ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Role of *Zingiber officinale* in the Treatment of Alzheimer 's disease: *In-Vitro* and *In-Vivo* Evidences.

Magda M Fathy[1], Hanaa H Eid[1], Marwa A Hussein[2,*], Hanaa H Ahmed[3], and Ahmed A Hussein[4]

[1]Pharmacognosy Department, Cairo University, Faculty of Pharmacy, Kasr El-Aini, 11562, Cairo, Egypt
[2]PhytochemistryDeptartment, National Research Centre, 33 El Bohouth st. (former El Tahrir st.) Dokki, Giza, Egypt, P. O. 12622.
[3]Departmentof Hormones, National Research Centre, 33 El Bohouth st.(former El Tahrir st.) Dokki, Giza, Egypt.
[4]ChemistryDepartment, University of Western Cape, Private Bag X17,Belle ville 7535, South Africa.

ABSTRACT

Zingiber officinale commonly called ginger is being used in Arabian folk medicine for treatment of different diseases including improvement of memory impairment. The present study was designed to evaluate the role of ginger in management/treatment of Alzheimer's disease *in-vitro* and *in-vivo*. The phytochemical study was carried out using different chromatographic methods including semi-preparative HPLC. Ginger methanolic extract (GME), the isolated pure compounds and ginger essential oil (GEO) were tested for their inhibiting activity *in-vitro* against acetyl cholinesterase enzyme (AChE) using a multi-well plate Ellman's assay and the antioxidant activity was tested with diphenylpicrylhydrazyl (DPPH) radical scavenging assay. In addition, the methanolic extract and the essential oil were studied *in-vivo* using Alzheimer's disease model induced in rats using oral $AlCl_3$. Six known compounds were isolated from the methanolic extract and identified as 6-gingerol(**1**), methoxy-[6]-gingerol(**2**), methoxy-[10]-gingerol(**3**), 6-shogaol(**4**), 8-shogaol(**5**) & diacetoxy-[6]-gingerdiol (**6**). All compounds showed antioxidant activity against the DPPH free radical. However, none of these compounds showed inhibitory activity against AChE *in-vitro* at 200 μg/ml. GME showed more antioxidant power than GEO in scavenging DPPH free radical. GME and GEO showed moderate AChE inhibitory activity *in-vitro.* On the other hand, the treatment with GME and GEO showed improvement in the learning and memory in Alzheimer model induced in rats also they showed significant inhibitory activity against acetylcholinesterase as compared to Alzheimer's disease group (positive control group). Moreover, they showed an improvement of the morphological structure of the brain tissue with disappearance of most amyloid plaques.

Keywords: *Zingiber officinale*, Ginger, Alzheimer's disease, acetylcholinesterase, antioxidant.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and cognitive impairment [1].The cause of Alzheimer's disease is not clearly understood but there is a severe loss of cholinergic neurons in some brain areas [2]. Current treatment strategy is to slow down the progression of deterioration in AD patients by using synthetic cholinesterase inhibitors that work by increasing levels of a chemical messenger (acetylcholine) involved in memory and judgment function [3]. These drugs cause serious side effects so; the researchers become concerned by botanicals and nutritional supplements, in order to search for new modalities for protection from AD or even to stop progression and deterioration of the disease in its early stages [4].

Zingiber officinale (Ginger) is one of the famous species that belong to family Zingiberaceae and it is native to Asia. It is cultivated in tropical countries such as India, China, Australia, Brazil, Jamaica and parts of the USA [5]. In traditional herbal medicine, the fresh rhizomes of ginger are used for colds, headaches, vomiting, flatulence and the dried rhizomes are used for stomach ache, lumbago, diarrhea and improvement of memory [6, 7]. Ginger was also proved to be one of the most effective medicinal plants as an antiemetic (in motion sickness), antioxidant, anti-inflammatory, antibacterial, antitussive, antihepatotoxic, antimutagenic, diuretic, spasmolytic and immune stimulant [5,8].

The present study evaluates the effectiveness of ginger methanolic extract (GME) and ginger essential oil (GEO) in inhibiting acetyl cholinesterase (AChE) activity *in-vitro* and *in-vivo* and to improve learning and memory in rat AD model *in-vivo*. The antioxidant activity of these constituents and the isolated compounds were also evaluated.

MATERIALS and METHODS

Plant material:

The fresh rhizomes were purchased from the local market and identified by Prof. Dr. Ebrahim Ahmed El-Garf, Department of Botany, Faculty of Science, Cairo University. *Zingiber officinale* essential oil was purchased from Sigma-Aldrich, Germany.

Phytochemical study of methanolic extract:

Ten kg o f fresh *Zingiber officinale* rhizomes were cut into slices and mixed with 5 liters 95% methanol, the mixture was blended in an electric blender and left for maceration at room temperature for 2 days, the extract was filtered and the filtrate was concentrated at 40 ^{o}C to yield 100g residue. Sixty gram of the residue were fractionated using a glass column of 33 X 4 cm packed with 400 gm silica gel 60 (0.063–0.2 mm) Merck using dry method. A mixture of hexane and ethyl acetate was used by increasing polarity by 5% ethyl acetate regularly till reaching 30% ethyl acetate then 40%, 60% and 100% ethyl acetate. Ten main fractions were collected and were further subjected to semi-preparative HPLC using HPLC Agilent 1100 series. The HPLC column used was reversed phase C18 (25 X 2.1) Discovery® Sigma-Aldrich. HPLC condition of separation: mobile phase: Solvent A: acetonitrile, Solvent B: water. Flow rate: 2ml/min. Gradient elution: at 0 min 50% Solvent (B), at 10 min 30%Solvent (B), at 33 min 0%Solvent (B) and 50%Solvent (B) at 40 min.

GC/MS analysis of ginger oil:

The GC/MS analysis of the essential oil sample was carried in the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. A TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) was used. The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 mL/min and split ratio of 1:10 using the following temperature program: 60 ^{o}C for 1 min; rising at 3 ^{o}C/min to 240 ^{o}C and held for 1min. The injector and detector were held at 220 ^{o}C. Diluted samples (1:10 hexane, v/v) of 1μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

The identification of volatile components was assigned by comparison of their retention indices (RI) in reference to (C9–C22) *n*-alkanes with those of literature or with those of authentic compounds available in the authors' laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley spectral library and other published data [9]. Determination of the percentage composition was based on peak area normalization without using correction factors.

***In-vitro* biological study:**

Chemicals and Reagents for *in-vitro* biological study:

Tris–HCl was purchased from MP Biomedicals, Inc. Solon, Ohio. Bovine serum albumin was purchased from Loba Chemie PVT. LTD, Mumbai, India. Acetyl cholinesterase enzyme (ACHE) from electric eel (type V-S, lyophilized powder, 658 U/mg solid, 1210 U/mg protein), acetylthiocholine iodide (ATCI), 5,5´-dithiobis-(2-nitrobenzoic acid) (DTNB), galantamine hydrobromide, DPPH(2,2-diphenyl-1-picryl hydrazyl), quercetin were purchased from Sigma-Aldrich, Germany.

Evaluation of AChE inhibiting activity:

The assay was based on previously published method [10] with minor modifications [11]. Acetyl cholinesterase enzyme (AChE) hydrolyzes the substrate acetylthiocholine iodide (ACTI) to produce thiocholine iodide and acetate. Thiocholine iodide in turn reduces colouring reagent 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] liberating thionitrobenzoate (yellow color) which is visible and is kinetically monitored at 450 nm in a microplate reader Floustar Optima, BMG lab technologies, Germany.

Evaluation of the antioxidant activity using DPPH free radical scavenging assay:

The assay was based on previously published method [12] with minor modifications [13]. DPPH is a stable free radical with violet color and has maximum absorbance at 540 nm in a microplate reader Floustar Optima, BMG lab technologies, Germany. When DPPH is mixed with a phenolic compound that can donate a hydrogen atom, it will transfer into a reduced form with loss of color. The degree of discoloration indicates the antioxidant power of the compounds.

***In-vivo* biological study:**

Chemicals and Reagents for *in-vivo* biological study:

Aluminium chloride ($AlCl_3$) was purchased from Sigma-Aldrich, Germany. Rivastigmine: (Exelon) 1.5 mg was purchased from Novartis Co. Germany and Acetylcholinesterase (AChE) assay kit was purchased from Quimica Clinica Aplicada S.A Co., Amposta, Spain.

Experimental Animals:

The present study was conducted on 56 adult male *Sprague Dawley* rats weighing from 150 to 200 g, 4 months old, obtained from the Animal House Colony of the National Research Centre (NRC), Cairo, Egypt. They were kept in plastic cages at controlled room temperature (25 ± 2°C) and humidity (55%) under a 12 h dark-light cycle. All animals were accommodated with laboratory conditions for at least two weeks before the experiment and maintained under the same conditions all over the experiment. Diet and water were allowed *ad libitum*. All animals received human care and use according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt (09093).

Experimental Design:

After an acclimatization period of two weeks, the animals were classified into 7 main groups (8 rats /group). The first group received saline solution orally and served as negative control group (normal group)[14]. The second group was received ginger methanolic extract (GME) in a dose of 100 mg/kg b.wt. intraperitoneal[15]daily for 16 weeks. The third group was received ginger essential oil (GEO) in a dose of 100 mg/kg b.wt. intraperitoneal[16]daily for 16 weeks. The fourth group was orally administered with aluminum

chloride ($AlCl_3$) in a dose of 17 mg/kg[14]daily for one month to induce AD (Positive control group) (AD group). The fifth group was orally administered with $AlCl_3$ for one month and then treated orally with Rivastigmine in a dose of 0.3 mg/kg b.wt[14]daily for other 12 weeks. The sixth group was orally administered with $AlCl_3$ for one month and then treated with GME in a dose of 100 mg/kg b.wt. intraperitoneal daily for 12 weeks(AD+GME). The seventh group was orally administered with $AlCl_3$ for one month and then treated with GEO in a dose of 100 mg/kg b.wt. intraperitoneal daily for 12 weeks(AD+GEO).

Behavioral paradigm:

Test for cognitive abilities using rewarded T-maze:

The method used in this study is according to previously published method [17].Only 6 groups (8 rats /group) were subjected to the rewarded T-maze test (as the single group acts as a negative control group at zero time) which was done thrice: at zero time before starting oral induction with $AlCl_3$, after induction by 24hrs, and finally 24hrs after the last oral treatment with the tested materials GME, GEO and Rivastigmine were given.

Biochemical analysis: estimation of AChE activity:

At the end of the experiment blood samples were collected and the clear sera were obtained and frozen at -20°C for biochemical analysis [18].The principle of AChE inhibitory activity was mentioned before in *in-vitro* section. Evaluation of AChE inhibitory activity in serum was determined by colorimetric method using AChE assay kit [19].

Histopathological investigation:

After blood collection, the rats were rapidly killed by decapitation and the whole brain of each animal was rapidly dissected and fixed in formalin buffer 10% and embedded in paraffin. Tissue sections of 4 microns were stained with hematoxylin and eosin (H&E) for histopathological examination [20].

Statistical Analysis:

All values of *in-vivo* biological study are presented as means ± standard error (means ± S.E). The comparison between more than two different groups was carried out using the ANOVA followed by Tukey Kramer multiple comparisons test. Difference was considered significant at $P<0.05$.

RESULTS

I-Phytochemical study:

Methanolic extract of *Zingiber officinale* rhizomes was subjected to intensive purification using different chromatographic techniques including semi-prep HPLC and resulted in six known compounds. Structural elucidation was achieved using NMR (^1H,^{13}CNMR, and DEPT-135) and mass spectrometry (EI/MS). The data obtained were compared with literatures [21].

Compound 1 was purified as pale yellow oil. Its structure (Figure 1) was identified as [6]-gingerol. EI-MS spectrum showed peak at 294(m/z) corresponding to $C_{17}H_{26}O_4$, which is typical [6]-gingerol. Its NMR (^1H,^{13}CNMR) data are presented in Table 1[21].

Compound 2 is pale yellow oil. Its structure (Figure 1) was identified as methoxy-[6]-gingerol. EI-MS spectrum showed peak at 308 (m/z) corresponding to $C_{18}H_{28}O_4$, which is typical methoxy-[6]-gingerol. The spectroscopic data (Table 1) showed typical data for [6]-gingerol except the existence of extra methoxy group at 3.26 s ($^{\delta}$c 55.8). The structure was confirmed by comparing the obtained data with those published in literature [21].

ISSN: 0975-8585

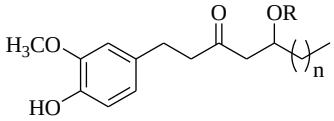
Compound 3 was purified as pale yellow oil. Its structure (Figure 1) was assigned as methoxy-[10]-gingerol. The spectral data (Table 1) showed identical signals like compound 2 with extra four methylene groups. The mass spectra and ^{13}C NMR confirmed the structure as methoxy-[10]-gingerol [21].

Compound 4 was purified as pale yellow oil. Its structure (Figure 1) was assigned as [6]-shogaol (-H2O molecule from [6]-gingerol). EI-MS spectrum showed peak at 276 (m/z) corresponding to $C_{17}H_{24}O_3$, which is typical [6]-shogaol.^{1}H, and ^{13}CNMR data (Table 1) confirmed the structure [21].

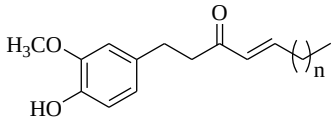
Compound 5 was purified as pale yellow oil. Its structure (Figure 1) was assigned as [8]-shogaol. EI-MS spectrum showed peak at 304 (m/z) corresponding to $C_{19}H_{28}O_3$, The NMR spectroscopic data (Table 1) is typical like the previous compound ([6]-shogaol). The only difference could be detected through the mass spectral analysis which showed difference of 28 amu corresponding to extra two methylene group. The ^{13}C NMR also supported this fact [21].

Compound 6 is pale yellow oil. Its structure (Figure 1) was assigned as diacetoxy-[6]-gingerdiol. EI-MS spectrum showed peak at 380 (m/z) corresponding to $C_{21}H_{32}O_6$, which is typical diacetoxy-[6]-gingerdiol. ^{1}H and ^{13}C NMR analysis (Table 1) were similar to [6]-gingerol, the only difference exist is the presence of two extra acetyl groups in compound 6, and the absence of the carbonyl group from [6]- gingerol [21].

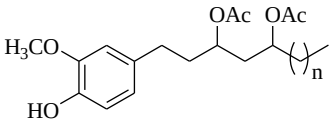
Figure 1: Chemical structures of the isolated compounds (1 -6) from ginger.



1- 6-Gingerol, R = H, n = 4
2- Methoxy-6-gingerol, R = CH_3, n = 4
3- Methoxy-10-gingerol, R = CH_3, n = 8



4- 6-Shogaol, n = 4
5- 8-Shogaol, n = 6



6- Diacetoxy-6-gingerdiol, n = 4

Table 1:^{1}H and ^{13}C NMR chemical shifts of compounds 1-6 isolated from ginger in $CDCl_3$

Position	6-gingerol		methoxy-[6]-gingerol		diacetoxy-[6]-gingerdiol		6-shogoal		8-shogoal		methoxy-[10]-gingerol	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
C-1	29.1*t*	2.84 *m*	31.8 *t*	2.81 *t*,*J*=6.8	31.2*t*	2.6 *t*, *J*=12.6	29.9 *t*	2.82 *brs*	31.7 *t*	2.83 *m*	31.8 *t*	2.81 *m*
C-2	45.3*t*	2.73 *m*	45.8 *t*	2.75 *t*,*J*=6.8	35.9*t*	2.5 *t*, *J*=12.6	41.9 *t*	2.82 *brs*	41.9 *t*	2.86 *m*	45.8 *t*	2.77 *m*
C-3	211.4 *s*		209.1 *s*		70.9 *d*	4.9 *m*	199.8 *s*		199.8 *s*		209.1 *s*	
C-4a, b	49.2*t*	2.43-2.60 *m*	47.6 *t*	2.43-2.60 *m*	38.4*t*	1.47-1.51 *m*	130.3 *d*	6.81 *d*, *J*= 15.8	130.3 *d*	6.11 *d*, *J* = 15.8	47.7 *t*	2.43-2.60 *m*
C-5	67.6 *d*	4.02 *m*	76.4 *d*	3.64 *m*	71.2*d*	4.9 *m*	147.9 *d*	6.09 *dt*, *J*= 15.8	147.9 *d*	6.83 *dt*, *J*=15.8, 3	76.4 *d*	3.64 *m*

ISSN: 0975-8585

								, 1.4				
C-6	36.3*t*	1.48-1.20 *m*	33.8 *t*	1.46 - 1.25 *m*	34.2*t*	1.94-1.23 *m*	32.4 *t*	2.19 *m*	32.5 *t*	2.22 *m*	33.8 *t*	1.46 - 1.40 *m*
C-7	31.6*t*		24.7*t*		24.8*t*		31.3 *t*	1.45 *m*	29.0 *t*	1.45 *m*	25.1 *t*	
C-8	25.0*t*		29.2*t*		31.6*t*		27.7 *t*	1.24 *m*	29.1 *t*	1.42-1.19 *m*	29.7 *t*	
C-9	22.5*t*		22.6 *t*		22.5*t*		22.4 *t*	1.39 *m*	28.1 *t*		29.5 *t*	
C-10	13.9*q*	0.88 *t*,*J*=6.8	13.9 *q*	0.88 *t*,*J*=6.8	13.9*q*	0.85 *t*, *J*=6.2	13.9 *q*	0.87 *t*, *J*= 6.6	29.7 *t*		29.6 *t*	
C-11									22.6 *t*		29.2 *t*	
C-12									14.1 *q*	0.86 *t*, *J*= 6.2	29.3 *t*	
C-13											22.7 *t*	1.24 *t*
C-14											14.1*q*	0.88 *t*, *J*=6.4
C-1'	133.5*s*		132.9 *s*		133.2 *s*		133.2 *s*		133.2 *s*		133 .0 *s*	
C-2'	110.9*d*	6.64 *br s*	111.1 *d*	6.77 *br s*	110.9 *d*	6.78 *br s*	111.1 *d*	6.76 *br s*,	111.1 *d*	6.73 *d*,*J*= 1.6	111.0 *d*	6.78 *brs*
C-3'	146.42 *s*		146.4 *s*		146.4 *s*		146.3 *s*		146.4 *s*		146.3 *s*	
C-4'	143.9*s*		143.8 *s*		143.8 *s*		143.8 *s*		143.8 *s*		143.8 *s*	
C-5'	114.4*d*	6.67 *d*, *J*= 7.2	114.3 *s*	6.81 *d*,*J*=8	114.2 *d*	6.82 *d*, *J*=8	114.3 *d*	6.84 *d*,*J*= 7.6	114.3 *d*	6.84 *d*,*J*= 7.6	114.3 *d*	6.82 *d*, *J*=8
C-6'	120.6*d*	6.62 *br d*, *J*= 7.2	120.8 *d*	6.66 *d*,*J*=8	120.9 *d*	6.64 *br d*, *J*=8	120.8 *d*	6.67 *d*,*J*= 7.6	120.8 *d*	6.69 *dd*,*J*=7.6, 1.6	120.7 *d*	6.66 *d*, *J*=8
C-3'-$\underline{OCH_3}$	55.7*q*	3.83*s*	56.9 *q*	3.84 *s*	55.9 *q*	3.86 *s*	55.8 *q*	3.85 *s*	55.9 *q*	3.85 *s*	56.9 *q*	3.85 *s*
C-5-$\underline{OCH_3}$			55.8 *q*	3.26 *s*							55.8 *q*	3.26 *s*
C-3-O$\underline{C}$OCH$_3$					170.0 *s*							
C-5-O$\underline{C}$OCH$_3$					170 .0 *s*							
C-3-OCO$\underline{CH_3}$					21.0 *q*	2.0 *s*						
C-5-OCO$\underline{CH_3}$					21.0 *q*	2.0 *s*						
ArOH		5.83 *br s*		5.53 *br s*		5.45 *br s*		5.49 *br s*		5.49 *br s*		5.40 *br. s*

Chemical shifts are in ppm downfield from tetramethylsilane, and referenced to solvent signal at δ 7.24 (for ^{1}H NMR) and 77.0 (for ^{13}C NMR), respectively

ISSN: 0975-8585

Chemical composition of *Zingiber officinale* essential oil:

Totally 39 compounds were identified by GC/MS analysis of *Zingiber officinale* essential oil and they are given in (Table 2). The principal constituent of ginger oil was *α*-Zingiberene (33.95%), a sesquiterpene hydrocarbon, followed by *β*-Sesquiphellandrene (15.28%), *β*-Bisabolene (9.85%) and *α*-Curcumene (8.74%) Most prominent monoterpenes are Camphene (6.65%) and *β*-Phellandrene (4.10%). Both oxygenated monoterpenes and sesquiterpenes are present in small proportions (4.7% and 0.65%, respectively) (Table 3). *α*-Zingiberene has been reported to be the major constituent in previously published literature [22] and this is in agreement with our findings.

Table 2: Chemical composition of the ginger essential oil.

No.	RT	KI	Components	Relative%
1	4.62	903	Tricyclene	0.15
2	4.86	912	α-Pinene	2.12
3	5.32	928	Camphene	6.65
4	6.07	955	β-Pinene	0.28
5	6.33	965	β-Myrcene	0.67
6	6.93	986	β-Thujene	0.26
7	7.66	1009	Limonene	1.28
8	7.75	1011	β-Phellandrene	4.10
9	7.79	1012	1,8-Cineole	3.39
10	9.64	1061	Terpinolene	0.12
11	10.29	1078	Linalool	0.17
12	13.31	1151	endo-Borneol	0.89
13	14.30	1174	α-Terpineol	0.25
14	17.90	1254	Bornyl acetate	0.13
15	21.23	1328	α-Himachalene	0.27
16	21.54	1335	Copaene	0.45
17	21.99	1345	Geranyl acetate	0.41
18	22.16	1348	Farnesol, acetate	0.94
19	22.68	1360	*cis*-α-Bergamotene	0.28
20	23.37	1375	Caryophyllene	0.11
21	23.93	1388	*trans*-α-Bergamotene	0.13
22	24.84	1408	*cis*-β-Farnesene	0.50
23	25.03	1413	Aromandendrene	0.25
24	25.60	1426	α-Guaiene	0.15
25	25.71	1429	γ-Muurolene	0.21
26	25.83	1431	Acoradiene	0.13
27	25.94	1434	Germacrene D	0.65
28	26.04	1436	α-Curcumene	8.74
29	26.29	1442	β-Selinene	0.13
30	26.44	1446	(*Z*,*E*)-α-Farnesene	0.16
31	26.62	1449	*α*-Zingiberene	33.95
32	26.97	1458	α-Farnesene	4.80
33	27.07	1460	β-Bisabolene	9.85
34	27.47	1469	γ-Gurjunene	0.76
35	27.73	1476	β-Sesquiphellandrene	15.28

ISSN: 0975-8585

No.	RT	KI	Components	Relative%
36	28.78	1500	Elemol	0.26
37	29.03	1506	γ-Elemene	0.44
38	31.94	1577	Isoaromadendrene epoxide	0.17
39	32.88	1599	β-Selinenol	0.22
			Total	99.7

RT = retention time; KI = Kovats indices

Table 3: Classifications of compounds of the ginger essential oil.

Classes of compounds	Relative%
Monoterpene hydrocarbons	15.63
Oxygenated monoterpenes	4.7
Sesquiterpene hydrocarbons	77.24
Oxygenated sesquiterpenes	0.65
Others	1.48

Biological study:

***In-vitro* study:**

Acetylcholinesterase inhibiting activity assay:

The preliminary screening of different concentrations (0.25, 0.50, 1 mg/ml) of GME & GEO showed inhibitory activity in a dose dependent manner. However, at high concentration of 1 mg/ml, GME and GEO gave 41.4±0.9% and 35.0 ±1.9% inhibition of AChE, respectively (Figure 2). Therefore, the values of IC_{50} couldn't be reached up to 1 mg/ml due to the turbidity of the GEO-buffer solution or precipitate formed in the more concentrated solution of the GME which caused inaccurate calculations. The six isolated compounds showed no inhibiting activity against acetylcholinesterase at tested concentration (200µg/ml). Galantamine was used as standard acetylcholinesterase inhibitor with IC_{50} value of 7.8 µM.

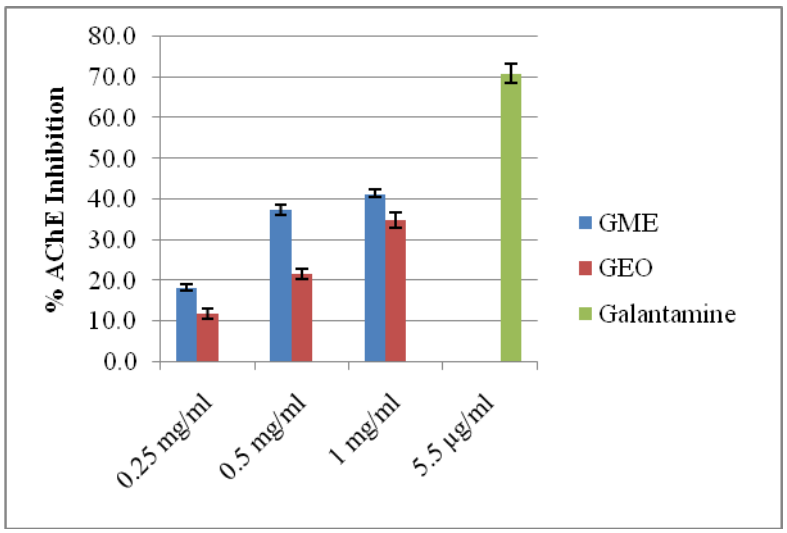


Figure 2: Inhibiting activity of different doses of ginger methanolic extract (GME) and ginger essential oil (GEO) against AChE using galantamine as standard acetylcholinesterase inhibitor. Data are means ± SEM (n=4)

ISSN: 0975-8585

Antioxidant activity assay:

Antioxidant activity of the GME & GEO was determined by using DPPH assay, compared with quercetin as standard antioxidant agent.

GME and GEO caused a concentration-dependent scavenging of DPPH radical at concentration 100-200-400 µg/ml (Figure 3). Essential oil, showed weaker free radical scavenging activity than the methanolic extract at the same tested concentrations. The EC_{50} of GME was 116.4 µg/ml.

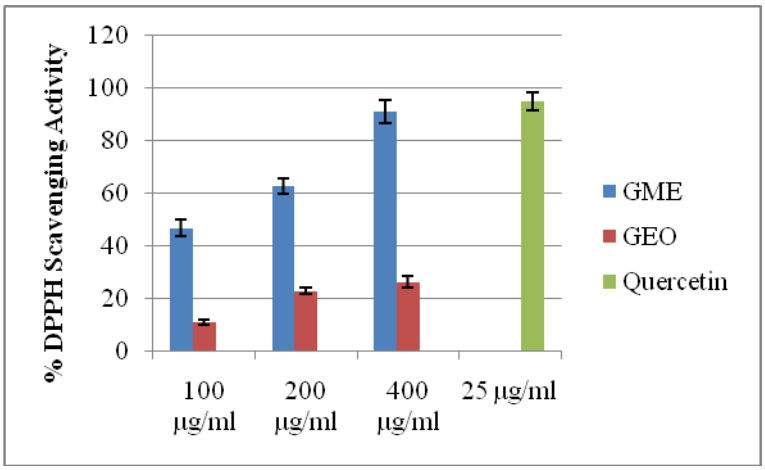


Figure 3: Percentage of antioxidant activity of different doses of ginger methanolic extract (GME) and ginger essential oil (GEO) using quercetin as antioxidant standard. Data are means ± SEM (n=4).

Figure (4) showed concentration-dependent scavenging of DPPH radical at concentration 0.4,1.6,6.25,25,100 µg/ml of isolated ginger compounds. The assay showed that [6]-shogaol had the highest antioxidant activity followed by [6]-gingerol, while methoxy-[10]-gingerol showed lowest antioxidant activity when compared with [6]-shogaol. Results are shown in (Figure 5).

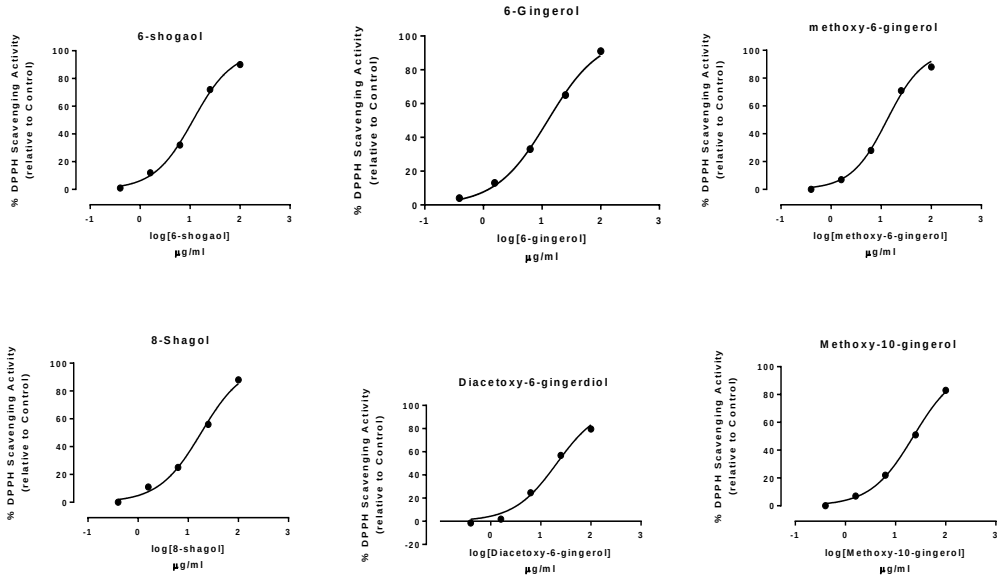


Figure 4: Antioxidant activity of isolated ginger compounds in relation to their different log concentrations (0.4, 1.6, 6.25, 25 and 100 µg/ml).

ISSN: 0975-8585

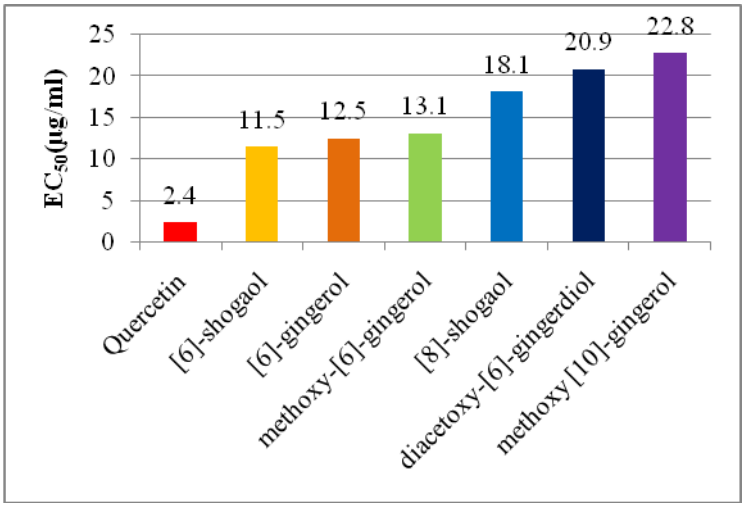


Figure 5: EC_{50} of isolated ginger compounds in comparison with quercetin.

***In-vivo* study:**

a- Effect of treatment with GME and GEO on behavioral status of AD-induced rats using rewarded T-maze test represented by the average time, elapsed until reaching food, calculated in seconds (Table 4).

Table 4: Effect of ginger methanolic extract (GME) and ginger essential oil (GEO) on behavioral status of Alzheimer's disease (AD) induced-rats using rewarded T-Maze test

Group (n=8)	Dose (mg/kg)	Time duration (sec.)		
		Baseline (0 weeks)	Induction (four weeks)	Treatment (twelve weeks)
AD group	17	15.11±1.76	115.75±13.54[a]	117.56±0.9[a]
AD+GME	100	20.67±1.21	104.67±9.97 [a]	50±2.34[abcd]
AD+GEO	100	15.67±1.55	97.04±4.89 [a]	79.22±1.54[abcd]
GME	100	13.38±1.15	18.93±1.03	23.33±1.03
GEO	100	21.38±0.52	20.56±1.72	30.09±0.62
AD+Rivastigmine	0.3	18.25±1.66	98.62±12.5 [a]	24.67±1.17[bc]

Results are expressed as means ± standard error (SE) for 8 animals/group. [a]Significant difference compared to baseline duration of each group. [b]Significant difference of treated groups compared to their corresponding induced groups. [c]Significant difference compared to AD-treated group. [d]Significant difference between AD+Rivastigmine-treated group and AD group treated with either GME or GEO.

Baseline duration:

The average time before starting treatment (zero weeks) was 15.11±1.76 for AD group, 20.67±1.21 for AD group treated with ginger extract group , 15.67±1.55 for AD group treated with ginger oil , 13.38±1.15 for ginger extract- treated group , 21.38±0.52 for ginger oil-treated group and 18.25±1.66 for AD group treated with Rivastigmine.

AD group:

Daily oral administration of $AlCl_3$ alone for four successive weeks resulted in a significant increase in the average time for rats to recognize food compared to baseline duration of the same group at P <0.05, as it became 115.75±13.54. Later on, when $AlCl_3$ was stopped for 12 weeks the average time for rats to recognize food increased to 117.56±0.9, which was significantly higher than the baseline duration of the same group at P <0.05, but there was no significant difference in the average time when compared to the same group when $AlCl_3$ was used continuously for 4 successive weeks.

AD group treated with ginger extract:

Before treatment with ginger extract, this group showed a significant increase in the average time (104.67±9.97) to recognize food compared to baseline duration of the same group at P < 0.05. After treatment, it showed a significant decrease in the average time to recognize food (50±2.34) compared to the same group before treatment, but still more than the baseline duration of the same group.

AD group treated with ginger oil:

Before treatment with ginger oil, this group showed a significant increase in the average time (97.04±4.89) to recognize food compared to baseline duration of the same group at P < 0.05. After treatment, it showed a significant decrease in the average time to recognize food (79.22±1.54) compared to the same group before treatment, but still more than the baseline duration of the same group.

Normal healthy group treated with ginger extract:

Before treatment with ginger extract, this group showed an insignificant change (P >0.05) in the average time (18.93±1.03) to recognize food compared to baseline duration of the same group. Also, after treatment, this group showed an insignificant change (P >0.05) in the average time (23.33±1.03) compared to the same group before treatment, but still more than the baseline duration of the same group.

Normal healthy group treated with ginger oil:

Before treatment with ginger oil, this group showed an insignificant change (P > 0.05) in the average time (20.56±1.72) to recognize food compared to baseline duration of the same group. Also, after treatment, this group showed an insignificant change (P > 0.05) in the average time (30.09±0.62) compared to the same group before treatment, but still more than the baseline duration of the same group.

Rivastigmine treated group:

Before treatment with rivastgmine, this group showed a significant increase in the average time (98.62±12.5) to recognize food compared to baseline duration of the same group at P < 0.05. After treatment, Rivastigmine group, showed a significant decrease in the average time to recognize food (24.67±1.17) compared to the same group before treatment, but this group didn't show significant change from the baseline duration of the same group.

b-The data in (Table 5) showed the effect of treatment with GME and GEO and Rivastigmine on cholinergic marker represented by serum AChE activity in AD-induced in rats.

Table5: Effect of ginger methanolic extract (GME) and ginger essential oil (GEO) on serum AChE activity in (Alzheimer's disease) AD-induced in rats.

Groups (n=8)	Dose (mg/kg)	Serum AChE (U/L)	% Change
Normal group	1ml saline (0.1%)	706.88±25.56	–
AD-group	17	916.03±4.79[a]	(29.58%)

ISSN: 0975-8585

AD+GME	100	677.09±53.32[b]	(-26.08%)
AD+GEO	100	781.09±26.27[b]	(-14.73%)
GME	100	675.20±50.22	(-4.48%)
GEO	100	762.63±60.71	(7.89%)
AD+Rivastigmine	0.3	732.27±20.67[b]	(-20.06%)

Results are expressed as means ± standard error; .[a]Significant difference as compared to normal group , [b]Significant difference as compared to AD-group.

In comparison with the normal group, $AlCl_3$ administration produced significant elevation ($p< 0.05$) in serum AChE activity (29.58%). Treatment of AD-induced rats with either GME or GEO as well as Rivastigmine resulted in significant decrease ($p< 0.05$) in serum AChE activity (-26.0818% for GME, -14.727% for GEO & -20.0578% for Rivastigmine) as compared to AD-induced group**.**

In comparison with Rivastigmine treated group, treatment with either GME or GEO resulted in insignificant change ($p> 0.05$) in serum AChE activity.

In comparison with the normal group, insignificant change ($p> 0.05$) in serum AChE activity was detected in groups treated with GME or GEO.

Histopathological Examination:

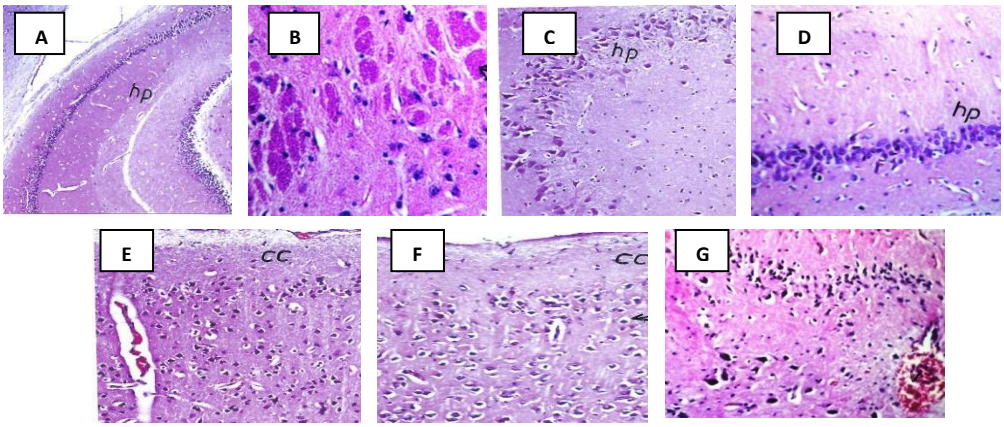


Figure 6: Photomicrographs of brain tissue section of rats in **A**: normal healthy group; **B**: AD group; **C**: AD group treated with GME; **D**: AD group treated with GEO; **E**: normal healthy group treated with GME; **F**: normal healthy group treated with GEO; **G:** AD group treated with Rivastigmine.

The photomicrograph of brain tissue section of rats in the negative control group (normal group) showing normal intact histological structure of the hippocampus (hp) (H&E X16) (Figure 6A). While, the photomicrograph of brain section of AD group showing various sizes of amyloid plaques formation (arrow) in the hippocampus (H&E X40)(Figure 6B). The photomicrograph of brain tissue section of AD group treated with GME showing atrophy of neuronal cells in hippocampus (hp) (H&E X40) (Figure 6C). Also, photomicrograph of brain tissue section of AD group treated with GEO showing intact histological structure of the hippocampus (hp) (H&E X40) (Figure 6D).The photomicrograph of brain section of normal healthy group treated with GME showing congestion in cerebral blood vessels (v) with normal histological structure of the cerebral cortex (cc) (H&E X40) (Figure 6E). Also, the photomicrograph of brain section of normal healthy group treated with GEO showing neuronal cellular oedema (arrow) in cerebrum (H&E X40) (Figure 6F). The photomicrograph of brain section of AD group treated with Rivastigmine showing focal gliosis (g) in cerebral cortex with congestion in cerebral blood vessels (v) (H&E X40) (Figure 6G).

ISSN: 0975-8585

DISCUSSION

Alzheimer's disease (AD) is a deadly vigorous disease which attacks elderly all over the world. Definitely, this disease is making disturbances in memory as well as behavior and it is characterized by cognitive dysfunction and deficits in daily living activities [23].

Due to the increase in the middle aged population, most researchers have focused on searching and developing cognitive enhancers from medicinal plants. It was reported in the Arabian folk medicine that ginger is used to improve memory [24].

AD management/treatment can be accomplished not only by acetycholinesterase inhibitors but also by using other treatment strategies. These strategies include the use of antioxidant [25]. Neurodegerative diseases have been linked to oxidative stress arising from peroxidation of membrane biomolecules and high levels of iron have been reported to play an important role in neurodegenerative diseases and other brain disorders. In this study we measured the antioxidant activity of GME and GEO. DPPH scavenging assay *in-vitro* revealed that the powerful antioxidant activity of GME over GEO as the methanolic extract produced 50% scavenging activity against DPPH radical at 116.4 µg/ml while the GEO showed lower inhibitory activity at the same concentration. Our results are in agreement with previously published data [22, 26]. The antioxidant activity of GME and GEO may be attributed to the presence of phenolic compounds. In addition, the isolated compounds showed powerful antioxidant activity, [6]- shogoal showed strong antioxidant effect. The superior effect of [6]-shogaol as an antioxidant scavenger over gingerols ([6]-gingerol, [8]-gingerol and [10]-gingerol) could be attributed to the presence of α,β-unsaturated ketone moiety[27,28]. In addition the short carbon chain of [6]-shogaol and [6]-gingerol interpret their superior potency over the rest of longer carbon chain compounds [28].

On the other hand, we measured inhibiting activity of GEO and GME against acetylcholinesterase *in-vitro*, they showed moderate inhibiting activity and these results are in agreement with previously published data [29,30]. However, the isolated compounds from GME showed very weak activity when tested separately. Therefore, we deduced that the activity of ginger extract may be due to synergistic effect of these compounds or presence of other compounds that exert the inhibitory activity against AChE enzyme. The phytochemical study for the chemical constituents of the GEO using GC/MS proved the presence of ,α-pinene, β-pinene, 1,8-cineole, α-terpineol, caryophyllene, limonene, terpinolene, linalool and camphene which were reported to have anti-acetyl cholinesterase activity [29, 31, 32, 33] and may contribute a major role in inhibition of acetyl cholinesterase enzyme.

To get comprehensive view of the ability of ginger in treatment of AD we extended our study on AD model in rats. This is done by exposure of AD group of rats to $AlCl_3$. In this study, the cognitive abilities of rats were evaluated by using the rewarded T-maze test.

The AD group treated with GME demonstrates a significant improvement after 12 successive weeks of treatment which showed reduction in time for reaching food by tested rats this in agreement with previously published data [34].Also, in AD group treated with GEO revealed a significant effect after 12 successive weeks of treatment. GEO constituents have an important role in AChE inhibition; also, it has significant cognition and memory improving effect. Monoterpenes which are present in GEO also, isolated from salvia species have been found to inhibit cholinesterase as well as nicotinic and muscarinic activities of cholinergic system that are involved in the memory retention process. *Salvia lavandulaefolia* constituents and/or their metabolites have been suggested to reach the brain (crossing the gastrointestinal and blood–brain barriers) and exert an effect on cognition [31]. The biochemical/neuronal systems affected are likely to include the cholinergic system since the essential oil constituents inhibit erythrocyte AChE *in vitro* [31] and rat brain AChE *in vivo* [35].

The cholinergic neurons play a major role in memory and attention. The dysfunction and death of these neurons, especially in the hippocampus, are thought to contribute to the pathophysiology of memory deficits associated with AD. In the present study, after four successive weeks of induction of AD it was revealed that $AlCl_3$ administration induces significant elevation in serum AChE activity [36].

The treatment of AD group with GME produced a significant decrease in serum AChE activity in studied group compared to AD group, but no significant change compared to Rivastigmine group. Acetyl

cholinesterase inhibitory activity of ginger has been previously reported in literature that ginger alcoholic extract significantly increases whole brain AChE inhibition activity which was reflected in improvement of learning and memory [37].

Essential oils have the strong potency to inhibit acetyl cholinesterase in brain because of their lipophilicity and small molecular size constituents [32]. In the current study, treatment of AD rats with GEO produced a significant decrease in serum AChE activity compared to AD group but no significant change compared to Rivastigmine group.

The microscopic investigation of brain rat in AD group revealed the plaque formation in the hippocampus. The mechanism of aluminum induced neurodegeneration is not clearly known. However, it has been reported that aluminum potentiates the activity of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions to cause oxidative damage leading to neurodegeneration [38]. Moreover, aluminum promotes the formation of amyloid-β plaque [39].

The microscopic investigation of both AD groups that treated with GME and GEO showed an improvement of the morphological feature of the brain tissue with the disappearance of most of amyloid plaques. This could be due to the anti-oxidant and anti-inflammatory effect of both GME and GEO [30,40].

In conclusion, our study revealed the ability of GME and GEO to improve the symptoms of AD induced in rats and counters the progress of the disease. The inhibitory activity of both could be attributed to the presence of bioactive phytochemicals with anti-acetylcholinesterase activity and antioxidant activity which justify its use in folk medicine for the management/treatment of Alzheimer's disease. The obtained results support the traditional use of ginger to alleviate ageing and memory problems.

ACKNOWLEDGEMENT

This work is extracted from a thesis entitled Phytochemical and Biological Evaluation of *Zingiber officinale* for the Treatment of Alzheimer's disease by M.A.H. The authors are thankful to Dr.Ahmed R. Hamed & Dr. Maha M. Soltan, Phytochemistry Department, NRC for their help in performing *in-vitro* anti-Alzheimer & anti-oxidant assays. The authors are grateful to Science and Technology Development Fund (STDF), Egypt (project number 251) for financial support.

REFERENCES

[1] Long CO, Dougherty J. Home Healthc Nurse 2003;21(1): 8-14.
[2] Perry E, Court J, Goodchild R, Griffiths M, Jaros E, Johnson M, Lloyd S, Piggott M, Spurden D, Ballard C, Mckeith I, Perry R. J Neural Transm 1998;105: 915-933.
[3] Hogan DB, Bailey P, Black S, Carswell A, Chertkow H, Clarke B, Cohen C, Fisk JD, Forbes D, Man-Son-Hing M, Lanctot K, Morgan D, Thorpe L. CMAJ 2008;179: 1019-1026.
[4] Wollen KA. Altern Med Rev 2010;15(3): 223-44.
[5] Langner E, Greifenberg S,Gruenwald J. Adv Ther 1998;15(1): 25-44.
[6] Dedov VN, Tran VH, Duke CC, Connor M, Christie MJ, Mandadi S, Roufogalis BD. Br J Pharmacol 2002;137(6): 793-798.
[7] Falch B, Reichling J, Saller R. Dtsch Apothek Zeitung 1997;137: 47-60.
[8] Blumenthal M, Goldberg A, Gruenwald J, Hall T, Riggins CW, Ristser RS, et al. American Botanical Council (1998): Austin, Texas.
[9] Adams RP. Allured Publishing Crop, Carol Stream, Illinois, USA 2007; 432.
[10] Ellman GL, Courtney KD, Andres JrV, Featherstone RM. Biochem Pharmacol 1961;**7**: 88-95.
[11] Lee JH, Lee KT, Yang JH, Baek NI, Kim DK. Arch Pharm Res 2004;27: 53-56.
[12] Nara K, Miyoshi T, Honma T, Koga H. Biosci Biotechnol Biochem 2006; 70: 1489-1491.
[13] Hamed, A. Investigation of multiple cytoprotective actions of some individual phytochemicals and plant extracts. Chapter 7 : Development of cell-free and intracellular screening systems for testing antioxidant properties of plant extracts. PhD, The University of Nottingham, United Kingdom (2009).
[14] Ahmed MS, Gilane MS, Hanaa HA, Ahmed AH, Soheir EK.Int J Pharm Pharm Sci,2013;5:87-94.
[15] Waggas AM.Pak J Biol Sci 2009;12(3): 201-212.

[16] Felipe CFB, Fonsec KS, Barbosa ALR, Bezerra JNS, Neto MA, Franca Fonteles MM, Barros Viana GS.J. Med. Plants Res.2008;2: 163-170.
[17] Deacon RM, Rawlins JN. Nat Protoc 2006;1(1): 7-12.
[18] Van Herk R, Klazinga NS, Schepers RM, Casparie AF. Soc Sci Med 2001;53(12): 1721-1732.
[19] Den Blaauwen DH, PoppeWA, Tritschler W.J Clin Chem Clin Biochem 1983;21(6): 381-386.
[20] Bancroft JD, Stevens A, Turner DR. Theory and Practice of Histological Techniques. Churchill Livingston, N.Y., London, San Francisco, Tokyo 1996, 4th ed.
[21] Jolad SD, Lantz RC, Chen GJ, Bates RB, Timmermann BN. Phytochemistry 2005;66: 1614-1635 .
[22] Jeena K, Liju VB, Kuttan R. Indian J Physiol Pharmacol 2013;57: 51-62.
[23] Orhan I, Sener B, Choudhary MI, Khalid A. J Ethnopharmacol 2004;91: 57-60.
[24] Saenghong N, Wattanathorn J, Muchimapura S, Tongun T, Piyavhatkul N, Banchonglikitkul C, Kajsongkram T. Evid Based Complement Alternat Med 2012 ;2012:383062. doi: 10.1155/2012/383062.
[25] Sonnen JA, Breitner JC, Lovell MA, Markesbery WR, Quinn JF, Montine TJ.Free Radic Biol Med 2008;45: 219-230.
[26] El-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. J Agric Food Chem 2010;58: 8231-8237.
[27] Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. J Ethnopharmacol 2010;127: 515-520.
[28] Guo J, Wu H, Du L, Zhang W, Yang J. J. Agr. Sci. Tech2014;16:1063-1072.
[29] Chaiyana W, Okonogi S. Phytomedicine 2012;19: 836-839.
[30] Oboh G, Ademiluyi AO, Akinyemi AJ. EXP Toxicol Pathol 2012;64: 315-319.
[31] Perry NS, Bollen C, Perry EK, Ballard C. Pharmacol Biochem Behav 2003;75: 651-659.
[32] Worawan K, Kittima W, Pattamapan L, Phanida P, Natthinee A, Veena N, Krit T,Nuntavan B. JAASP 2012;1: 58-67.
[33] Lopez MD, Campoy FJ, Pascual-Villalobos MJ, Munoz-Delgado E, Vidal C. J Chem Biol Interact 2015;229: 36-43.
[34] Jun W, Qifu H, Shiqi J. J Med Res, 2008;8: 33-37.
[35] Perry NS, Houghton PJ, Jenner P, Keith A, Perry EK. Phytomedicine 2002;9: 48-51.
[36] Yellamma K, Saraswathamma S, Kumari BN. Toxicol Int 2010;17: 106-112.
[37] Joshi H, Parle M. Afr. J. Trad. CAM 2006; 3: 64-74.
[38] Xie CX, Yokel RA.Arch Biochem Biophys 1996;327: 222-226.
[39] Baldeiras I, Santana I, Proenca MT, Garrucho MH, Pascoal R, Rodrigues A, Duro D, OliveiraCR. J Alzheimers Dis 2008;15: 117-128.
[40] Ha SK, Moon E, Ju MS, Kim DH, Ryu JH, Oh MS, Kim SY.Neuropharmacology 2012;63(2): 211-223.