Evaluation of Anti-Alzheimer Activity of Ampelopsis brevipedunculata and the Isolated Compounds

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ABSTRACT

Medicinal plants are main target for discovery of new drugs for treatment of many diseases. This study evaluated anti-Alzheimer activity of methanol (80%) extract of Ampelopsis brevipedunculata aerial parts and the isolated compounds. Anti-Alzheimer activity for (1) 5, 7-dihydroxy flavanone (pinocembrin), (2) 5, 7-dihydroxy-3, 6, 4'-trimethoxy flavone (santin), (3) Acacetin (4'-methoxy apigenin), (4) Acacetin 7-O-a-rhamnoside, (5) Acacetin 7-O-β-glucoside, (6) Luteolin 7-O-a-rhamnoside, (7) Apigenin 7-O-β-glucoside, (8) Apigenin 7-O-β-glucuronide and A. brevipedunculata extract was detected according to Ellman’s method. The results showed that A. brevipedunculata extract and the compounds 2, 6 and 8 were active as anti-Alzheimer agents. The extract showed IC$_{50}$ = 0.62 μg mL$^{-1}$ while IC$_{50}$ = 0.44 μg mL$^{-1}$, IC$_{50}$ = 0.32 μg mL$^{-1}$ and IC$_{50}$ = 0.56 μg mL$^{-1}$ for the compounds 2, 6 and 8, respectively, neostigmine (IC$_{50}$ = 1.87 μg mL$^{-1}$) was used as positive control. These results showed that A. brevipedunculata extract and the isolated compounds can be promising agents for treatment of Alzheimer’s disease in the future.

Key words: Ampelopsis brevipedunculata, aerial parts, Alzheimer’s disease, flavonoids

INTRODUCTION

Alzheimer’s Disease (AD) is a neurodegenerative disorder that affects memory, behavior and ability to work. It has become the fourth leading cause of death in the elderly population (over 65 years of age) as a result of different biochemical pathways (Racchi et al., 2004). The therapy of early and moderate stages of AD is mainly based on acetylcholine esterase inhibitors such as synthetic donepezil and galantamine. However, these licensed medicines have drawbacks of inducing severe peripheral and central side effects, including gastrointestinal disturbances, insomnia, fatigue or depression (Cummings, 2004). The serious side effects caused by licensed drugs used to treat AD have forced researchers to investigate safer AChE inhibitors from natural sources. One of the best sources of new substances to treat AD is natural products and their derivatives. Traditionally, plants have been used to enhance memory and to alleviate other symptoms associated with AD (Chattipakorn et al., 2007). Plants are considered as good sources for discovering new drugs and traditional medicine is one of the best ways to introduce effective plants. Ampelopsis brevipedunculata is an ornamental plant from Vitaceae family and it is native to temperate areas of Asia (China and Japan) (Huxley et al., 1992). It has flowers from July to
August and the seeds ripen from October to November. The fresh fruits, roots and leaves are antiphlogistic, depurative and febrifuge. It is used externally in the treatment of boils, abscesses and ulcers, traumatic bruises and aches (Huxley et al., 1992). Very few reports about phytococonstituents of the plant (Xu et al., 1995; Yoshiteru et al., 1990) and no reports about biological activities from this plant. In this current study, we evaluated for the first time anti-Alzheimer activity of *A. brevipedunculata* aerial parts methanol (80%) extract and also the isolated compounds.

**MATERIALS AND METHODS**

**General experimental procedures:** UV/Vis, Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt) was used. 1H-NMR and 13C-NMR (Varian Unity Inova); (Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals); Thin Layer Chromatography (TLC) F254 plates; Paper Chromatography (PC) Whatman No. 1 (Whatman Led. Maid Stone, Kent, England) sheets for qualitative detection of flavonoids and sugars were used in this study. Solvent mixtures were; BAW (n-butanol: Acetic acid: Water 4:1:5 upper phase) and 15% acetic acid.

**Plant identification and collection:** *Ampelopsis brevipedunculata* aerial parts were collected from Al-Zohiriya garden, Giza, Egypt in May 2012. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeeza Labib consultant of plant taxonomy at the ministry of agriculture and director of Orman Botanical Garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

**Plant extract preparation:** Air dried powdered of *A. brevipedunculata* aerial parts (1.2 kg) was extracted with methanol (80%) (methanol: distilled water, 80:20, v/v) several times at room temperature by maceration method. The extract was concentrated under reduced pressure to give 41 g. The extract was phytochemically screened according to methods described by Yadav and Agarwala (2011).

**Isolation of the bioactive compounds from methanol (80%) extract of *A. brevipedunculata***: *Ampelopsis brevipedunculata* aerial parts methanol (80%) extract (40 g) was subjected to silica gel column chromatography eluting with n-hexane, dichloromethane, ethyl acetate and methanol gradually. One hundred and fifty fractions of 100 mL conical flask were collected. The fractions that showed similar Paper Chromatography (PC) in two solvent systems butanol-acetic acid-water (4:1:5) and 15% acetic acid were combined to give 4 fractions (I, II, III and IV):

- Fraction I (6.25 g) was subjected to sub-column of silica gel eluted with n-hexane: dichloromethane (1:1) gave compound 1, further elution with dichloromethane gave compounds 2 and 3
- Fraction II (4.38 mg) was subjected to sub-column of silica gel eluted with dichloromethane: ethyl acetate (1:1) yielded compound 4 and further elution with ethyl acetate: dichloromethane (4:1) gave compound 5
- Fraction III (3.85 g) was subjected to sub-column of silica gel eluted with ethyl acetate: methanol (95: 5) afforded compound 6
Compounds 7 and 8 were obtained from fraction IV (2.95 g) by elution with ethyl acetate: methanol (90:10).

All the isolated compounds were purified on sephadex LH-20 column using different systems of methanol and distilled water.

**General method for acid hydrolysis of flavonoid glycosides:** Five milligrams of each flavonoid glycoside 4, 5, 6, 7 and 8 in 5 mL 10% HCl was heated for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-Paper Chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (n-BuOH-AcOH-H$_2$O, 4:1:5, upper layer).

**Acetylcholinesterase inhibition assay:** The methanol (80%) extract of *A. brevipedunculata* was dissolved in methanol to prepare solution of 10 mg mL$^{-1}$. Then, 1.5 μL of the extract was spotted on silica gel TLC plate and developed with chloroform: methanol (9:1) after which the enzyme inhibitory activity was detected using Ellman’s method “in situ” on the plate (Ellman et al., 1961; Rhee et al., 2001). The developed plate was sprayed with 1 mM of Ellman’s reagent, 5, 5'-ditiobis [2-nitrobenzoic acid] (DTNB) and 1 mM of acetylthiocholine iodide (ATCI) in buffer A. It dried for 3-5 min, then an enzyme solution of AChE from an electric eel (type VI-s lyophilized, 261 U mg$^{-1}$ solid, 386 U mg$^{-1}$ protein) dissolved in buffer A (500 U mL$^{-1}$ stock solution) was diluted with buffer A to obtain 5 U mL$^{-1}$ enzyme and was then sprayed on the plate (Rhee et al., 2001). Yellow background with white spot for inhibiting extract was visible after about 5 min (Fig. 1). This observation must be recorded within 15 min because they fade after 20-30 min. To observe whether the positive results of the extract in TLC or the microplate assay are due to enzyme inhibition or to the inhibition of the chemical reaction between DTNB and thiocholine (the product of the enzyme reaction), 5 U mL$^{-1}$ of AChE was premixed with 1 mM ATCI in buffer A and incubated for 15 min at 37°C. This enzyme-substrate mixture was used as thiocholine spray (Rhee et al., 2001). The extract was spotted on the silica gel TLC plate developed as described above and sprayed with 1 mM solution DTNB followed by the thiocholine spray. White spot on a yellow background was observed for false positive extract. The inhibitory quantitative effect of the extract on acetylcholinesterase activity was evaluated using and adaptation of the spectrophotometric method of Ellman et al. (1961) modified by Rhee et al. (2001). Five different concentrations were prepared in triplicate, starting from *A. brevipedunculata* methanol extract (1, 0.5, 0.25, 0.125 and 0.0625 mg mL$^{-1}$). The reaction was monitored at 412 nm for 5 min in spectrophotometer. In test tube is placed 100 μL of sample (concentration 0.1% solution in 50 mM Tris-HCl pH 8 and methanol 10%) was mixed with 100 μL of AChE 0.22 U mL$^{-1}$ (22 U of enzyme diluted in 100 mL of 50 mM Tris-HCl pH 8, 0.1% BSA) and 200 μL of buffer (50 mM Tris-HCl, pH 8, BSA 0.1%). Incubated the mixture for 5 min at 30°C and subsequently added 500 μL of DTNB (concentration of the 3 mM in Tris-HCl pH 8, 0.1 M NaCl, 0.02 M MgCl$_2$) and 100 μL of ATCI (4 mM in water).

A blank should also be prepared by substituting AChE with 100 μL of buffer (50 mM Tris-HCl buffer pH 8, 0.1% BSA). The reaction is monitored for 5 min at 412 nm and initial velocity (Vo) recorded. Anticholinesterase activity (%) was calculated:

$$I (%) = \frac{1-Vo\text{ sample}}{Vo\text{ white}} \times 100$$
where, Vo and Vo represent the initial rates blank samples and white, respectively. Inhibition concentration 50% (IC₅₀) values so obtained by plotting Log-Probit. Neostigmine (commercial acetylcholinesterase inhibitor) is used as positive control at the same concentration of the extract.

RESULTS

The present study evaluated anti-Alzheimer activity of *A. brevipedunculata* aerial parts methanol (80%) extract and also the isolated compounds. The results showed that *A. brevipedunculata* extract and the compounds 5, 7-dihydroxy-3, 6, 4’-trimethoxy flavone (Santin), luteolin 7-O-α-rhamnoside and apigenin 7-O-β-glucuronide were active as anti-Alzheimer agents, the extract showed IC₅₀ = 0.62 μg mL⁻¹ while IC₅₀ = 0.44 μg mL⁻¹, IC₅₀ = 0.32 μg mL⁻¹ and IC₅₀ = 0.56 μg mL⁻¹ for these compounds, respectively neostigmine (IC₅₀ = 1.87 μg mL⁻¹) was used as positive control.

Phytochemical investigation of the plant extract revealed the presence of triterpenes, flavonoids, tannins and carbohydrates (Table 1) and further phytochemical analysis demonstrated the identification of 5, 7-dihydroxy flavanone (pinocembrin) (1), 5, 7-dihydroxy-3, 6, 4’-trimethoxy flavone (santin) (2), acacetin (4-methoxy apigenin) (3), acacetin 7-O-α-rhamnoside (4), acacetin 7-O-β-glucoside (5), luteolin 7-O-α-rhamnoside (6), apigenin 7-O-β-glucoside (7), apigenin 7-O-β-glucuronide (8) as shown in Fig. 2.

Table 1: Phytochemical analysis of methanol (80%) extract of *A. brevipedunculata* aerial parts

| Chemical constituents | Methanol (80%) extract |
|-----------------------|------------------------|
| Carbohydrates and/or glycosides | + |
| Tannins | |
| Condensed tannins | + |
| Hydrolysable tannins | + |
| Alkaloids and/or nitrogenous bases | - |
| Flavonoids | + |
| Sterols and triterpenes | + |
| Saponins | - |
| Coumarins | - |

(+) Presence of constituents, (-) Absence of constituents

Fig. 1(a-b): Inhibition of acetylcholinesterase compounds 2, 6 and 8; 10 = methanol (80%) extract of *Ampelopsis brevipedunculata* aerial parts and p = caffeine
DISCUSSION

Structure elucidation of the isolated compounds

The 5, 7-dihydroxy flavanone (Pinocembrin) (1): Twenty two milligram, white needles. UV \( \lambda_{max} \) (nm): (MeOH): 230sh, 292, 338sh; (NaOMe): 248, 330; (AlCl\(_3\)): 314, 378; (AlCl\(_3\)/HCl): 312, 378; (NaOAc): 234, 330; (NaOAc/H\(_3\)BO\(_3\)): 237, 295, 338sh. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \( \delta \) ppm 12.18 (1H, s, 5-OH), 7.54-7.36 (5H, m, H-2', 3', 4', 5', 6'), 5.94 (1H, d, \( J = 2 \) Hz, H-8), 5.92 (1H, d, \( J = 2 \) Hz, H-6), 5.58 (d, \( J = 1.2 \) Hz, H-2), 3.26 (1H, dd, \( J = 12.4, 16.6 \) Hz, H-3\( \alpha \)), 2.76 (1H, d, \( J = 16.9 \) Hz, H-3\( \beta \)). \(^1\)C-NMR (DMSO-d\(_6\), 100 MHz): \( \delta \) ppm 195.78 (C-4), 166.74 (C-7), 163.36 (C-5), 162.56 (C-9), 138.58 (C-1'), 128.54 (C-3', 4', 5'), 126.50 (C-2', 6'), 101.78 (C-10), 95.96 (C-6), 95.24 (C-8), 78.38 (C-2), 42.15 (C-3). It was isolated as a deep purple spot under UV light and on exposure to ammonia or spraying with AlCl\(_3\) reagent, respectively it gave a florescent yellow colour and its spectral signals proved the structure of 5, 7-dihydroxy flavanone.

The 5, 7-dihydroxy-3, 6, 4'-trimethoxy flavone (Santin) (2): Eighteen milligram, yellow powder. UV \( \lambda_{max} \) (nm): (MeOH): 274, 345; (NaOMe): 279, 298, 372; (AlCl\(_3\)): 282, 307sh, 362, 410; (AlCl\(_3\)/HCl): 284, 307sh, 356, 407sh; (NaOAc): 276, 298sh, 368; (NaOAc/H\(_3\)BO\(_3\)): 275, 344. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \( \delta \) ppm 12.70 (1H, s, 5-OH), 7.95 (2H, d, \( J = 8.9 \) Hz, H-2', 6'), 7.06 (2H, d, \( J = 8.9 \) Hz, H-3', 5'), 6.48 (1H, s, H-8), 3.82 (3H, s, OCH\(_3\)-6), 3.78 (3H, s, OCH\(_3\)-3), 3.74 (3H, s, OCH\(_3\)-4'). \(^1\)C-NMR (DMSO-d\(_6\), 100 MHz): \( \delta \) ppm 178.18 (C-4), 166.74 (C-7), 163.36 (C-5), 162.56 (C-9), 138.58 (C-1'), 128.54 (C-3', 4', 5'), 126.50 (C-2', 6'), 101.78 (C-10), 95.96 (C-6), 95.24 (C-8), 78.38 (C-2), 42.15 (C-3). It was isolated as a deep purple spot under UV light and on exposure to ammonia or spraying with AlCl\(_3\) reagent, respectively it gave a florescent yellow colour and its spectral signals proved the structure of 5, 7-dihydroxy flavanone.

Acacetin (4'-methoxy apigenin) (3): Sixteen milligram, yellow powder. UV \( \lambda_{max} \) (nm): (MeOH): 269, 328; (NaOMe): 276, 365; (AlCl\(_3\)): 278, 302, 345, 382; (AlCl\(_3\)/HCl): 278, 302, 342, 380; (NaOAc): 20
Acacetin 7-O-α-rhamnoside (4): Fifteen milligram, yellow amorphous powder. ¹H-NMR (DMSO-d₆, 300 MHz): δ ppm 7.82 (2H, d, J = 8.2 Hz, H₂', H-6'); 6.74 (2H, d, J = 8.2 Hz, H-3', H-5'); 6.54 (1H, s, H-3), 6.46 (1H, d, J = 2.5 Hz, H-8); δ 6.42 (1H, d, J = 2.5 Hz, H-6); 5.32 (1H, d, J = 2.5 Hz, H-1''), 4.12 (3H, s, OCH₃), 3.94-3.22 (Rhamnose sugar protons), 1.28 (3H, d, J = 6.2 Hz, CH₃ of rhamnose). ¹³C-NMR (DMSO-d₆, 100 MHz): δ ppm 176.28 (C-4), 160.74 (C-2), 160.64 (C-4'), 160.38 (C-7), 159.48 (C-9), 127.62 (C-6'), 127.16 (C-2'), 121.28 (C-1'), 115.82 (C-3'), 115.64 (C-5'), 108.14 (C-3), 105.46 (C-10), 99.86 (C-1''), 97.86 (C-6), 96.78 (C-8), 77.38 (C-3''), 76.26 (C-5''), 73.14 (C-2''), 69.56 (C-4''), 69.52 (C-6''), 56.54 (OCH₃). It gave a deep purple colour under UV light. With ammonia or spraying with AlCl₃ reagent, respectively it afforded florescent yellow colour. Acid hydrolysis afforded acacetin as an aglycone and rhamnose as sugar moiety. Spectral data of compound 4 was in agreement with those described by Joshi and Verma (2012).

Acacetin 7-O-β-glucoside (5): Twelve milligram, yellow amorphous powder. ¹H-NMR (DMSO-d₆, 400 MHz): δ ppm 12.94 (1H, s, 5-OH), 8.09 (2H, d, J = 9.2 Hz, H-2', H-6'); 7.16 (2H, d, J = 9.2 Hz, H-3', H-5'); 6.98 (1H, s, H-3), 6.88 (1H, d, J = 2.4 Hz, H-8), 6.48 (1H, d, J = 2.4 Hz, H-6), 5.09 (1H, d, J = 7.8 Hz, H-1''), 3.87 (3H, s, OCH₃), 4.12-3.28 (rest of sugar protons). ¹³C-NMR (DMSO-d₆, 100 MHz): δ ppm 181.84 (C-4), 163.74 (C-2), 163.12 (C-7), 161.78 (C-4), 161.12 (C-5), 156.82 (C-9), 128.46 (C-2'), 128.42 (C-6'), 122.68 (C-1'), 114.64 (C-3'), 114.64 (C-5'), 105.38 (C-3), 103.88 (C-5'), 99.84 (C-1''), 99.54 (C-6), 94.86 (C-8), 77.18 (C-3''), 76.32 (C-5''), 72.94 (C-4''), 69.58 (C-6''), 56.54 (OCH₃). It gave a deep purple colour under UV light. With ammonia or spraying with AlCl₃ reagent, respectively it afforded florescent yellow colour. Acid hydrolysis afforded acacetin as an aglycone and glucose as sugar moiety. Spectral data of compound 5 was in agreement with those described by Li et al. (2008).

Luteolin 7-O-α-rhamnoside (6): Eighteen milligram, yellow amorphous powder. UV λ max (nm): (MeOH): 256, 268sh, 349; (NaOMe): 264, 301sh, 395; (AlCl₃): 275, 300sh, 330, 433; (AlCl₃/HC1): 274, 294sh, 358, 388; (NaOAc): 260, 268sh, 366sh, 406; (NaOAc/H₃BO₃): 260, 374. ¹H-NMR (DMSO-d₆, 300 MHz): δ ppm 7.38 (1H, dd, J = 2.1, 7.8, H-6'); 7.35 (1H, d, J = 2.1, H-2'), 7.35 (1H, d, J = 2.1, H-2'), 6.86 (1H, d, J = 7.8, H-5'), 6.72 (1H, s, H-3), 6.68 (1H, d, J = 2.1, H-8), 6.45 (1H, d, J = 2.1, H-6), 5.08 (1H, d, J = 7.9, H-1''), 4.24-3.12 (rest of sugar protons). It gave a deep purple spot under UV light. With ammonia or spraying with AlCl₃ reagent, respectively it gave florescent yellow colour. Acid hydrolysis afforded luteolin as an aglycone and rhamnose as sugar moiety. Spectral data of compound 6 was in agreement with those described by Li et al. (2008).

Apigenin 7-O-β-glucoside (7): Nineteen milligram, yellow powder. UV λ max (nm): (MeOH): 270, 335; (NaOMe): 276, 386; (AlCl₃): 281, 345, 360; (AlCl₃/HC1): 281, 342; (NaOAc): 269, 392;
(NaOAc/H$_3$BO$_3$): 267, 352. $^1$H-NMR (DMSO-d$_6$, 400 MHz): $\delta$ ppm 7.92 (2H, d, $J = 8.9$ Hz, H-2, H-6), 6.96 (2H, d, $J = 8.9$ Hz, H-3, H-5), 6.82 (1H, s, H-3), 6.64 (1H, d, $J = 2.2$ Hz, H-8), 6.54 (1H, d, $J = 2.2$ Hz, H-6). 5.22 (1H, d, $J = 7.2$ Hz, H-1", glucose), 4.12-3.18 (remaining sugar protons). $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): $\delta$ ppm 182.48 (C-4), 164.72 (C-2), 162.86 (C-7), 161.96 (C-5), 161.38 (C-4), 157.45 (C-9), 128.86 (C-2, C-6), 121.28 (C-1), 116.42 (C-3, C-5), 105.78 (C-10), 104.78 (C-8), 103.45 (C-3), 100.02 (C-1", glucose), 95.18 (C-6), 76.18 (C-5"), 75.24 (C-3"), 73.98 (C-2"), 71.18 (C-4"), 66.74 (C-6"). It gave a deep purple spot under UV light. On exposure to ammonia or spraying with AlCl$_3$ reagent, respectively it afforded fluorescent yellow colour. Acid hydrolysis afforded apigenin as an aglycone and glucose as sugar moiety. Spectral data of compound 7 was in agreement with those described by Mabry et al. (1970).

Apigenin 7-O-β-glucuronide (8): Twenty two milligram, yellow powder. UV $\lambda_{max}$ (nm): (MeOH): 269, 333; (NaOMe): 275, 379; (AlCl$_3$): 266sh, 275, 399, 376sh; (AlCl$_3$/HCl): 266sh, 275, 299, 342, 374sh; (NaOAc): 255sh, 269, 390; (NaOAc/H$_3$BO$_3$): 268, 339. $^1$H-NMR (DMSO-d$_6$, 400 MHz): $\delta$ ppm 7.92 (2H, d, $J = 8$ Hz, H-2, H-6), 6.94 (2H, d, $J = 8$ Hz, H-3, H-5), 6.82 (1H, s, H-3), 6.78 (1H, d, $J = 1.6$ Hz, H-8), 6.54 (1H, d, $J = 1.6$ Hz, H-6); 5.28 (1H, d, $J = 8$ Hz, H-1"), 4.22-3.25 (remaining sugar protons). $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): $\delta$ ppm 182.42 (C-4), 171.82 (C-6", glucuronide), 164.75 (C-2), 163.18 (C-7), 162.28 (C-5), 157.35 (C-9), 128.94 (C-2, C-6, 121.18 (C-1), 116.44 (C-3, C-5), 105.78 (C-10), 103.28 (C-3), 100.35 (C-1", glucuronide), 99.78 (C-6), 95.18 (C-8), 76.32 (C-5"), 73.26 (C-3"), 72.18 (C-2"). It gave a deep purple spot under UV light. On exposure to ammonia or spraying with AlCl$_3$ reagent, respectively it afforded florescent greenish yellow colour. Acid hydrolysis afforded apigenin as an aglycone and glucuronic acid as sugar moiety. Spectral data of compound 8 was in agreement with those described by Moussaoui et al. (2010).

Anti-Alzheimer activity of Ampelopsis brevipedunculata methanol (80%) extract and the isolated compounds: Ampelopsis brevipedunculata extract inhibited a progression of carbon tetrachloride-induced hepatic injury in the mice (Yabe and Matsui, 2000). The synthetic drug tacrine (Cognex) was the first AChEI to be licensed but its routine use has been largely restricted due to its hepatotoxicity (Watkins et al., 1994). Thus, the plants that have demonstrated hepatoprotective activity are relevant in terms of searching for novel formulations or compounds for AD treatment.

The qualitative results of inhibition of enzyme acetylcholinesterase in Thin Layer Chromatography (TLC) showed that methanol (80%) extract of A. brevipedunculata inhibited the enzyme by the appearance yellow backgrounds with white spots for inhibiting compounds were visible after about 5 min. These are the results of the first tests, yellow backgrounds with white spots for inhibiting compounds and for A. brevipedunculata extract were visible after about 5 min apparently tested positive enzyme inhibition in concentration of 10 mg mL$^{-1}$ (Fig. 1). The results of acetylcholinesterase inhibition quantitative for the methanol extract of A. brevipedunculata (IC$_{50}$ = 0.62 μg mL$^{-1}$) that presented strong activity in both tests, the IC$_{50}$ values were determined (IC$_{50}$ = 0.44 μg mL$^{-1}$; IC$_{50}$ = 0.32 μg mL$^{-1}$ and IC$_{50}$ = 0.56 μg mL$^{-1}$ for 2, 6 and 8, respectively. Neostigmine (IC$_{50}$ = 1.87 μg mL$^{-1}$) was used as positive control. The concentration of inhibition 50% (CI$_{50}$) compounds was tested starting at five different concentrations (1.0, 0.5, 0.25, 0.125, 0.0625 mg mL$^{-1}$) tested in triplicate, shows the specie that showed higher inhibition activity for compounds, in comparison to commonly used drug neostigmine (IC$_{50}$ = 1.87 μg mL$^{-1}$).
Acetylcholinesterase inhibitors are successfully used to treat the symptoms of Alzheimer’s disease (Feitosa et al., 2011). Other studies indicated flavonoids inhibitors of acetylcholinesterase, the ethyl acetate extract of *Kalanchoe brasiliensis* Camb. (Crassulaceae) leaves was studied and showed inhibition of enzyme associated Alzheimer’s disease, acetylcholinesterase (AChE). Microplate assays, presented with 100% of inhibition, to a concentration of 2 mg mL$^{-1}$ and in the Thin Layer Chromatography (TLC), showed a white spot in the yellow field in the plate, confirmed positive result of the inhibition, it was based from the assay described by Ellman modified by Rhee. The bioassay guided fractionation of extracts of the most active extracts (*K. brasiliensis*) which resulted in the isolation of an active mixture of three flavonoids: 8-methoxyquercetin, 3, 7-di-O-rhamnopyranoside and 8-methoxykaempferol-3, 7-di-O-rhamnopyranoside (Trevisan et al., 2006). The alcoholic extract of the fresh leaves of *K. brasiliensis* showed inhibitory property of cholinesterase with rectus abdominis isolated frogs in experiments by Fonteles et al. (1982). In such experiments, the inhibitory effect of d-tubocurarine in the contractile responses induced by acetylcholine was effectively blocked by the extract, the same occurring with prostigmine, a well-known anticholinesterase. So the anti-Alzheimer action of these flavonoid glycosides were confirmed both in vivo and in vitro.

**CONCLUSION**

The natural compounds tested herein, especially luteolin 7-O-α-rhamnoside could be a good candidate as lead molecule for development of new drugs against anti-Alzheimer action.

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