INTRODUCTION

Plants have been used for therapeutic purposes by people that live in many Brazilian regions, mainly those located in the North and Northeast regions, where poverty is endemic. In Umbuzeiro do Manu, a community located in the semi-arid section of Sergipe state, Brazil, several medicinal plants are used in the formulation of stimulants of Central Nervous System (CNS), teas to treat insomnia, migraine, as sedatives, among other finalities [1]. The genus *Maytenus* is the most important inside the Celastraceae family. The term *Maytenus* is derived from the word, “Maytén,” a name firstly used by the “Mapuches” (“men of the land”), which live in Chile. According to South American folklore, many medicinal uses have been attributed to different species of *Maytenus* [2, 3]. Omena and co-workers studying the pharmacological potential of *Maytenus* species proved the CNS stimulant property of *M. rigidia* [1]. Neurodegenerative diseases are a group of sickness conditions that result from chronic breakdown associated to progressive loss of function or structure of neurons, particularly those of the CNS. The accumulation of aggregated proteins in neurons is involved in these types of diseases and its progression is caused by neurodegeneration processes [4]. The neurodegeneration that occur in Alzheimer’s disease (AD) is one of the most well studied processes. The AD is characterized by loss of memory, language deterioration, poor judgment, and human motor and perceptual-spatial skills. Dysfunction of cholinergic neurotransmission in the brain contributes to the salient cognitive decline in AD. The loss of cholinergic cells, particularly in the basal forebrain, is accompanied by a decrease in the concentration of the neurotransmitter acetylcholine (ACh). Elevated levels of β-amyloid (Aβ) are associated with alterations of synaptic function and neural network activity that probably underlie the cognitive deficits in AD. Moreover, Aβ accumulation leads to its deposition into plaques and is thought to drive a pathologic cascade, which ultimately culminate in neuronal death [5]. One of the most accepted strategies in AD treatment is the use of acetylcholinesterase (AChE) inhibitors [6]. Drugs that act inhibiting AChE generate limited therapeutic results against AD, but primarily provide a short-term alleviation of the symptoms, without blocking the progression of the disease [7]. Therefore, the structural diversity of AChE inhibitors used to explore different modes of action, have stimulated the interest related to identification of natural compounds as potential new types of bioactive compounds.

In this context, *Maytenus* sp. that has CNS stimulant effect can be considered as potential source of AChE inhibitors. Therefore, it is interesting to determine its potential for AChE inhibition. The ethanol extract from leaves of *Centella asiatica* was reported as being a tranquillizing for rat, and this activity was attributed to asiatic acid. In assays performed in vitro, this pentacyclic triterpene and its derivatives protect the cortical neurons against excitotoxicity induced by glutamate [8]. Pentacyclic triterpenes are secondary plant metabolites widespread in fruits peel, leaves, branches, stem barks and roots.

Some compounds of the lupane, oleanane and ursane series exhibit different pharmacological properties, while being devoid of prominent toxicity [9]. Ursolic acid, taraxerol (Ursane series) and lupeol (Lupane series) showed great AChE inhibition activity [10, 11]. In view of the potential of plants for AChE inhibition, we have screened in this study the extracts and eleven pentacyclic triterpenes (Figure 1) from *M. imbricata* and *M. gonocladia*. The outcomes of this work may contribute to bring about new active secondary metabolites, which can be used as prototypes or even as drugs against the effects on CNS caused by AD. The results also confirm some traditional uses of Brazilian medicinal plants of the *Maytenus* genus.

**Fig. 1:** Chemical structures of pentacyclic triterpenes isolated from *Maytenus* genus

MATERIALS AND METHODS

**Plant materials**

Roots of *M. imbricata* were carefully collected to prevent damage to the specimen. The collection area is located on Morro de Santana,
Ouro Preto, Minas Gerais (MG), Brazil. The plant material was identified by the botanists Dra. Rita Maria de Carvalho Okano, Departamento de Botânica of the Universidade Federal de Viçosa (UFV) and Dra. Maria Cristina Teixeira Braga Messias, Departamento de Botânica of the Universidade Federal de Ouro Preto (UFOP). A voucher specimen (Nº 27780) was deposited in the collection of the Herbarium of Departamento de Botânica, UFV, MG, Brazil. Branches and roots of *M. gonoclada* were collected in Serra da Piedade, Caeté, MG, Brazil also avoiding damages to the specimen. A voucher specimen (Nº 60280) was deposited in the Herbarium of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. Both collected plant material were dried over kraft paper, at room temperature.

**Preparation of extracts**

The collected roots of *M. imbricata* were powdered in a mill. The powder (1.5 kg) was submitted to extractions in a Soxhlet apparatus with hexane-ethyl ether (1:1). The roots of *M. gonocladua* were separated into barks (250.0 mg) and heartwoods (750.0 mg). The powdered material from branches (1.0 kg) was exhaustively and sequentially extracted in a Soxhlet apparatus, with hexane and chloroform. The heartwood and the bark of roots were subjected to exhaustive extraction in a Soxhlet apparatus, with hexane/ethyl ether (1:1). All extract solutions were eluted with hexane/CHCl3 (9:1) and, after eluent evaporation it was sprayed over the plates for TLC bioautography assay. In the TLC assay it was not observed AChE inhibition induced by the extracts of any *Myrtus* species. In the order hand, this preliminary assay however was able to show that 3-oxo-11α-hydroxy-29(29)-ene (1), 3-oxo-29-hydroxyfriedelane (5) and 3,7-dioxo-friedelane (7) were able to inhibit AChE under the same assay conditions (Figure 2).

**Results**

Aiming the discovery of a new drug that leads to acetylcholinesterase inhibition as a treatment for Alzheimer’s disease, extracts from *M. gonoclada*, and *M. imbricata* and the pentacyclic triterpenes 1 to 11 (Figure 1) were assayed for their AChE inhibitory property by means of TLC bioautography assay. In the TLC assay it was not observed AChE inhibition induced by the extracts of any *Myrtus* species. In the order hand, this preliminary assay however was able to show that 3-oxo-11α-hydroxy-29(29)-ene (1), 3-oxo-29-hydroxyfriedelane (5) and 3,7-dioxo-friedelane (7) were able to inhibit AChE under the same assay conditions (Figure 2).

**Phytochemical investigation**

Phytochemical methods were applied to low polarity extracts of *M. imbricata* and *M. gonoclauda* for the isolation of compounds. According to the TLC analysis, the pentacyclic triterpenes of the lupane [3-oxo-11α-hydroxy-29(29)-ene (1) and 3β,11α-dihydroxy-29(29)-ene (2)], friedelane [3-oxo-friedelane (3), 3β-hydroxy-friedelane (4), 3-oxo-29-hydroxy-friedelane (5), 3-oxo-12α-hydroxy-friedelane (6), 3,7-dioxo-friedelane (7), 3,11-dioxo-friedelane (8) and 3,16-dioxo-friedelane (9)], and quinonemethide skeleton [tingenone (10) and 6-oxo-tingenol (11)] were subjected to AChE inhibition assay.

**AChE Bioautography Assay**

Evaluation of AChE property of the extracts from *M. imbricata* and *M. gonoclauda* and the triterpenes isolated were conducted according to the qualitative methodology Thin Layer Chromatography (TLC) as described by Rhee et al. [14]. The samples were solubilized in CHCl3 at a concentration of 20 μg/mL and 100 μL of respective solutions were applied in silica gel thin layer chromatography plates. The extracts were eluted with hexane/CHCl3 (9:1) and, after eluent evaporation it was sprayed over the plates a solution of n-naphthyl acetate (1 mM) and a solution of Salt Fast Blue B (1 mM). Subsequently AChE (3 U/mL) solubilized in 50 mM Tris/HCl pH 8 containing 0.1% bovine serum albumin (BSA) fraction also was sprayed in the plate. The acetylcholinesterase inhibition was detected by the appearance of white halos 10 min after enzyme addition. The halos persisted for 20-30 min.

**Microplate assay**

The triterpenes that gave positive inhibition of AChE in the TLC bioautography method were subjected to microplate assay. Thus, the buffers A (50 mM Tris- HCl, pH 8, containing 0.1 M NaCl and 0.02 M MgCl2·6H2O), B (50 mM Tris- HCl, pH 8, containing 0.1% bovine serum albumin), and C (50 mM Tris- HCl, pH 8) were prepared to study the *in vitro* AChE inhibitory activity. This activity was measured using a 96-well microplate reader based on Ellman’s method [14, 15]. The enzyme hydrolyzes the substrate acetylthiocholine. The obtained product, thiocholine, decomposes the Ellman’s reagent, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), providing 2-nitrobenzene-5-mercaptop-thiolocline and 5-thio-2- nitrobenzoate, which can be quantified at 405 nm. The volumes of 25 μL of acetylthiocholine iodide (15 mM in water), 125 μL of DTNB (3 mM in buffer A), 50 μL of buffer B, and 25 μL of sample (10 mg/mL in MeOH diluted by 10 times with buffer C, resulting on a concentration of 1 mg/mL) were added into each well of 96-well microplate. Instead of adding the sample solution, a volume of 25 μL of buffer C was employed to prepare the blank sample. The positive control was prepared under the same conditions, using physostigmine (eserine) as standard. Tests were carried out in quintuplicate. The absorbance was measured at 405 nm every 60 s by eight times using an Elisa Thermoplate microplate reader. After addition of 25 μL of acetylcholinesterase solution (0.226 U/mL in buffer B), the absorbance was again read every 60 s for ten times. The increase in absorbance relative to substrate spontaneous hydrolysis was corrected by reaction rate variation before and after addition of the enzyme. The inhibition percentage was calculated by comparing the rates of the sample with the blank.

**DISCUSSION**

The results found for these compounds were in the same level that those found for physostigmine, the positive control. Therefore, triterpenes from skeletal lupane or friedelane, may be included among potential compounds for obtaining biologically active derivatives to be used to treat CNS disorders caused by Alzheimer’s disease. Secondary metabolites isolated from higher plants active toward AChE enzyme, e.g. galanthamine and huperzine A are, routinely used as drugs for the treatment of different forms of dementia. However, the available drugs for the AD treatment are frequently associated with adverse effects and present bioavailability problems. Alternatively, the literature describes the relation between pentacyclic triterpenes and treatments for AD, e.g. ginkgolide B indicated as anti- AD effect [4]. Recent studies showed that oral administration of retinoid X receptor (RXR) agonist benoxarotene, using a mouse model of AD, resulted in an enhanced clearance of soluble Aβ oligomers within hours in an apol-
dependent manner. Soluble Aβ oligomers are now recognized as key pathogenic structures in Alzheimer’s disease. Aβ plaque area was reduced more than 50 % within just 72 hours. Furthermore, bexarotene stimulated the rapid reversal of cognitive, social, and olfactory deficits and improved neural circuit function. Consequently, RXR agonist activation stimulates the physiological Aβ clearance mechanisms, resulting in the rapid reversal of a broad range of Aβ-induced deficits [5]. Thus, the pentacyclic triterpenes 1, 5 and 7 (Fig. 1) may be considered as promising compounds with different possibilities of pharmacological effect, acting not only as inhibitors of AChE, but probably also as anti-Aβ effect. Given that these compounds can act in different regions of the brain responsible for the causes of the disease, it is important the continuity of the studies involving pentacyclic triterpenes.

CONCLUSION

In this work it was observed that the pentacyclic triterpenes 1, 5 and 7 have ACHE inhibition property. The results open possibilities to the employment of these compounds as drug leads to be used in the treatment of Alzheimer’s disease.

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REFERENCE