

CONCLUSION

The *C. cinnamomifolium*, *N. membranaceae* and *O. longifolia* extracts, all belonging to Lauraceae family, showed high DPPH antiradical scavenging efficiency. Of these, *O. longifolia* leaves extract present the lowest IC_{50}. On the other hand, five extracts of *Zanthoxylum* and *Ocotea* genus exhibited strong anticholinesterase activity against AChE, they were characterized by the presence of isoquinoline alkaloids. By comparison of antioxidant and anticholinesterase activities, was possible to identify at least five extracts which have activity in the both assays. Therefore, further studies should be conducted to isolate and characterize potential neuroprotective compounds.

SIGNIFICANCE STATEMENTS

This study discovers the possible relationship between the presence of isoquinolone alkaloids and anticholinergic activity in species of *Ocotea* and *Zanthoxylum* genus. Thus, they can be considered potential multitarget therapeutic agents for the treatment of neurodegenerative diseases. Therefore, which gives steadiness to the investigations in order to make rational isolation of active alkaloids and extend it with molecular docking studies to explore the detailed interaction with targets.

ACKNOWLEDGEMENT

The authors are thankful for the financial support provided by the Dirección de Investigación de Sede Bogotá, Universidad Nacional de Colombia (grant number 201010026356) at the project entitled "Búsqueda de agentes para el tratamiento del Alzheimer en especies vegetales de la flora colombiana (fase I)" to carried out this research.

REFERENCES

1. Muangpaisan, W., A. Mathews, H., Hori and D. Seidel, 2011. A systematic review of the worldwide prevalence and incidence of Parkinson's disease. J. Med. Assoc. Thailand, 94: 749-755.
2. Sugino, H., A. Watanabe, N. Amada, M. Yamamoto, Y. Ohgi, D. Kostic and R. Sanchez, 2015. Global trends in Alzheimer disease clinical development: Increasing the probability of success. Clin. Ther., 37: 1632-1642.
3. Galimberti, D. and E. Scarpini, 2014. Neurodegenerative Diseases: Clinical Aspects, Molecular Genetics and Biomarkers. Springer-Verlag, Germany.
4. Bertram, L. and R.E. Tanzi, 2005. The genetic epidemiology of neurodegenerative disease. J. Clin. Invest., 115: 1449-1457.
5. Palop, J.J., J. Chin and L. Mucke, 2006. A network dysfunction perspective on neurodegenerative diseases. Nature, 443: 768-773.
6. Scrivo, R., M. Vasile, I. Bartosiewicz and G. Valesini, 2011. Inflammation as "common soil" of the multifactorial diseases. Autoimmun. Rev., 10: 369-374.
7. Sheikh, S., E. Haque and S.S. Mir, 2013. Neurodegenerative diseases: Multifactorial conformational diseases and their therapeutic interventions. J. Neurodegenerat. Dis., 10.1155/2013/563481.
8. Jucker, M. and L.C. Walker, 2013. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature, 501: 45-51.
9. Chen, X., C. Guo and J. Kong, 2012. Oxidative stress in neurodegenerative diseases. Neural Regenerat. Res., 7: 376-385.
10. Liu, Z., T. Zhou, A.C. Ziegler, P. Dimitrion and L. Zuo, 2017. Oxidative stress in neurodegenerative diseases: From molecular mechanisms to clinical applications. Oxidat. Med. Cell. Longevity, 10.1155/2017/2525967.
11. Li, J., W. Li, Z.G. Jiang and H.A. Ghanbari, 2013. Oxidative stress and neurodegenerative disorders. Int. J. Mol. Sci., 14: 24438-24475.

12. Caccamo, D., L.R. Pisani, P. Mazzocchetti, R. Ientile, P. Calabresi, F. Pisani and C. Costa, 2016. Neuroprotection as a potential therapeutic perspective in neurodegenerative diseases: Focus on antiepileptic drugs. Neurochem. Res., 41: 340-352.
13. Yin, F., J. Yao, R.D. Brinton and E. Cadenas, 2017. The metabolic-inflammatory axis in brain aging and neurodegeneration. Front. Aging Neurosci., Vol. 9. 10.3389/fnagi.2017.00209.
14. Bolognesi, M.L., 2013. Polypharmacology in a single drug: Multitarget drugs. Curr. Med. Chem., 20: 1639-1645.
15. Cavalli, A., M.L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini and C. Melchiorre, 2008. Multi-target-directed ligands to combat neurodegenerative diseases. J. Med. Chem., 51: 347-372.
16. Rosini, M., E. Simoni, M. Bartolini, A. Cavalli and L. Ceccarini *et al*., 2008. Inhibition of acetylcholinesterase, β-amyloid aggregation and NMDA receptors in Alzheimer's disease: A promising direction for the multi-target-directed ligands gold rush. J. Med. Chem., 51: 4381-4384.
17. Holzgrabe, U., P. Kapkova, V. Alptuzun, J. Scheiber and E. Kugelmann, 2007. Targeting acetylcholinesterase to treat neurodegeneration. Expert Opin. Ther. Targets, 11: 161-179.
18. Da Rocha, M.D., F.P.D. Viegas, H.C. Campos, P.C. Nicastro and P.C. Fossaluzza *et al*., 2011. The role of natural products in the discovery of new drug candidates for the treatment of neurodegenerative disorders II: Alzheimer's disease. CNS Neurol. Disorders-Drug Targets, 10: 251-270.
19. Campos, H.C., M.D. da Rocha, F.P.D. Viegas, P.C. Nicastro and P.C. Fossaluzza *et al*., 2011. The role of natural products in the discovery of new drug candidates for the treatment of neurodegenerative disorders I: Parkinson's disease. CNS Neurol. Disorders-Drug Targets, 10: 239-250.
20. Howes, M.J.R. and P.J. Houghton, 2009. Acetylcholinesterase inhibitors of natural origin. Int. J. Biomed. Pharm. Sci., 3: 67-86.
21. Wiart, C., 2013. Lead Compounds from Medicinal Plants for the Treatment of Neurodegenerative Diseases. Vol. 2, Academic Press, Elsevier Inc., New York.
22. Kim, Y.C., 2010. Neuroprotective phenolics in medicinal plants. Arch. Pharm. Res., 33: 1611-1632.
23. Ciesla, L., J. Kryszen, A. Stochmal, W. Oleszek and M. Waksmundzka-Hajnos, 212. Approach to develop a standardized TLC-DPPH test for assessing free radical scavenging properties of selected phenolic compounds. J. Pharm. Biomed. Anal., 70: 126-135.
24. Ciesla, L.M., M. Waksmundzka-Hajnos, K.A. Wojtunik and M. Hajnos, 2015. Thin-layer chromatography coupled with biological detection to screen natural mixtures for potential drug leads. Phytochem. Lett., 11: 445-454.
25. Komsta, L., M. Waksmundzka-Hajnos and J. Sherma, 2013. Thin Layer Chromatography in Drug Analysis. Chromatographic Science Series. CRC Press, USA.
26. Deng, J., W. Cheng and G. Yang, 2011. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chem., 125: 1430-1435.
27. Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
28. Carpinella, M.C., D.G. Andrione, G. Ruiz and S.M. Palacios, 2010. Screening for acetylcholinesterase inhibitory activity in plant extracts from Argentina. Phytother. Res., 24: 259-263.
29. Rahman, A., M. Choudhary and W. Thomsen, 2001. Bioassay Techniques for Drug Development. Harwood Academic Publishers, Amsterdam.
30. Nitola, Y., D. Munoz, O. Patino and J. Prieto, 2016. Caracterizacion fitoquimica y evaluacion de actividad inhibitoria sobre acetilcolinesterasa de hojas de *Piper pesaresanum* C. DC. Rev. Cubana Plant. Med., 21: 1-10.
31. Pena, L.A., E. Avella and A. de Diaz, 2000. Benzoquinona e hidroquinona preniladas y otros constituyentes aislados de *Piper bogotense* C.DC. Rev. Colomb. Quim., 29: 25-37.
32. Quettier-Deleu, C., B. Gressier, J. Vasseur, T. Dine and C. Brunet *et al*., 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol., 72: 35-42.
33. Patino, O.J. and L.E. Cuca, 2010. Isoquinoline alkaloids of *Zanthoxylum quinduensis* (Rutaceae). Biochem. Syst. and Ecol., 38: 853-856.
34. Pabon, L.C. and L.E. Cuca, 2010. Aporphine alkaloids from *Ocotea macrophylla* (Lauraceae). Quim. Nova, 33: 875-879.
35. Konrath, E.L., C.D.S. Passos, L.C. Klein, Jr. and A.T. Henriques, 2013. Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease. J. Pharm. Pharmacol., 65: 1701-1725.
36. Kulkarni, S.K. and A. Dhir, 2010. Berberine: A plant alkaloid with therapeutic potential for central nervous system disorders. Phytother. Res., 24: 317-324.
37. Apak, R., S. Gorinstein, V. Bohm, K.M. Schaich, M. Ozyurek and K. Guclu, 2013. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC technical report). Pure Applied Chem., 85: 957-998.
38. Al-Mamun, R., A. Hamid, M.K. Islam, J.A. Chowdhury and A.Z. Azam, 2011. Lipid lowering activity and free radical scavenging effect of *Cinnamomum tamala* (fam: Lauraceae). Int. J. Nat. Sci., 1: 93-96.
39. Saab, A.M., R. Tundis, M.R. Loizzo, I. Lampronti and M. Borgatti *et al*., 2012. Antioxidant and antiproliferative activity of *Laurus nobilis* L. (Lauraceae) leaves and seeds essential oils against K562 human chronic myelogenous leukaemia cells. Nat. Prod. Res., 26: 1741-1745.
40. Yamaguchi, K.K., J.M. Alcantara and V.F. Veiga, 2012. Investigacao do potencial antioxidante e anticolinesterasico de 20 especies da familia Lauraceae. Acta Amazonica, 42: 541-546.

41. Prieto, J.A., L.C. Pabon, O.J. Patino, W.A. Delgado and L.E. Cuca, 2010. Constituyentes quimicos, actividad insecticida y antifungica de los aceites esenciales de hojas de dos especies colombianas del genero *Ocotea* (Lauraceae). Rev. Colomb. Quim., 39: 199-209.
42. Patino, O.J., J.A. Prieto and L.E. Cuca, 2012. *Zanthoxylum genus* as Potential Source of Bioactive Compounds. In: Bioactive Compounds in Phytomedicine, Rasooli, I. (Ed.)., InTech, Croatia.
43. Rodriguez-Guzman, R., L.C.J. Fulks, M.M. Radwan, C.L. Burandt and S.A. Ross, 2011. Chemical constituents, antimicrobial and antimalarial activities of *Zanthoxylum monophyllum*. Planta Med., 77: 1542-1544.
44. Patino, O.J. and L.E. Cuca, 2004. Alcaloides benzofenantridinicos de *Zanthoxylum quinduensis*. Rev. Colomb. Quim., 33: 13-20.
45. Brunhofer, G., A. Fallarero, D. Karlsson, A. Batista-Gonzalez, P. Shinde, C.G. Mohan and P. Vuorela, 2012. Exploration of natural compounds as sources of new bifunctional scaffolds targeting cholinesterases and beta amyloid aggregation: The case of chelerythrine. Bioorg. Med. Chem., 20: 6669-6679.
46. Avila, M.C., L.T. Albarracin, L.E. Cuca, O. Patino, J.D. Guzman, T. Mchugh and N. Begum, 2017. Alcaloides aporfinicos con actividad antituberculosa aislados de *Ocotea discolor* Kunth (Lauraceae). Rev. Colomb. Quim., 46: 22-27.