Anti-Alzheimer’s and Anti-inflammatory Activities of Compounds Isolated from Solanum Mauritianum

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ABSTRACT
Solanum mauritianum, commonly known as “Tabaquillo”, was one of the most used plants by tribes from South America as a remedy for headaches. Based on this ethnopharmacological use, a bioguided isolation of compounds with anti-inflammatory and anti-Alzheimer’s activities from S. mauritianum was carried out by measuring the inhibition of NF-κB in C8D1A, Neuro-2a, and EOC 13.31 cells, and by measuring the inhibition of acetylcholinesterase and β-amyloid. This allowed the isolation and characterisation by nuclear magnetic resonance and mass spectrometry of four compounds (1–4). Compounds 1–4 showed NF-κB inhibitory activity with IC_{50} values of 9.13–9.96, 17.17–17.77, 2.41–2.79, and 1.59–1.93 µM, respectively, while celastrol (the positive control) had an IC_{50} value of 7.41 µM. Likewise, compounds 1–4 showed anti-Alzheimer’s activity, inhibiting the acetylcholinesterase by 40.33, 20.57, 61.26, and 83.32 %, respectively, while galantamine (positive control) showed an inhibition of 90.38 %. In addition, concerning the inhibition of β-amyloid aggregation, compounds 1–4 showed an inhibition of 47, 23, 65, and 93 %, respectively, while curcumin (positive control) had an inhibition of 71.19 %.
Introduction

Alzheimer’s disease (AD) is a worldwide health problem that was noted as the sixth top cause of death in the United States in 2015 [1]. In 2015, an estimated 46.8 million people worldwide suffered from AD, and this number is expected to rise to 131.5 million in 2050 with a subsequent increase in social and financial burdens [2].

AD is a progressive and neurodegenerative disorder among the elderly, characterised by progressive loss of memory and cognition [3]. In the early stages of AD, people have trouble remembering recent events, also called short-term memory loss. In the following stages, other symptoms arise, such as problems with communication through language, disorientation, loss of motivation, neglect in self-care, sleep disorders, and behavioural issues increasing in intensity [4]. In the late stage of AD, patients may not recognise family, and gradually the brain functions are lost due to damage at the level of the central nervous system, leading to death [5].

This is caused by brain damaged neurons and neurites, and highly insoluble β-amyloid (Aβ) peptide deposits and intracellular neurofibrillary tangles (NFTs), providing stimuli for inflammation [6]. This inflammation in the nerve tissue is characterised by the activation of astrocytes and microglia, and by the activation of cytokines and chemokines. Microglia are the primary carriers of the inflammatory reaction, forming approximately 10% of all brain cells. The activation of microglia is based on extracellular deposition of Aβ plaques, neuronal damage induced by the toxicity of the T-protein, or after ischemic or traumatic brain injury during AD progression [7].

Moreover, the macrophage-like resident immune cells in the brain also play an inflammation role in the development of AD. Thus, the microglia are activated by oligomeric and fibrillar particles of Aβ and by substances of degenerated neurons that increase their migration and phagocytosis. These main neurotropic molecules produced by activated microglia are reactive oxygen species, glutamate, and proinflammatory cytokines (TNF-α and IL-1β) [8].

The current diagnosis of AD is made by mental and cognitive examinations. From a histopathological point of view, this means the presence of senile Aβ plaques and NFTs in brain tissue, the two main hallmarks of AD [9]. Although several compounds undergoing research appear to be protective and have therapeutic effects in the AD model, none of these drugs was able to stop or reverse the course in patients with AD [10]. Acetylcholinesterase (AChE) inhibitors are the main existing therapeutic agents for AD treatment, which temporarily decrease dementia by raising the neurotransmitter level [11]. Despite extensive studies on new drugs for AD treatment, none has been successful from this point of view, leading to the need for further research [12].

Natural compounds (natural extracts and bioactive compounds) are an emerging approach for AD therapy [13]. The first natural product studied in a clinical trial was nicotine in 1992. However, no new clinical trials were performed in the last two decades for this compound [14]. During the ‘90s, several other compounds were studied in clinical trials for AD therapy, such as vitamins [15]. These compounds are still being tested in human trials today. Other natural compounds have been moved to the clinical trials phase, such as curcumin (Curcuma longa), huperzine A (Huperzia serrata), and bryostatin (Bugula neritina), whose effects started to be evaluated in humans in 2017 [16].

In this context, Solanum (Solanaceae) is a genus of approximately 1230 species. Its extracts are obtained from different parts, such as leaves, stems, bark, and roots, and have been used in the ethnopharmacology of different cultures [17]. Different compounds with anti-Alzheimer activity have been isolated from these species, e.g., Solanum asperum [18], Solanum nigrum, Solanum macrocarpon [19], and Solanum betaceum [20].

In this report, we analyse a different and less known species of this genus, Solanum mauritianum Scop., commonly known as “Tabaquillo”. This species is native from north-eastern Argentina, Southern Brazil, Paraguay, and Uruguay, and the infusions from its leaves have been used in traditional medicine against fever and headaches [21, 22]. To date, only four compounds present in this species have been characterised, all of them of an alkaloid structure (solasodine, solasonine, solamargine, and caulophyllum-A) [23]. Recently, Pelo et al. [21] mentioned the presence of phenols, flavonoids, quinones, saponins, and cardiotonics through qualitative colouration reactions. Finally, with respect to the pharmacological activities of the species S. mauritianum, only antioxidant and antitumoral effects have been reported [24, 25].

This study reports the isolation of four compounds (1–4) with an inhibitory effect on NF-kB as well as their inhibitory effect on cholinesterase and Aβ aggregation.

Results and Discussion

Using hexane (HEX), dichloromethane (DCM), MeOH, and distilled water (DH2O), four extracts of increasing polarity were obtained from dry powdered leaves of S. mauritianum. To evaluate their complexity and to perform an initial identification of the metabolite groups present in each of the extracts, 1H NMR spectra of each of them was recorded (Figs. 15–55, Supporting Information). The n-HEX and DCM extracts showed a group of intense signals in the aliphatic zone (δH 2.5–0.5 ppm), corresponding to hydrogens located in aliphatic chains. Moreover, the 1H NMR spectra of n-HEX, DCM, and MeOH showed signals between δH 5.5–5.0 ppm, which correspond to the presence of olefinic-type protons, and around δH 4.5–3.7 ppm, corresponding to hydrogens close to heteroatoms such as ethers, amines, or alcohols. Finally, less intense signals were observed around δH 8.0–6.0 ppm, corresponding to the presence of aromatic compounds. In addition, the MeOH extract presented a group of signals at δH 1.2–0.7 ppm, which corresponds to shorter alkyl chains. Finally, the aqueous extract presented a group of signals around δH 4.5–3.5 ppm, which corresponds to glycosylated compounds.

According to the observed signals, the n-HEX and DCM extract contain a high concentration of fatty acids, with aromatic compounds as secondary components, while the aqueous extract contains mostly glycosides and the MeOH extract contains a variety of compounds, both free and linked to sugars.

In this report, we only include a detailed characterisation of compounds 3 and 4, since they have not been previously reported as natural compounds, only as synthesised compounds. For compounds 1 and 2, only a brief characterisation has been included since they have been fully described in previous works (Fig. 1).
Subsequently, a comparison was made between the $^1H$ NMR spectra of the DCM extract and those of the resulting compounds (Fig. 6S, Supporting Information). Given the low relative mass of the extract, it was to be expected that the signals of the compounds tended to be weaker and less defined. Signals around $\delta_{\text{H}}$ 7.2 ppm were shown in both the extract and compounds 1 and 4. Signals around $\delta_{\text{H}}$ 1.7–1.2 ppm in the DCM extract masked the aliphatic multiplets of compound 4. Also, the signals of compound 1 were not easily distinguishable in the extract spectrum.

The $^1H$ NMR spectrum of the DCM extract and those of the resulting compounds showed the presence of fifteen distinct signals, corresponding to five quaternary carbon atoms, eight CH groups, and two CH$_2$ groups. Signals at $\delta_{\text{C}}$ 127.3 and 128.3 were found in greater intensity, thus supporting the hypothesis of the existence of a monosubstituted phenyl. Likewise, the presence of a signal at $\delta_{\text{C}}$ 191.0 indicated the existence of a carbonyl group, and the signals at $\delta_{\text{C}}$ 21.2 and 17.2 confirmed the presence of two methyl groups.

The final assignment of the compound was done using the $^1H$–$^1H$ COSY, HSQC, and HMBC spectra (Fig. 2). The $^1H$–$^1H$ COSY spectrum showed the presence of three independent spin systems, constituted by an olefin, a phenyl, and an aniline ring. The multiplicities of H-3', H-4', and H-6' protons, and the appearance of weak $^1H$–$^1H$ COSY correlations between the pairs (H-2' CH$_3$ with H-3' and H-5' CH$_3$ with H-6' and H-4') allowed the placement of the methyl groups within the aniline ring. Finally, the information from the HMBC spectrum reinforced the proposed structure, showing two groups of main correlations. On the one hand, the olefin and phenyl systems showed correlations with the carbonyl group (H-2, H-3, H-1'–C-1), while the H-3 proton showed a correlation with C-1', confirming the amine bond. On the other hand, the aniline ring showed an abundance of signals that were correlated to both the atoms forming the ring and those with the methyl groups. Each of these methyl groups showed three correlations, one strong (H-2' CH$_3$/C-2' and H-5' CH$_3$/C-5') and two weaker (H-2' CH$_3$/C-1' and H-5' CH$_3$/C-4', C-6').

1-(3,4,5-Trihydroxyphenyl)-nonan-1-one (4) was obtained as an amber solid; $R_t = 0.44$ (Hex/AcOEt; 2:1); m.p. 156°C; ≥ 98% purity (Figs. 32S–40S and Table 4S, Supporting Information). HR-ESI-MS yielded peaks for the protonated ion ([M + H]$^+$ $m/z = 267.1584$) and the sodium adduct ([M + Na]$^+$ $m/z = 289.1404$), accounting for a molecular formula of C$_{15}$H$_{23}$O$_4$, with a degree of unsaturation of five.

The analysis of the $^1H$ NMR spectrum shows seven multiplets, with one aromatic singlet at $\delta_{\text{H}}$ 7.21, which integrates for two protons, suggesting a symmetrical tetrasubstituted phenyl ring, and two broad singlets at $\delta_{\text{C}}$ 6.14 and 5.89, suggesting the existence of two different exchangeable protons (3'-OH and 4'-OH). Likewise, a triplet type signal (2H, $J = 7.4$) was found at $\delta_{\text{H}}$ 2.88, corresponding to H-2 in position a with the carbonyl group, while the following signal, corresponding to a multiplet at $\delta_{\text{H}}$ 1.75–1.62, was correlated with H-3 in the $\beta$ position with the carbonyl group. In the shift between $\delta_{\text{C}}$ 1.40–1.23, there was a multiplet signal corresponding to the rest of the CH$_2$ groups of the aliphatic chain. Finally, the last signal, a triplet at $\delta_{\text{H}}$ 0.88 (3H, $J = 7.0$), was assigned to a terminal methyl group.

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The $^{13}$C NMR and DEPT-135 spectra showed the presence of thirteen distinct signals, corresponding to four quaternary carbon atoms, a CH group, seven CH$_2$ groups, and a CH$_3$ group, signalling an aliphatic chain of eight carbon atoms. The signals at $\delta_C$ 108.9 and 143.7 that show a higher intensity reinforced the hypothesis of the symmetrically tetra-substituted phenyl. The signal at $\delta_C$ 201.0 pointed to the existence of a carbonyl group, and the high shifts of the quaternary aromatic carbons (C-3’ and C-4’) pointed to the existence of a trihydroxy derivative. The shifts of carbons ($\delta_C$ 29.5) and C-6 ($\delta_C$ 29.5) appeared very close together due to their placement in the middle of the non-functionalised aliphatic chain, which implies similar chemical environments, and thus, chemical shifts due to the absence of any significant inductive effect.

The final compound assignment was performed with the data extracted from the $^1$H-$^1$H COSY, HSQC, and HMBC spectra (Fig. 3). The $^1$H-$^1$H COSY spectrum was consistent with the assignment, no further information could be extracted from it, as the CH$_2$ multiplet does not have the required resolution to differentiate between the individual alkyl protons. However, the HMBC spectrum showed correlations between the H-3/C-4, H-9/C-7, and H-9/C-8 pairs, which allowed their assignment. There were also signals that correlated H-2’ with the rest of the aromatic carbons in addition to the carbon-yl group.

Regarding the cytotoxicity of the $S$. mauritianum extracts, the results showed that the DCM (CC$_{50}$ = 95.30–97.20 µg/mL) and aqueous (CC$_{50}$ = 95.34–96.67 µg/mL) extracts did not show relevant cytotoxicity ($p < 0.089$) when compared to the actinomycin D (ACT) positive control (CC$_{50}$ = 0.01 µg/mL) in any of the cell lines (CBD1A, Neuro-2a, and EOC 13.31) (Table 1).

Concerning the anti-inflammatory capacity, the results showed that the MeOH (IC$_{50}$ = 22.35–25.73 µg/mL) extract presented a higher inhibitory activity of the production of NF-$\kappa$B than the DCM extract (IC$_{50}$ = 26.15–27.65 µg/mL) (compared to the positive control, celestrol, IC$_{50}$ = 3.34 µg/mL) (Table 2). However, the MeOH extract did show cytotoxicity in any of the tested cell lines.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Cytotoxicity (CC$_{50}$ µg/mL) at 72 h (CI 95 %, R2)</th>
<th>Neuro-2a</th>
<th>EOC 13.31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>98.23 (93.95 to 103.55, 0.9723)</td>
<td>98.61 (93.20 to 103.80, 0.9896)</td>
<td>98.92 (93.94 to 103.75, 0.9947)</td>
</tr>
<tr>
<td>DMSO</td>
<td>20.28 (15.02 to 25.19, 0.9682)</td>
<td>20.29 (15.24 to 25.25, 0.9766)</td>
<td>20.58 (15.45 to 25.52, 0.9684)</td>
</tr>
<tr>
<td>ACT</td>
<td>0.01 (3.384 to 3.358, 0.9192)</td>
<td>0.01 (3.319 to 3.332, 0.9422)</td>
<td>0.01 (3.310 to 3.396, 0.9574)</td>
</tr>
<tr>
<td>EHEX</td>
<td>85.66 (80.55 to 90.38, 0.9382)</td>
<td>86.06 (81.13 to 91.23, 0.9643)</td>
<td>86.42 (81.38 to 91.40, 0.9425)</td>
</tr>
<tr>
<td>EDCM</td>
<td>95.30 (90.68 to 100.73, 0.9355)</td>
<td>96.52 (91.61 to 101.57, 0.9271)</td>
<td>97.20 (92.39 to 102.55, 0.9702)</td>
</tr>
<tr>
<td>EMeOH</td>
<td>75.35 (70.32 to 80.95, 0.9245)</td>
<td>77.54 (72.55 to 82.89, 0.9153)</td>
<td>77.99 (72.31 to 82.63, 0.9622)</td>
</tr>
<tr>
<td>ED$<em>{2}$H$</em>{2}$O</td>
<td>95.34 (90.14 to 100.18, 0.9441)</td>
<td>96.56 (91.33 to 101.99, 0.9577)</td>
<td>96.67 (91.37 to 101.21, 0.9915)</td>
</tr>
</tbody>
</table>

Cl 95 %: confidence interval 95 %/Tukey’s multiple comparisons test (”" $p < 0.001$). ACT = actinomycin D, EHEX = n-hexane extract, EDCM = dichloromethane extract, EMeOH = methanol extract, ED$_{2}$H$_{2}$O = aqueous extract.

Regarding the ability to inhibit AChE by $S$. mauritianum extracts, only the DCM extract inhibited AChE by 53.30 % (IC$_{50}$ = 26.94 µg/mL) compared to galantamine (positive control), which inhibits it by 90.38 % (IC$_{50}$ = 4.31 µg/mL) (Fig. 4).

On the other hand, regarding the inhibition of Aβ aggregation by the extracts of $S$. mauritianum, only the DCM extract showed an inhibition of the Aβ aggregation by 59.02 % (IC$_{50}$ = 26.94 µg/mL) compared to curcumin (positive control), which inhibits it by 71.19 % (IC$_{50}$ = 3.68 µg/mL) (Fig. 5).

The DCM extract was fractionated using HEX/AcOEt as the mobile phase, producing eight fractions that were subjected to cytotoxicity, anti-inflammatory, and anti-Alzheimer’s assays. Table 5S shows the cytotoxicity results; fractions F1–F6 showed low cytotoxicity while the last fractions (F7 and F8) were highly cytotoxic. The anti-inflammatory results showed that fractions F2 (IC$_{50}$ = 14.99–15.94 µg/mL), F4 (IC$_{50}$ = 15.28–15.79 µg/mL), and F5 (IC$_{50}$ = 12.27–12.56 µg/mL) have the highest inhibitory activity of NF-$\kappa$B production (Table 5S, Supporting Information). Concerning the anti-Alzheimer’s activity of the fractions obtained from the DCM extract, the results showed that fractions F2 (IC$_{50}$ = 15.57 µg/mL), F4 (IC$_{50}$ = 15.51 µg/mL), and F5 (IC$_{50}$ = 12.37 µg/mL) inhibit AChE by 55.46, 59.70, and 56.61 %, respectively, compared to galantamine, which inhibits it by 90.38 % (IC$_{50}$ = 4.31 µg/mL) (Fig. 4, Supporting Information). Additionally, the results on the inhibition of Aβ aggregation confirmed that fractions F2 (IC$_{50}$ = 15.57 µg/mL), F4 (IC$_{50}$ = 15.51 µg/mL), and F5 (IC$_{50}$ = 12.37 µg/mL) have inhibition percentages of 60.51, 60.59, and 64.83 %, respectively, while curcumin has an inhibition rate of 71.19 % (IC$_{50}$ = 3.68 µg/mL) (Fig. 5, Supporting Information).

A total of four subfractions (F2A–F2D) were obtained through the chromatographic separation of fraction F2, which were subsequently tested through cytotoxicity, anti-inflammatory and anti
Alzheimer’s assays. Table 75, Supporting Information, shows the cytotoxicity results; subfractions F2A and F2B showed low cytotoxicity when compared to the others subfractions. The anti-inflammatory results showed that subfraction F2B (IC50 = 9.06–9.93 μg/mL) has the highest inhibitory activity of NF-κB production (Table 85, Supporting Information). Concerning anti-Alzheimer’s activity of the subfractions, the results showed that subfraction F2B (IC50 = 9.47 μg/mL) inhibits AChE by 64.76% compared to galantamine, which inhibits it by 90.38% (IC50 = 4.31 μg/mL) (Fig. 43S, Supporting Information). Additionally, the results on the inhibition of Aβ aggregation confirmed that subfraction F2B (IC50 = 9.47 μg/mL) has an inhibition percentage of 66.51%, while curcumin has an inhibition rate of 71.19% (IC50 = 3.68 μg/mL) (Fig. 44S, Supporting Information).

A total of five subfractions (F4A–F4E) were obtained through the chromatographic separation of fraction F4, which were subsequently tested through cytotoxicity, anti-inflammatory, and anti-Alzheimer’s assays. Table 95, Supporting Information, shows the cytotoxicity results; subfractions F4A and F4B showed low cytotoxicity when compared to the others subfractions. The anti-inflammatory results showed that subfraction F4B (IC50 = 13.26–13.65 μg/mL) has the highest inhibitory activity of NF-κB production (Table 105, Supporting Information). Concerning the anti-Alzheimer’s activity of the subfractions, the results showed that subfraction F4B (IC50 = 13.46 μg/mL) inhibits AChE by 66.87% compared to galantamine, which has an inhibition rate of 90.38% (IC50 = 4.31 μg/mL) (Fig. 45S, Supporting Information). Additionally, the results on the inhibition of Aβ aggregation confirmed that subfraction F4B (IC50 = 13.46 μg/mL) has an inhibition percentage of 69.26%, while curcumin has an inhibition rate of 71.19% (IC50 = 3.68 μg/mL) (Fig. 46S, Supporting Information). A total of four subfractions (F5A–F5D) were obtained through the chromatographic separation of fraction F5, which were subsequently tested through cytotoxicity, anti-inflammatory, and anti-Alzheimer’s assays. Table 11S, Supporting Information, shows the cytotoxicity results; subfractions F5B and F5C showed low cytotoxicity when compared to the others subfractions. The anti-inflammatory results showed that subfraction F5C (IC50 = 6.81–7.02 μg/mL) has the highest inhibitory activity of NF-κB production.
Concerning anti-Alzheimer’s activity of the compounds, the results showed that compound 4 (IC$_{50}$ = 1.74 µM) inhibited AChE by 83.32 % compared to galantamine with an inhibitory capacity of 90.38 % (IC$_{50}$ = 11.70 µM) (Fig. 6).

Additionally, the results on the inhibition of Aβ aggregation confirmed that compound 4 (IC$_{50}$ = 1.74 µM) has an inhibition percentage of 71.19 % compared to curcumin, which has an inhibition percentage of 71.19 % (IC$_{50}$ = 3.68 µg/mL) (Table 4, Supporting Information).

Regarding the cytotoxicity of the pure compounds, Phenylacetic acid (compound 2, CC$_{50}$ = 9.56–96.15 µM) and 4-[(2-hydroxyimino)-methyl]-N,N-dimethylaniline (compound 1, CC$_{50}$ = 86.63–88.93 µM) had lower cytotoxicity than the compounds (2)-3-(2,5-dimethylphenyl)-amino)-1-phenylprop-2-en-1-one (compound 3, CC$_{50}$ = 71.25–71.65 µM) and 1-(3,4,5-trihydroxyphenyl)-nonan-1-one (compound 4, CC$_{50}$ = 69.41–70.95 µM)

Two of the four compounds showed an inhibitory capacity of NF-κB production higher than celastrol (IC$_{50}$ = 3.68 µg/mL), while IC$_{50}$ values of 1.59–1.93 µM (compound 4) and 2.41–2.79 µM (compound 3), while the other two had a smaller inhibitory capacity than the control, with values of 9.13–9.96 µM (compound 1) and 17.17–17.77 µM (compound 2) (Table 4).

Concerning anti-Alzheimer’s activity of the compounds, the results showed that compound 4 (IC$_{50}$ = 1.74 µM) inhibited AChE by 83.32 % compared to galantamine with an inhibitory capacity of 90.38 % (IC$_{50}$ = 11.70 µM) (Fig. 6).
showed cytotoxic activity, reporting that solasodine isolated from CHCl₃/AcOEt (6:4) showed cytotoxicity in MCF-7 cells, with IC₅₀ values of 24.8, 47.3, and 87.4 µg/mL at 72, 48, and 24 h respectively, whereas, caulophillumine-A (C-A), isolated from the chloroform extract, had values of 35.6 (72 h), 54.1 (48 h), and 88.3 µg/mL (24 h). However, our results show that the IC₅₀ (cytotoxicity) values of the obtained extracts (HEX, DCM, MeOH, and DΗ₂Ο) are in the range of 85.66–96.67 µg/mL at 72 h of treatment in the C8D1A, Neuro-2a and EOC 13.31 cell lines. Analysing the differences in the cytotoxicity values, we can conclude that the composition of the extracts influences their cytotoxic activity, with the extracts rich in alkaloids being the most cytotoxic, followed by those rich in saponins and finally those extracts rich in polyphenolic compounds, which have the lowest cytotoxicity, thus confirming the analysis of our extracts from which polyphenolic compounds were isolated while presenting low cytotoxicity (Figs. 15–55, Supporting Information).

On the other hand, there are no reported studies related to the anti-inflammatory activity of S. mauritianum extracts. However, there are reports on the anti-inflammatory in vivo (murine model) activity of species of the genus Solanum, for example, the MeOH extract of S. nigrum showed activity at a dose of 375 mg/kg b.w. [30], the hydroethanol extract of Solanum lycocarpum exhibited in vivo activity at the dose of 75–150 µg/kg b.w. in the later phase of inflammation [31], and the n-HEX and chloroform extracts of Solanum pubescens showed activity at a dose of 200 mg/kg b.w. [32]. These results suggest that extracts of the species S. mauritianum have anti-inflammatory potential.

Finally, although there are no reports on S. mauritianum and its effect against AD, there are reports on extracts of Solanum gilo, Solanum kumba, and Solanum aethiopicum that modulate the activities of enzymes in purinergic, monoaminergic, and cholinergic systems associated with AD-like symptoms, with an IC₅₀ between 10–15 µg/mL [33]. Reports on the ethanol extract from S. betaceum show a positive effect in the cognitive function of rats fed with doses between 100–400 mg/kg b.w., causing a decrease of levels of the N-methyl-D-aspartate receptor [20]. Moreover, there are reports on the MeOH extract of Solanum virginianum showing memory-enhancing effects on rats fed with doses between 25–100 mg/kg b.w. This effect can be partly attributed to the AChE inhibition, with an IC₅₀ of 386.25 µg/mL [34]. In this sense, we can conclude that the extracts of S. mauritianum have potential against AD as confirmed by previous reports on these related plants.

The four isolated compounds have been described in previous works. In relation to compound 1 (4-[(2S)-(2-hydroxyimino)ethyl]-N,N-dimethylaniline), it has been synthetically obtained from hydroxymine hydrochloride and 4-(dimethylamino)-benzaldehyde [35]. Compound 2 (phenylactic acid) is an intermediate compound of the shikimic acid pathway present in plant species [28, 36]. Regarding compound 3 ((Z)-3-((2,5-dimethylphenyl)-amino)-1-phenylprop-2-en-1-one), it was obtained synthetically from (Z)-3-(dimethylamino)-1-phenylprop-2-en-1-one and 2,5-dimethylaniline [37]. Finally, compound 4(1-(3,4,5-trihydroxyphenyl)-nonan-1-one) is a derivative of gallic acid, previously isolated from the species Rhodiola crenulata [38]. However, this is the first report of the isolation of these compounds in S. mauritianum.

Analysing the results of the tested activities (cytotoxicity, anti-inflammatory, and anti-Alzheimer’s) of the four compounds, we can observe that there is a significant difference in their pharmacological potential due to their lipophilic capacity. This is because the higher the partition coefficient (cLog P), the more lipophilic the compound is and, thus, it is better distributed in hydrophobic environments such as the lipid bilayers that make up cells [39]. Based on this premise, compounds 4 (cLog P = 4.08) and 3 (cLog P = 3.67) exhibit higher lipophilicity compared to compounds 1 (cLog P = 1.91) and 2 (cLog P = 1.72).

Regarding the cytotoxic effects of the isolated compounds, there are no reports of their effects on cell viability. On the other hand, regarding the anti-inflammatory activity, there are only reports on compounds 2, 3, and 4. In the case of compound 2, its anti-inflammatory effects were reported as inhibiting the induction of nitric oxide synthase, which subsequently inhibits the TNF-α and IL-1β inflammatory mediators in astrocyte, microglial, and macrophages cell lines at an IC₅₀ of 5000 µM. However, in our study, the IC₅₀ value obtained for this compound was 280 times less when tested in the C8D1A, Neuro-2a, and EOC 13.31 cell lines. For compound 3, there are reports on its anti-inflammatory effects through the inhibition of CD40-TRAF6 (a protein involved in the NF-κB pathway) in RAW 264.7 cells, with an IC₅₀ of 16 µM [40]. In our study, we report that compound 3 inhibited the production of NF-κB at an IC₅₀ five times less (2.41–2.79 µM) when tested in the C8D1A, Neuro-2a, and EOC 13.31 cell lines. Lastly, although compound 4 has been reported to have no substantial activity as an inhibitor of NF-κB at an IC₅₀ of 1.6 µM in HEK293T (human embryonic kidney) and RAW 264.7 (mouse macrophage) cells [41], our study shows that compound 4 showed an inhibition of NF-κB production in the C8D1A, Neuro-2a and EOC 13.31 cell lines, with IC₅₀ values between 1.59–1.93 µM. Therefore, if we consider that the mechanism of action of celastrol (positive control) is through the suppression of the degradation of IκBα and inhibition of the translocation of p65.
of the nucleus [42], we can conclude that all compounds act on these factors on the NF-κB inhibition pathway.

Lastly, with regard to anti-Alzheimer’s activity, there are no reports on the isolated compounds. In our case, the assay of anti-Aβ42 aggregation activity was performed by the thioflavin T (ThT) method. This assay depends on the measurement of the fluorescence emitted from incubating the mixture of Aβ42 with ThT at various intervals of times. Over time, the intensity of the fluorescence increases in correspondence with the degree of amyloid aggregation in the presence of ThT. The degree at which the fluorescence is quenched in the presence of an anti-Alzheimer’s agent is indicative of the inhibition of the Aβ aggregation and, hence, the anti-Alzheimer’s efficacy.

Although the percentage of AChE and Aβ42 inhibition observed for the isolated compounds is equal to or less than the positive controls (galantamine and curcumin), the concentration at which they have produced such an effect is significantly lower. To determine whether they are more or less effective than the positive controls, the assays should have been performed using the same concentrations. However, this study was not carried out to determine only anti-Alzheimer’s activity, but also to determine anti-inflammatory activity. In this sense, the concentrations that were tested for anti-Alzheimer’s activities were those that showed inhibition of NF-κB.

In our study, the incubation of a mixture of 25 μM of monomeric Aβ42 and the compounds (at a concentration obtained from NF-κB inhibition) revealed that compound 4 has a higher pharmacological potential in comparison to curcumin and the other isolated compounds, due to its binding to amyloid fibrils β42, inhibiting their aggregation [43]. Regarding the inhibition of AChE by the isolated compounds, compound 4 showed a higher activity due to its interaction with amino acid residues defining the active site of AChE via a hydrogen bond, hydrophobic, and n-n interaction of its structure [44]. On the other hand, with respect to compound 1, there is only one report that shows its reactivating activity of AChE (16 %) via a hydrogen bond, hydrophobic, and n-n interaction of its structure [44].

Material and Methods

Plant material

*S. mauritianum* was collected from the Cerro de Pasco community, Pasco province, Peru (10°39’53.0” S 76°15’40.5” W), in July 2019, at an altitude of 4380 m. The botanical identification was confirmed by the International Papa Centre (No. ARV 5643).

Reagents

First grade organic solvents were used for isolating the compounds and they were purchased from Sigma-Aldrich. Column chromatography was performed with silica gel (20–45 μm and 40–63 μm; Merck). TLC was performed using Merck Silica gel 60-F254 plates. Chromatograms thus obtained were visualised by UV absorbance (254 nm) and through heating a plate stained with phosphomolybdic acid (H3PMo12O40) solution in EtOH.

NMR and MS analysis

NMR experiments were performed on Bruker Advance DRX 300 and 500 spectrometers operating at 300, 500 (1H) or 76 MHz, and 126 MHz (13C) with tetramethylsilane (TMS) as the internal standard. Spectra were calibrated by assignment of the residual solvent peak to δH 7.26, δC 3.31, and δH 77.16 for CDCl3 and δC 49.00 for MeOD. The complete assignment of protons and carbons was done by analysing the correlated 1H-1H COSY, HSQC, and HMBC spectra.

HR-ESI-MS (High-resolution electrospray ionisation mass spectrometry) analyses were performed using a mass spectrometer with a hybrid quadrupole time-of-flight analyser (model MAXIX II) from the commercial house Bruker, S.A. Samples were analysed using the electrospray ionisation technique by direct infusion at a flow of 3 µL/min using MeOH with 0.1 % formic acid as the ionising phase. The source parameters were as follows: end plate offset: 500 V; capillary: 3500; nebuliser: 0.2 bar; dry gas: 2.0 L/min; dry temp.: 250 °C; and mass range of 50–3000 Da.

Extraction and isolation

The dry powder of the leaves of *S. mauritianum* (200 g) was extracted by repeated maceration (3 times/24 h/25 °C) with 800 mL of different solvents, increasing the polarity: HEX, DCM, MeOH, and D2O. Subsequently, the extracts were filtered, and the respective solvents were removed by vacuum rotary evaporation at room temperature (25 °C). As a result, four extracts of 2.8, 3, 2.5, and 13 g, respectively, were obtained.

The DCM extract (3 g) was selected as the most active one and was fractionated using a chromatographic column (12 × 60 cm) with Si-60 Silica gel (40–63 μm; Merck) as a stationary phase and a HEX/AcOEt gradient (9:1→1:1 v/v) as the eluent. A total of eight fractions (F1–F8) were obtained, where fractions F2 (120 mg), F4 (213 mg), and F5 (110 mg) showed higher biological activity.

Subsequently, based on the biological activity data, a second separation of F2 was carried out by using a chromatographic column (2 × 50 cm) with Si-60 Silica gel (20–45 μm; Merck) as a stationary phase and HEX/AcOEt (9:1) as the eluent. A total of 12 subfractions (2A–2Δ) were obtained, where fraction F2B showed higher biological activity. Finally, F2B was purified by a microcolumn using silica gel (20–45 μm) as the stationary phase and HEX/AcOEt (3:1) as the mobile phase, obtaining compound 1 (6.8 mg).

Regarding F4, the separation was carried out using a chromatographic column (2 × 50 cm) with Si-60 Silica gel (20–45 μm; Merck) as a stationary phase and HEX/AcOEt (3:1). A total of five subfractions (4A–4E) were obtained, where F4B showed higher biological activity. Finally, F4B was purified by a microcolumn using
silica gel (20–45 μm) as the stationary phase and HEX/AcOEt (3:1) as the mobile phase, obtaining compound 2 (5.3 mg).

Subsequently, the separation of F5 was carried out by using a chromatographic column (2 × 50 cm) with Si-60 Silica gel (20–45 μm; Merck) as a stationary phase and HEX/AcOEt (3:1). A total of four fractions (5A–5E) were obtained. FSC (53 mg) showed higher biological activity. Finally, FSC was purified by a microcolumn using silica gel (20–45 μm) as the stationary phase and HEX/AcOEt (3:1) as the mobile phase, obtaining compounds 3 (6.7 mg) and 4 (4.1 mg).

A summary of the bioguided fractionation process can be observed in Fig. 49S,

**Cell culture reagents and drugs**

Three *Mus musculus* cell lines were used in this study. CB11A (mouse astrocyte, CRL-2541), Neuro-2a (mouse neuroblasts, CCL-131), and EOC 13.31 (mouse microglia, CRL-2468) cells were used as a negative control to evaluate the cytotoxicity of the samples. All cell lines were obtained from the ATCC. Cells were cultured in specific media according to ATCC recommendations. The incubation condition for all cells was in an atmosphere of 95 % air and 5 % CO₂ at 37 °C.

DMEM (Sigma-Aldrich), FBS (Summit Biotechnology), and PBS (SAFC Biosciences, Inc.) were used as culture mediums. L-glutamine was obtained from Applichem. Penicillin and streptomycin were purchased from Fisher Scientific. For cytotoxicity and activity assays, the compounds were dissolved in DMSO (Merck) at a concentration of 10 mM, while extracts and fractions were dissolved at 20 mg/mL in DMSO.

**Cytotoxicity assay**

Cells were seeded in 96-well plates at a density of 5 × 10⁴ cells/well and incubated overnight at 37 °C in a humidified atmosphere of 5 % CO₂. Stock solutions of the samples were prepared by dissolving them in DMSO at a concentration of 20 μg/mL for the fractions and 10 μM for the compounds. Subsequently, from the stock solutions, a series of dilutions were made until a final DMSO concentration of 0.1 % was obtained in each of the wells of the plate for each of the tested concentrations [45]. The tested concentrations were 100, 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, and 0.2 μg/mL (extracts and fractions) or μM (compounds). The reaction mixture contained 150 μL of (100 mM) sodium phosphate buffer (pH = 7.8), 8 mM MgCl₂, 1 mM dithiothreitol, 1 % Triton X-100, and 7 % glycerol, during 15 min at room temperature in a horizontal shaker. Luciferase activity was measured using a GloMax 96 microplate luminometer (Promega) following the instructions of the luciferase assay kit (Promega). The RLU was calculated, and the results are expressed as percentage of inhibition of NF-κB activity induced by TNF-α (100 % activation). The experiments for each concentration of the test elements were performed in triplicate wells.

**Acetylcholinesterase inhibitory activity**

AChE inhibition was determined by the method described by Khan et al. [48]. Samples were solubilised in MeOH and the tested concentrations were 100, 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, and 0.2 μg/mL (extracts and fractions) or μM (compounds). The reaction mixture contained 150 μL of (100 mM) sodium phosphate bufferer (pH = 8.0), 10 μL of 5,5′-dithiobis-(2-nitrobenzoic acid) (≥ 98 % DTNB; Sigma-Aldrich, CAS Number 69–78–3), 10 μL of test sample solution, and 20 μL of AChE solution (Sigma-Aldrich, CAS Number 9000–81–1). This mixture was homogenised and incubated for 15 min at 37 °C followed by the addition of 10 μL of acetylthiocholine iodide (≥ 99 %; Sigma-Aldrich, CAS Number 1866–15–5) to initiate the reaction. Galantamine hydrobromide (≥ 94 %; Sigma-Aldrich, CAS Number 1953–04–4) was used as a positive control at a concentration of 4.31 μg/mL for the extract and fractions, equivalent to 11.70 μM. The assay was based on ATCh hydrolysis by AChE, obtaining 5-thio-2-nitrobenzoate anion, a mixture which turns yellow when forming complexes with DTNB. Finally, absorbance of the samples was recorded with a UV-Vis Shimadzu spectrophotometer at a wavelength of 412 nm (15 min).

**Assay of β-amyloid aggregation inhibitory activity**

The Aβ aggregation was assessed using the ThT method described by Abouelela et al. [49]. Briefly, 25 μM Aβ42 (Peptide Institute, Inc.) were used; for the samples, the concentrations were the same as in the *in vitro* AChE assay, using a 50 mM sodium phosphate buffer, pH = 7.5, 100 mM NaCl, 1 % DMSO (v/v). The total fluid volume was 25 μL. Curcumin (≥ 94 %; Sigma-Aldrich, CAS Number 458–37–7) was used as a positive control at a concentration of 3.68 μg/mL for the extract and fractions, equivalent to 10 μM. Reactions were incubated at 37 °C for 72 h. Aliquots were diluted fourfold into 5 μM ThT

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and immediately evaluated for fluorescence (excitation = 445 nm, emission = 490 nm) to monitor the amount of Aβ aggregate.

**Statistical analysis**

Cytotoxic concentration 50 % (CC50) and inhibitory concentration 50 % (IC50) values were determined by nonlinear regression. All experiments were performed in triplicate. One-way ANOVA statistical analysis (Tukey’s multiple comparisons test, * p < 0.05; ** p < 0.001) was performed to evaluate the significant differences among values. All analyses were performed using GraphPad Prism, version 9.0.0.

**Supporting information**

1H NMR, 13C NMR, 1H-1H COSY, HSQC, HMBC, and MS spectra for extracts and isolated compounds are available as Supporting Information.

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**Conflict of Interest**

The authors declare they have no conflict of interest.

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