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

[1]Khaled Rashed, [1]Ataa Said, [2]Chistiane Feitosa and [2]Ana Carolina Cardoso Sucupira

[1]Department of Pharmacognosy, National Research Centre, 33 El-Bohouth st. Dokki, P.O. Box 12622, Giza, Egypt

[2]Department of Chemistry, Laboratory of Natural Products, Federal University of Piaui, Ininga, 64049-550, Teresina-Pi, Brazil

Corresponding Author: Khaled Nabih Zaki Rashed, Department of Pharmacognosy, National Research Centre, 33 El-Bohouth st. Dokki, P.O. Box 12622, Giza, Egypt Tel: 01003642233

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-Alzhemier 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-α-rhamnoside, (5) Acacetin 7-O-β-glucoside, (6) Luteolin 7-O-α-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 galanthamine. 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 phytoconstituents 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. ^{1}H-NMR and ^{13}C-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) F_{254} 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. Tereeza 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:

$$I\,(\%) = \frac{1\text{-Vo sample}}{\text{Vo white}} \times 100$$

where, Vo and Vo represent the initial rates blank samples and white, respectively. Inhibition concentration 50% (IC_{50}) 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 $IC_{50} = 0.62$ 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 these compounds, respectively neostigmine ($IC_{50} = 1.87$ 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/or triterpenes	+
Saponins	-
Coumarins	-

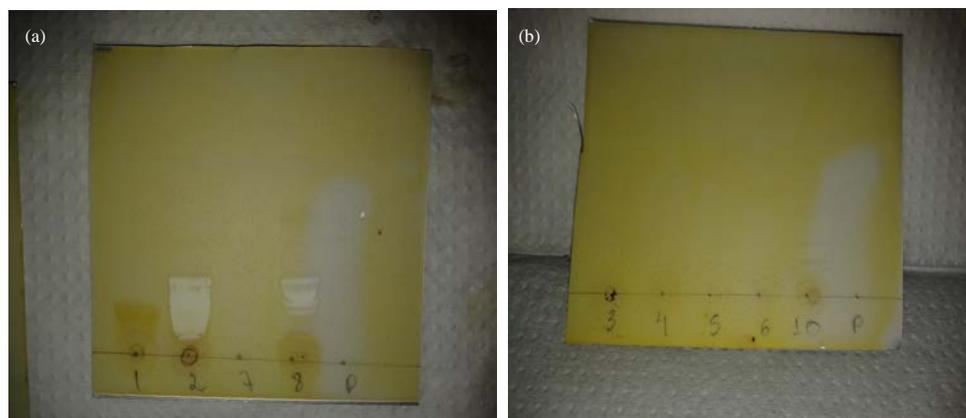(+) Presence of constituents, (-) Absence of constituents

Fig. 1(a-b): Inhibition of acetylcholinesterase compounds 2, 6 and 8; 10 = methanol (80%) extract of *Ampelopsis brevipedunculata* aerial parts and p = caffeine
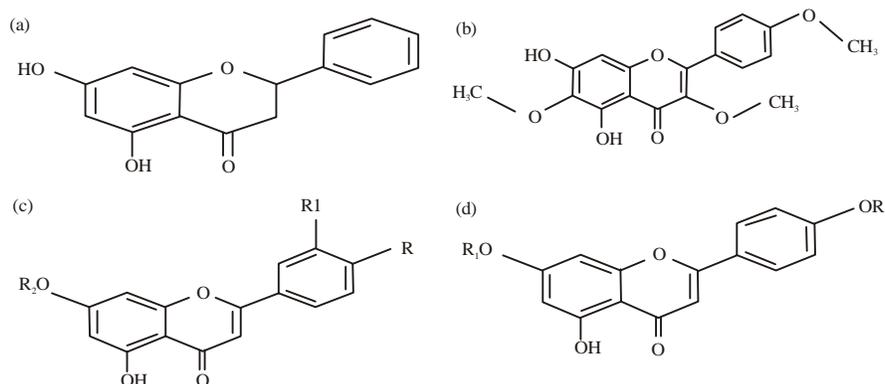
Fig. 2(a-d): Chemical structures of the compounds isolated from *A. brevipedunculata* methanol
(80%) extract, (a) 1: Pinocembrin, (b) 2: Santin, (c) 3: Acacetin (4'-methoxy apigenin),
(R = OCH₃, R1 = R2 = H) 4: Acacetin 7-O-α-rhamnoside (4), (R = OCH₃, R1 = H,
R2 = Rhamnose) 5: Acacetin 7-O-β-glucoside, (R = OCH₃, R1 = H, R2 = Glucose)
6: Luteolin 7-O-α-rhamnoside (6), (R = R1 = OH, R2 = Rhamnose) (d) 7: Apigenin
7-O-β-glucoside, (R = H, R1 = Glucose) 8: Apigenin 7-O-β-glucuronide (R = H,
R1 = Glucuronic acid)

DISCUSSION

Structure elucidation of the isolated compounds

The 5, 7-dihydroxy flavanone (Pinocembrin) (1): Twenty two milligram, white needles. UV
λmax (nm): (MeOH): 230sh, 292, 338sh; (NaOMe): 248, 330; (AlCl₃): 314, 378; (AlCl₃/HCl): 312, 378;
(NaOAc): 234, 330; (NaOAc/H₃BO₃): 237, 295, 338sh. ^{1}H-NMR (DMSO-d₆, 300 MHz): δ 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 2.4 Hz, H-2), 3.26 (1H, dd, J = 12.4, 16.6 Hz, H-3α), 2.76 (1H, d, J = 16.9 Hz,
H-3β). ^{13}C-NMR (DMSO-d₆, 100 MHz): δ 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 AlCl₃ 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 λmax (nm): (MeOH): 274, 345; (NaOMe): 279, 298, 372; (AlCl₃): 282, 307sh, 362, 410;
(AlCl₃/HCl): 284, 307sh, 356, 407sh; (NaOAc): 276, 298sh, 368; (NaOAc/H₃BO₃): 275, 344. ^{1}H-NMR
(DMSO-d₆,300 MHz): δ 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, OCH₃-6), 3.78 (3H, s, OCH₃-3), 3.74
(3H, s, OCH₃-4'). ^{13}C-NMR (DMSO-d₆, 100 MHz): δ ppm 178.18 (C-4), 161.18 (C-4'), 157.16 (C-7),
155.24 (C-2), 152.28 (C-5), 151.62 (C-9), 136.88 (C-3), 131.28 (C-6), 129.46 (C-2', 6'), 122.28 (C-1'),
114.18 (C-3', 5'), 104.66 (C-10), 93.94 (C-8), 59.88 (OCH₃-6), 59.64 (OCH₃-3), 55.38 (OCH₃-4'). It gave
a deep purple colour under UV light. On exposure to ammonia or spraying with AlCl₃ reagent,
respectively it afforded florescent yellow colour. Its spectral data was in agreement with
5, 7-dihydroxy-3, 6, 4'-trimethoxy flavone.

Acacetin (4'-methoxy apigenin) (3): Sixteen milligram, yellow powder. UVλmax (nm): (MeOH):
269, 328; (NaOMe): 276, 365; (AlCl₃): 278, 302, 345, 382; (AlCl₃/HCl): 278, 302, 342, 380; (NaOAc):

275, 358; (NaOAc/H_3BO_3): 270, 332. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ ppm 12.84 (1H, s, 5-OH); 8.05 (2H, d, J = 9.1, H-2', 6'), 7.12 (2H, d, J = 9.1, H-3', 5'), 6.86 (1H, d, J = 2.6, H-8), 6.76 (1H, s, H-3), 6.48 (1H, d, J = 2.6, H-6), 3.86 (3H, s, 4'-OCH$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 181.78 (C-4), 163.82 (C-7), 162.88 (C-2), 162.36 (C-5), 161.24 (C-4'), 156.84 (C-9), 128.26 (C-2', 6'), 122.68 (C-1'), 114.52 (C-3', 5'), 105.36 (C-10), 103.74 (C-3), 100.02 (C-6), 94.78 (C-8). It gave a purple colour under UV light. With ammonia or spraying with AlCl$_3$ reagent, respectively it afforded florescent yellow colour. Spectral data of compound 3 was in agreement with those described by Rodriguez *et al*. (1972).

Acacetin 7-O-α-rhamnoside (4): Fifteen milligram, yellow amorphous powder. ^{1}H-NMR (DMSO-d_6, 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, OCH$_3$), 3.94-3.22 (Rhamnose sugar protons), 1.28 (3H, d, J = 6.2 Hz, CH$_3$ of rhamnose). ^{13}C-NMR (DMSO-D_6, 100 MHz): δ ppm 176.28 (C-4), 160.74 (C-2), 160.64 (C-4'), 160.38 (C-7), 160.16 (C-5), 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 (OCH$_3$). It gave a deep purple colour under UV light. With ammonia or spraying with AlCl$_3$ 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. ^{1}H-NMR (DMSO-d_6, 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, 4-OCH$_3$), 4.12-3.28 (rest of sugar protons). ^{13}C-NMR (DMSO-d_6, 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 (H-6'), 122.68 (C-1), 114.64 (C-3 , C-5'), 105.38 (C-10), 103.88 (C-3), 99.84 (C-1"), 99.54 (C-6), 94.86 (C-8), 77.18 (C-3"), 76.32 (C-5"), 72.94 (C-2"), 69.58 (C-4"), 60.54 (C-6"), 56.12 (4-OCH$_3$). It gave a deep purple colour under UV light. With ammonia or spraying with AlCl$_3$ 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 λmax (nm): (MeOH): 256, 268sh, 349; (NaOMe): 264, 301sh, 395; (AlCl$_3$): 275, 300sh, 330, 433; (AlCl$_3$/HCl): 274, 294sh, 358, 388; (NaOAc): 260, 268sh, 366sh, 406; (NaOAc/H_3BO_3): 260, 374. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ ppm 7.38 (1H, dd, J = 2.1, 7.8, H-6'), 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 AlCl$_3$ 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 Babaei *et al*. (2008).

Apigenin 7-O-β-glucoside (7): Nineteen milligram, yellow powder. UV λmax (nm): (MeOH): 270, 335; (NaOMe): 276, 386; (AlCl$_3$): 281, 345, 360; (AlCl$_3$/HCl): 281, 342; (NaOAc): 269, 392;

(NaOAc/H_3BO_3): 267, 352. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 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): δ 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 florescent 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 λmax (nm): (MeOH): 269, 333; (NaOMe): 275, 379; ($AlCl_3$): 266sh, 275, 299, 349, 376sh; ($AlCl_3$/HCl): 266sh, 275, 299, 342, 374sh; (NaOAc): 255sh, 269, 390; (NaOAc/H_3BO_3): 268, 339. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 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): δ ppm 182.42 (C-4), 171.82 (C-6", glucuronide), 164.75 (C-2), 163.18 (C-7), 162.28 (C-5), 161.48 (C-4), 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"), 75.16 (C-4"), 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-Orhamnopyranoside (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.

REFERENCES

Babaei, H., O. Sadeghpour, L. Nahar, A. Delazar and H. Nazemiyeh *et al*., 2008. Antioxidant and vasorelaxant activities of flavonoids from *Amygdalus lycioides Var. horrid*. Turk. J. Biol., 32: 203-208.

Chattipakorn, S., A. Pongpanparadorn, W. Pratchayasakul, A. Pongchaidacha, K. Ingkaninan and N. Chattipakorn, 2007. *Tabernaemontana divaricata* extract inhibits neuronal acetylcholinesterase activity in rats. J. Ethnopharmacol., 110: 61-68.

Cummings, J.L., 2004. Alzheimer's disease. New Engl. J. Med., 351: 56-67.

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.

Feitosa, C., R.M. de Freitas, N. Luz, M. Bezerra and M. Trevisan, 2011. Acetylcholinesterase inhibition by somes promising Brazilian medicinal plants. Braz. J. Biol., 71: 783-789.

Fonteles, M.C., R.L. Capelo and V.S.N. Rao, 1982. VII Simposio de Plantas. Medicinais, Brasil.

Huxley, A.J., M. Griffiths and M. Levy, 1992. The New RHS Dictionary of Gardening. Vol. 4, Macmillan, New York, USA.

Joshi, N. and D.L. Verma, 2012. Flavonoids from the flowers and leaves of *Bergenia stracheyi*. Int. J. Res. Phytochem. Pharmacol., 2: 160-163.

Li, Y.L., J. Li, N.L. Wang and X.S. Yao, 2008. Flavonoids and a new polyacetylene from *Bidens parviflora* willd. Molecules, 13: 1931-1941.

Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York, USA., Pages: 354.

Moussaoui, F., A. Zellagui, N. Segueni, A. Touil and S. Rhouati, 2010. Flavonoid constituents from algerian *Launaea resedifolia* (OK) and their antimicrobial activity. Rec. Nat. Prod., 4: 91-95.

Racchi, M., M. Mazzucchelli, E. Porrello, C. Lanni and S. Govoni, 2004. Acetylcholinesterase inhibitors: Novel activities of old molecules. Pharmacol. Res., 50: 441-451.

Rhee, K.I., M.R. Van Rijn and R. Verpoorte, 2001. Qualitative determination of false-positive effects in the acetylcholinesterase assay using thin layer chromatography. Phytochem. Anal., 14: 127-131.

Rodriguez, E., N.J. Carman, G.V.V. Velde, J.H. Mcreynolds, T.J. Mabry, M.A. Irwin and T.A. Geissman, 1972. Methoxylated flavonoids from *Artemisia*. Phytochemistry, 11: 3509-3514.

Trevisan, M.T.S., M.Z.B. Bezerra, G.M. Pinheiro, C.M. Feitosa, R. Verpoorte and R.B. Filho, 2006. Larvicides and acetylcholinesterase inhibitors from *Kalanchoe* species. New Chem., 29: 415-418.

Watkins, P.B., H.J. Zimmerman, M.J. Knapp, S.I. Gracon and K.W. Lewis, 1994. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. J. Am. Med. Assoc., 271: 992-998.

Xu, Z., X. Liu and G. Xu, 1995. Chemical constituents of roots of *Ampelopsis brevipedunculata*. Zhongguo Zhong Yao Za Zhi, 20: 484-486.

Yabe, N. and H. Matsui, 2000. *Ampelopsis brevipedunculata* (Vitaceae) extract inhibits a progression of carbon tetrachloride-induced hepatic injury in the mice. Phytomedicine, 7: 493-498.

Yadav, R.N.S. and M. Agarwala, 2011. Phytochemical analysis of some medicinal plants. J. Phytol., 3: 10-14.

Yoshiteru, O., U. Yuji and H. Hiroshi, 1990. Ampelopsins A, B and C, new oligostilbenes of *Ampelopsis brevipedunculata* VAR. *Hancei*. Tetrahedron, 45: 5121-5126.