Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Neuroprotective effects of a combination of *Boswellia papyrifera* and *Syzygium aromaticum* on $AlCl_3$ induced Alzheimer's disease in male albino rat

Efeitos neuroprotetores de uma combinação de *Boswellia papyrifera* e *Syzygium aromaticum* em Alzheimer induzido por $AlCl_3$ em ratos albinos machos

R. M. Aljarari[a]*

[a]University of Jeddah, College of Science, Department of Biology, Jeddah, Saudi Arabia

Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by hippocampal, and cortical neuron deterioration, oxidative stress, and severe cognitive dysfunction. Aluminum is a neurotoxin inducer for cognitive impairments associated with AD. The treatment approaches for AD are unsatisfactory. *Boswellia papyrifera and Syzygium aromaticum are* known for their pharmacological assets, including antioxidant activity. Therefore, the current study explored the possible mitigating effects of a combination of *Boswellia papyrifera and Syzygium aromaticum* against aluminum chloride ($AlCl_3$) induced AD. The AD model was established using $AlCl_3$ (100 mg/kg), and the rats were orally administrated with *Boswellia papyrifera or Syzygium aromaticum* or a combination of them daily for 8 weeks. The Y-maze test was used to test cognition in the rats, while acetylcholinesterase (AChE) and oxidative stress markers were estimated in homogenates of the cerebral cortex and hippocampus. Also, the histopathological examination of the cortex and hippocampus were investigated. The results revealed that administration of either *B. papyrifera* or *S. aromaticum* extracts significantly improved the cognitive functions of AD rats, enhanced AChE levels, increased oxidative enzymes levels, including SOD and GSH, and reduced MDA levels in homogenates of the cerebral cortex and hippocampus and confirmed by improvement in histological examination. However, using a combination therapy gave better results compared to a single treatment. In conclusion, the present study provided primary evidence for using a combination of *B. papyrifera* and *S. aromaticum* to treat cognitive dysfunction associated with $AlCl_3$ Induced AD by improving the AChE levels and modulating oxidative stress in the brain.

Keywords: *Boswellia papyrifera*, *Syzygium aromaticum*, Alzheimer's disease (AD), oxidative stress, aluminium chloride ($AlCl_3$), cognitive functions.

Resumo

A doença de Alzheimer (DA) é a doença neurodegenerativa mais comum, caracterizada por hipocampo, deterioração dos neurônios corticais, estresse oxidativo e disfunção cognitiva grave. O alumínio é um indutor de neurotoxinas para deficiências cognitivas associadas à DA. As abordagens de tratamento para DA são insatisfatórias. *Boswellia papyrifera* e *Syzygium aromaticum* são conhecidos por seus ativos farmacológicos, incluindo atividade antioxidante. Portanto, o presente estudo explorou os possíveis efeitos atenuantes de uma combinação de *Boswellia papyrifera* e *Syzygium aromaticum* contra a DA induzida por cloreto de alumínio ($AlCl_3$). O modelo DA foi estabelecido usando $AlCl_3$ (100 mg/kg), e os ratos foram administrados por via oral com *Boswellia papyrifera* ou *Syzygium aromaticum* ou uma combinação deles diariamente por 8 semanas. O teste do labirinto em Y foi usado para testar a cognição nos ratos, enquanto a acetilcolinesterase (AChE) e marcadores de estresse oxidativo foram estimados em homogeneizados do córtex cerebral e hipocampo. Além disso, o exame histopatológico do córtex e hipocampo foram analisados. Os resultados revelaram que a administração de extratos de *B. papyrifera* ou *S. aromaticum* melhorou significativamente as funções cognitivas de ratos com DA, aumentou os níveis de AChE, aumentou os níveis de enzimas oxidativas, incluindo SOD e GSH, e reduziu os níveis de MDA em homogeneizados do córtex cerebral e hipocampo e confirmado pela melhora no exame histológico. No entanto, o uso de uma terapia combinada apresentou melhores resultados em comparação com um único tratamento. Em conclusão, o presente estudo forneceu evidências primárias para o uso de uma combinação de *B. papyrifera* e *S. aromaticum* para tratar a disfunção cognitiva associada à DA induzida por AlCl3, melhorando os níveis de AChE e modulando o estresse oxidativo no cérebro.

Palavras-chave: *Boswellia papyrifera*, *Syzygium aromaticum*, doença de Alzheimer (AD), estresse oxidativo, cloreto de alumínio ($AlCl_3$), funções cognitivas.

*e-mail: rmaljerary@uj.edu.sa
Received: February 28, 2023 – Accepted: June 24, 2023

1. Introduction

Alzheimer's disease (AD) is a neurological condition that progresses over time and places a high financial and psychological cost on society (Singh et al., 2016, 2018). Over 45 million individuals are estimated to be affected by AD globally, and by 2050, this figure is projected to quadruple every 20 years (Dos Santos et al., 2018; Scheltens et al., 2016). Cognitive impairment, particularly short-term memories, is the earliest presenting symptom of AD, but long-term memories are well-preserved. Executive decision-making and the capacity to do regular work drastically decline as the disease progresses and cognitive impairment becomes apparent. The reduction of cholinergic synapses in the hippocampus and neocortex is a recurrent observation in AD, emphasizing the importance of using an effective approach to control the acetylcholinesterase (AChE) function to overcome this problem (Heo et al., 2004; Loizzo et al., 2008). AD patients have lower levels of the neurotransmitter AChE, which was initially discovered as a synthetic substance in 1867 and is used to transfer nerve signals from one nerve cell to another or through other muscle fibers (Houghton et al., 2006). The U.S. Food and Drug Administration has given the green light to a few AChE inhibitors, including tacrine and rivastigmine, for relieving AD symptoms (J. K. Kim et al., 2009).

Aluminum (Al) is a lethal neurotoxin, and its deposition in the brain contributes to the emergence of neurodegenerative diseases, including AD (Campbell, 2002; Zatta et al., 2003; Kawahara, 2005). Epidemiological research revealed a connection between chronic Al exposure and neurological damage as well as cognitive impairment Long-term dialysis patients who received Al-containing dialysates acquired dialysis dementia (Gupta et al., 2019). A potential source of cognitive damage has been identified by miners' exposure to aluminum powder (Rifat et al., 1990). Animals treated by Al showed AD-like symptoms (Platt et al., 2001; Praticò, 2002). Prolonged exposure to Al disrupts the hippocampal synaptic plasticity due to its deposition in all parts of the rat brain, including the hippocampus, which is the site of learning and memory (Niu et al., 2007).

Currently, the treatment approaches for AD are unsatisfactory. They can only temporarily improve cognitive skills or alleviate symptoms while having numerous adverse effects. In order to treat not just the symptoms of AD but also to cure its pathology with lesser side effects, a class of drugs must be created that can target a wider range of targets (Cummings et al., 2019; Hukins et al., 2019; Yiannopoulou and Papageorgiou, 2020). Numerous active substances were extracted parts mainly from medicinal plants in Europe and Asia and showed promising pharmacological activity against AD (Uddin et al., 2019). *Boswellia papyrifera* is a floral plant species and frankincense that is indigenous to Ethiopia, and others in Africa. It is also described as Sudanese frankincense. Due to the tree's significant resin, Ethiopia cultivates it (Schmiech et al., 2021). Clove, also known as *Syzygium aromaticum*, is a dried flower bud from the Myrtaceae family that may be located all over the globe. The marketable component of the clove tree is made up of the leaves and buds (Batiha et al., 2020). There have been numerous reports of using *B. papyrifera* and *S. aromaticum* in medicine (Batiha et al., 2020; Schmiech et al., 2021).

There is a growing body of literature that recognizes *B. papyrifera* and *S. aromaticum* for their neuroprotective activity. Oja and his colleagues demonstrate the role of *S. aromaticum* in mitigating iron-mediated oxidative brain injury in rats (Ojo et al., 2022). Also, a combination of exercise and *S. aromaticum* reverse the memory deficits, apoptosis, and mitochondrial dysfunction of the hippocampus in AD (Panahzadeh et al., 2022). Additionally, *Genus Boswellia* acts as a good candidate for neurodegenerative disorders, including AD (Rajabian et al., 2020). Also, many studies reported the beneficial effect of *B. papyrifera* on learning and memory in rodents (Farshchi et al., 2010; Mahmoudi et al., 2011). However, the neuroprotectant activity of a combination of the two extracts against AD has remained unclear.

Therefore, the present work aims to investigate the neuroprotective roles of a combination of *B. papyrifera* and *S. aromaticum* extracts against $AlCl_3$-induced AD in male rats. This was achieved by measuring the cognitive impairment using the Y-maze test, AChE levels, and oxidative stress biomarkers, as well as investigating the histological picture of the cortex and hippocampus.

2. Material and Methods

2.1. Materials

$AlCl_3$ has been obtained from (Alpha Chemika, Mumbai, India) and prepared in saline (0.9%).

2.2. Plants and extraction

B. papyrifera dried parts of tree and *S. aromaticum* dried flowers were purchased from AL-Haraz store in Egypt. The plants were kindly identified by Prof. Dr. A. A. Fayed, Professor of plant taxonomy, Faculty of Science, Assiut University, Egypt. A voucher sample was kept in the Faculty of Science Herbarium, Assiut University, Assiut, Egypt. 500 g of both plants were washed by distill water, dried then blended by mortar. The powder of each plant was soaked in 5L of 80% methanol for 3 days in flasks that shake for 170 RPM. The extracts were filtered using 0.45µm filter paper. The methanol of each filtrate was removed using a rotary evaporator at 40 °C then frozen in the refrigerator for further experiments (Melesie Taye et al., 2020).

2.3. GC-Ms of B. papyrifera and S. aromaticum extracts

B. papyrifera and *S. aromaticum* methanol extracts were screened using a direct capillary column TG-%MS (30m*0.25mm*0.25m film thickness) and a trace GC1310-Isq mass spectrometer (Thermo Scientific, Austin, TX, USA). The column oven's temperature was initially kept at 60 °C, then increased by 5 °C/min to 230 °C and held for 3 min. The final temperature of 290 °C was increased by 30 °C/min and then maintained for three minutes. The injector and MS transfer line were kept at temperatures of 240 and 250 °C, respectively, and helium was used as the carrier gas

with a constant flow rate of 1 ml/min. A diluted sample of 1 µl was automatically fed into the GC's split mode using the Autosampler AS1300. Full scan mass spectra were collected in the range of m/z 40 to 1000 at an ionization voltage of 70 eV. The ion source's temperature was set at 200 °C. The compounds were recognized by comparing the retention timings and mass spectra of the compounds to those in the WILEY 09 and National Institute of Standards and Technology (NIST 11) databases (Deyab et al., 2021). Metabolite identity was reported only when the matching value of the mass spectra comparison was more than 70%.

2.4. Animals and experimental design

Forty male albino Wister rats (150-200 g body weight), were obtained from a breeding unit at the Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt. Rats were randomly divided into five groups after ten days of acclimatization (eight rats each). Group (1) act as negative control and was gavaged daily for 8 weeks with a saline solution using oral gavage. Group (2): act as positive control and was orally administrated with 100 mg/kg $AlCl_3$ for eight weeks (Singh et al., 2018). Group (3): act as *B. papyrifera* treated group and was administrated with $AlCl_3$ (100 mg/kg) and *B. papyrifera methanolic* extract *(*200 mg/kg) once daily for eight weeks using oral gavage (Khajehdehi et al., 2022). Group (4): act as *S. aromaticum* treated group and was administrated with $AlCl_3$ (100 mg/kg) and *S. aromaticum methanolic* extract *(*200 mg/kg) once daily for eight weeks using oral gavage (Agboola et al., 2022). Group (5): act as *B. papyrifera +S. aromaticum* treated group and was administrated with $AlCl_3$ (100 mg/kg) and *B. papyrifera + S. aromaticum* methanolic extracts *(*200 mg/kg) once daily for eight weeks using oral gavage. The experimental design was in strict accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Veterinary Institutional Animal Care and Use Committee (VET-IACUC).

2.5. Behavioral assessment (Y-maze memory test)

After the last doses of different drugs, rats were submitted to the behavioral analysis room, acclimatized for three h, and tested using the Y-maze test. The device was made of a white wooden maze with three arms that were each 16 cm broad, 50 cm long, and 32 cm high. Each rat from a different group was positioned in one arm and allowed to go vigorously through the maze for five minutes. Four paws had to be inside the arm for entry to be considered legal. The measuring parameters were the number of arm entries and the spontaneous alternation percentage (SAP). SAP is the ability of rats to alternate between different three arms, it was calculated using the Equation 1 below (Kitanaka et al., 2015; Khalil et al., 2020):

$$[(\text{number of variations}) / (\text{total number of arms entries}-2)] \times 100 \quad (1)$$

2.6. Euthanasia and sampling

24 h after the Y-maze test, rats were sacrificed by cervical dislocation following AVMA Guidelines. Brains were excised gently and washed with cold saline, then divided into two halves. One-half was preserved in 10% neutral buffered formalin for histopathological investigation. The other half was preserved in a deep freezer at -80 °c for subsequent biochemical analysis.

2.7. Biochemical assessment

Brain samples were rinsed with physiological buffer saline (100 mM Na2HPO4/NaH2PO4, 0.16 mg/ml heparin, pH 7.4) to get rid of RBCs and clot residues. One gram of tissue samples was homogenized in 5 ml of cold phosphate-buffered saline (50 mM potassium phosphate, 1 mM of ethylenediaminetetraacetic acid [EDTA], pH 7.5) using a sonic homogenizer. All homogenates were centrifuged at 14,000 ×g for 15 min at 4 °C. The supernatant was used to measure AChE content, lipid peroxidation marker (MDA), antioxidant enzymatic activities of SOD, and the levels of GSH. These parameters were measured according to manufacturer protocols (Oxis Research, Portland, USA) (Chang et al., 2013; W. Kim et al., 2014).

2.8. Histopathological examinations

The formalinized brains were washed in tap water followed by dehydration using serial dilutions of alcohol. Specimens were then cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for 24 h. Paraffin bees' wax tissue blocks were sectioned at 4 µm thickness using a slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E). Three brain regions were evaluated in these glass slides, including the prefrontal cortex and hippocampus (CA1 and CA3) areas, and photographed using a light microscope attached to a camera (Olympus BX-53 Olympus Corporation, Tokyo, Japan) (Sevastre-Berghian et al., 2017).

2.9. Statistical analysis

one-way analysis of variance followed by post hoc test Bonferroni test was done to analyze the difference between groups using SPSS 24 software (Chicago: SPSS Inc. IBM Corp.). where ($P \leq 0.05$ considered as significant). Histograms were plotted using GraphPad Prism Version 9 (GraphPad Software Inc., La Jolla, CA, USA). Data were expressed as mean ± standard error (SE).

3. Results

3.1. GC-Ms of B. papyrifera and S. aromaticum extracts

B. papyrifera methanol extract revealed the presence of 22 bioactive compounds upon analysis using GC-MS, including eight major compounds which were: Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol, 2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroper, 1-Isopropyl-5,9,13-trimethyl-4,16-dioxatricyclo[11.2.1.03,5]hexadec-8-en-12-ol, (3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one, Nerolidol isobutyrate, (-)-Spathulenol, 1-Isopropyl-

5,9,13-trimethyl-4,16-dioxatricyclo[11.2.1.03,5]hexadec-8-en-12-ol and Retinol, acetate as shown in Table 1. Furthermore, *S. aromaticum* had 41 different compounds upon analysis using GC-MS, including eight major compounds which were: Phenol, 2-methoxy-4-(1-propenyl)-, acetate, 3-Allyl-6-methoxyphenol, 4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4S), Caryophyllene oxide, Caryophylla-4(12),8(13)-dien-5.alpha.-ol, 1H-Cycloprop[e] azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. alpha.,4.beta.,4 2.70 2',3',4'Trimethoxyacetophenon, 3.alpha.,7.beta.-Dihydroxy-5.beta.,6.beta.-epoxycholestane and (3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta[8]annulene-3 as shown in Table 2.

3.2. Behavioral assessment (Y-maze memory test)

The Y-maze test is frequently used to assess the health of rats' hippocampus (Postu et al., 2019). AD rats displayed a substantial reduction in the number of arm entries and SAP% compared to the control group (Figure 1). There was no statistically significant difference between the treated groups and the control group in the number of arm entries. However, administration of *B. papyrifera* and *S. aromaticum* alone or in combination considerably increases the SAP% compared to the AD group. When compared to the *B. papyrifera* and *S. aromaticum* treated groups, the combination group showed a rise in the number of arm entries and SAP%, although There was no statistically significant difference.

3.3. Biochemical assessment

Cortical AChE levels were markedly elevated ($p< 0.05$) in AD rats compared to the control group. However, administration of *B. papyrifera* and *S. aromaticum* individually or in combination dramatically decreased ($p< 0.05$) these levels compared AD group. However,

Table 1. Different compounds present in *Boswellia papyrifera* extract using GC-MS analysis with their retention time (RT), area percentage, and molecular mass.

Peak#	RT (min)	Area	Area%	Name	m/z
1	15.649	544128	0.17	Acetic acid, octyl ester	43.00
2	25.290	1895579	0.59	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	41.05
3	38.131	10841587	3.37	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol	43.05
4	39.311	1105805	0.34	3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)-	43.00
5	40.515	616123	0.19	1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone	43.00
6	42.715	7699172	2.40	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroper	43.00
7	42.886	17848957	5.55	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetra	43.00
8	43.349	25401485	7.91	Nerolidol isobutyrate	43.00
9	43.895	21086450	6.56	1-Isopropyl-5,9,13-trimethyl-4,16-dioxatricyclo[11.2.1.03,5] hexadec-8-en-12-ol	43.00
10	44.134	39159240	12.19	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	43.00
11	44.520	11424568	3.56	Ethyl geranyl acetate	43.00
12	45.024	30424455	9.47	Nerolidol isobutyrate	43.00
13	45.151	18310770	5.70	(-)-Spathulenol	43.00
14	45.749	22108164	6.88	1-Isopropyl-5,9,13-trimethyl-4,16-dioxatricyclo[11.2.1.03,5] hexadec-8-en-12-ol	43.00
15	45.935	8915031	2.77	Unknown	43.00
16	46.155	9440885	2.94	Ethyl geranyl acetate	43.00
17	46.375	5557849	1.73	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a	43.00
18	46.703	6848096	2.13	Retinol, acetate	43.00
19	46.875	4536434	1.41	Oleoyl chloride	55.05
20	47.095	3183673	0.99	Unkonwn	43.00
21	54.126	807020	0.25	9,19-Cyclo-27-norlanostan-25-one, 3-(acetyloxy)-24-methyl-, (3.beta.,24R)-	123.15
22	56.416	5095573	1.59	24-Norursa-3,12-dien-11-one	232.15

Table 2. Different compounds present in *S. aromaticum* extract using GC-MS analysis with their retention time (RT), area percentage, and molecular mass.

Peak#	RT (min)	Area	Area%	Name	m/z
1	20.505	823932865	57.05	Phenol, 2-methoxy-4-(1-propenyl)-, acetate	164.10
2	20.710	441447768	30.75	3-Allyl-6-methoxyphenol	164.05
3	21.377	1134395	0.08	Benzaldehyde, 3-hydroxy-4-methoxy-	152.05
4	21.749	352330	0.02	Caryophyllene	93.05
5	24.551	75659551	5.24	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	164.05
6	25.157	400846	0.03	Cyclohexane, 1,5-dimethyl-2,3-divinyl-	41.00
7	25.317	284437	0.02	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	69.05
8	25.624	495853	0.03	Caryophyllenyl alcohol	111.10
9	25.947	12349774	0.86	Caryophyllene oxide	43.0
10	26.425	1374588	0.10	Ledol	43.0
11	26.570	702554	0.05	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	43.0
12	26.655	469193	0.03	Mandelic acid, 3,4-dimethoxy-, methyl ester	167.05
13	26.769	692034	0.05	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	43.0
14	26.988	2979224	0.21	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4S	119.05
15	27.112	2505413	0.17	Caryophyllene oxide	71.05
16	27.211	3595764	0.25	Caryophylla-4(12),8(13)-dien-5.alpha.-ol	136.05
17	27.319	1289093	0.04	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.a 0.11 .alpha.-Cadinol	43.00
18	27.406	608762	0.12	Andrographolide	161.10
19	27.615	1606041	0.26	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4 2.70 2',3',4'Trimethoxyacetophenone	95.10
20	27.694	1717535	0.06	Caryophyllene oxide	41.00
21	28.014	3797711	0.10	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-	41.00
22	29.482	828750	0.25	Caryophylla-4(12),8(13)-dien-5.alpha.-ol 0.09 .tau.-Muurolol	43.00
23	29.555	1377167	0.04	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.a 0.11 .alpha.-Cadinol	137.10
24	29.959	886479	0.06	Humulenol-II	85.05
25	30.130	869528	0.06	Benzyl Benzoate	105.00
26	30.257	865127	0.06	3-Allyl-6-methoxyphenol	137.10
27	30.341	1416105	0.10	2,4,6-Trimethoxyacetophenone	85.05
28	30.516	410249	0.03	Caryophyllene oxide	105.00
29	30.798	382291	0.14	3.alpha.,7.beta.-Dihydroxy-5.beta.,6.beta.-epoxycholestane	137.10
30	31.495	437839	0.02	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	137.10
31	32.547	1702214	0.12	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol	85.05
32	36.710	282777	0.05	Oleoyl chloride	43.00
33	38.082	1663251	0.03	[1,1'-Biphenyl]-2,2'-diol, 3,3'-dimethoxy-5,5'-di-2-propenyl-	67.00
34	38.512	224803	0.03	Phenol, 2-methoxy-4-(1-propenyl)-, acetate	43.00
35	44.115	4788212	0.05	Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis[2-methoxy-	43.00
36	44.594	719862	0.03	28-Norolean-17-en-3-ol	326.10
37	44.877	1067555	0.06	Humulenol-II	326.10
38	46.825	760765	0.06	Benzyl Benzoate	382.10
39	47.036	478433	0.06	3-Allyl-6-methoxyphenol	55.05
40	47.804	1386777	0.10	2,4,6-Trimethoxyacetophenone	326.10
41	56.399	1410044	0.12	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7 methanocyclopenta[8]annulene-3	232.15

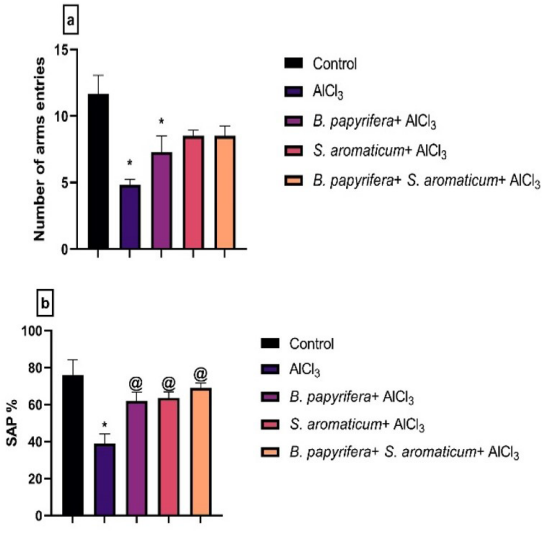

Figure 1. Effects of the *B. papyrifera* and *S. aromaticum* and/or combination on the spatial working memory of $AlCl_3$ induced rats using Y-maze test. (a) number of arm entries (b) and SAP%; spontaneous alternation. Values are means ± S.E.M. (n = 8 animals per group). Statistical significance was determined by one-way ANOVA Followed by Bonferroni's post hoc analyses – * compared to the control group; @ compared to $AlCl_3$ group ($p < 0.05$).

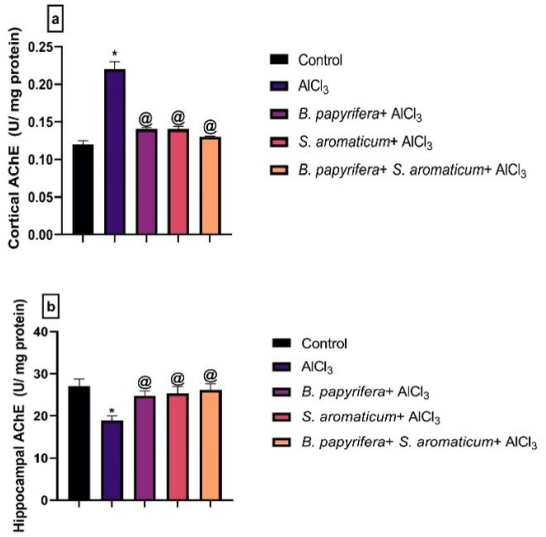

Figure 2. Effects of the *B. papyrifera* and *S. aromaticum* and/or combination on the (a) Cortical AChE; and (b) Hippocampal AChE on the brain homogenates of the $AlCl_3$ induced rats. Values are means ± S.E.M. (n = 8 animals per group). Statistical significance was determined by one-way ANOVA Followed by Bonferroni's post hoc analyses – * compared to the control group; @ compared to AlCl3 group ($p < 0.05$).

AChE levels were slightly decreased in hippocampal samples of the AD group compared to the control. While administration of these plants singly or in combination enhances AChE levels reaching normal levels as shown in Figure 2. Additionally, MDA levels were substantially elevated in cortical and hippocampal samples of AD rats ($p < 0.05$) and slightly reduced upon using *B. papyrifera* and *S. aromaticum* individually or in combination where treatment using *S. aromaticum* showed the lowest levels in the treatments as represented in Figure 3. Moreover, cortical and hippocampal SOD levels were dramatically decreased in the AD rats compared to the control group. However, the administration of *B. papyrifera* and *S. aromaticum* individually or in combination significantly increased these levels compared to AD rats (Figure 4). The cortical and hippocampal GSH levels dramatically reduced in AD rats compared to the control group. However, administration of *B. papyrifera* and *S. aromaticum* individually or in combination slightly elevated the GSH levels compared to AD rats (Figure 5).

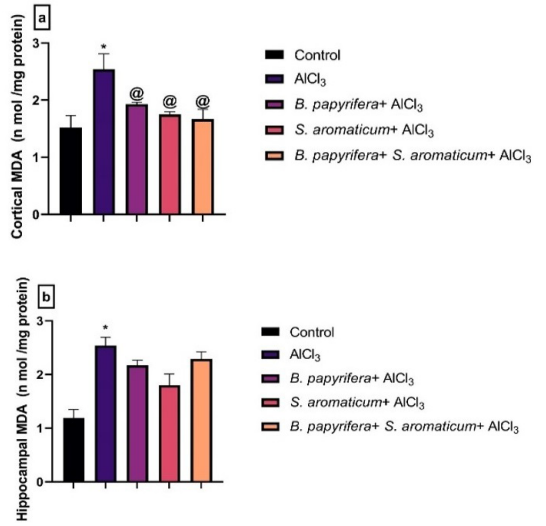

Figure 3. Effects of the B. papyrifera and S. aromaticum and/or combination on the (a) Cortical MDA, (b) and Hippocampal MDA on the brains of the AlCl3 induced rats. Values are means ± S.E.M. (n = 8 animals per group). Statistical significance was determined by one-way ANOVA Followed by Bonferroni's post hoc analyses – * compared to the control group; @ compared to $AlCl_3$ group ($p < 0.05$).

3.4. Histopathological examinations

The impact of *B. papyrifera* and *S. aromaticum* was confirmed by testing histopathological sections from both the cerebral cortex as well as hippocampus of the brains of different tested groups of animals. Rats in the control group displayed a normally distributed structure of neurons, neuroglia, and neuropil in H&E-stained cerebral cortex slices (Figure 6a). Contrarily, AD rat's cerebral cortex exhibited pyknotic pyramidal cells with neurofibrillary tangles, perineuronal gaps, and neuropil vacuolation (Figure 6b). While, examination of *B. papyrifera* rat brain sections revealed triangular-shaped pyramidal cells with nearly normal structure, vesicular stained nuclei, and some pyknotic neurons with neurofibrillary tangles could be seen (Figure 6c). Also, *S. aromaticum* rat brain sections showed deteriorated and pyknotic neuronal cells with neuropil

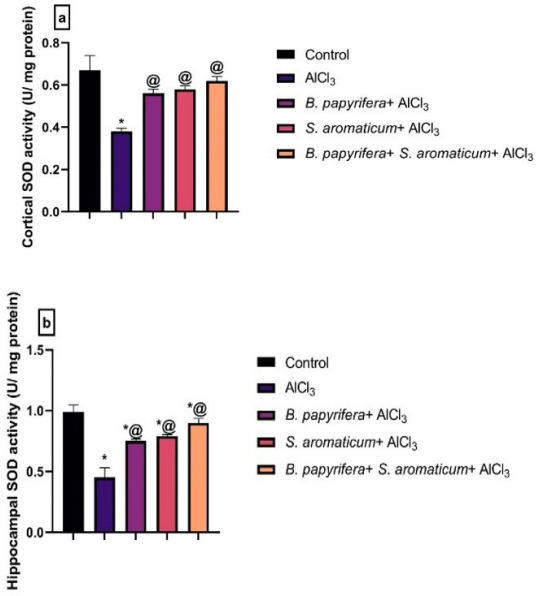

Figure 4. Effects of the B. papyrifera and S. aromaticum and/or combination on the (a) Cortical SOD; superoxide dismutase (b) and Hippocampal SOD on the brain homogenates of the $AlCl_3$ induced rats. Values are means ± S.E.M. (n = 8 animals per group). Statistical significance was determined by one-way ANOVA Followed by Bonferroni's post hoc analyses – * compared to the control group; @ compared to $AlCl_3$ group ($p < 0.05$).

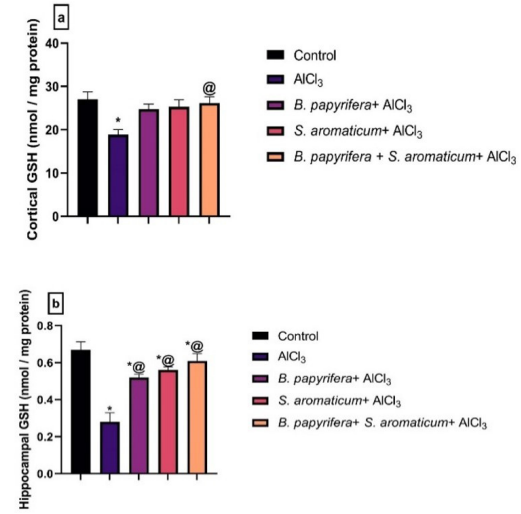

Figure 5. Effects of the B. papyrifera and S. aromaticum and/or combination on the (a) Cortical GSH, (b) and Hippocampal GSH on the brain homogenates of the $AlCl_3$ induced rats. Values are means ± S.E.M. (n = 8 animals per group). Statistical significance was determined by one-way ANOVA Followed by Bonferroni's post hoc analyses – * compared to the control group; @ compared to $AlCl_3$ group ($p < 0.05$).

vacuolation in their cerebral cortex (Figure 6d). Conversely, the cerebral cortex of rats treated with a combination of *B. papyrifera* and *S. aromaticum* displayed nearly normal triangular-shaped neurons with big vesicular nuclei and few neurons that looked pyknotic with pericellular space (Figure 6e).

Concerning the hippocampus, Rats in the control group displayed a normal three-layer structure. The neuropil's molecular layer was made up of neurons and neuroglia, the pyramidal layer was made up of triangular-shaped neurons with massive, vesicular nuclei, and the polymorphic layer was made up of neurons and neuroglia (Figure 7a). However, the hippocampus of AD rats showed numerous structural alterations and hippocampal three layers; molecular, pyramidal, and polymorphic layers respectively. The molecular layer had neuropil vacuolation. The pyramidal layer showed neuronal cells with neurofibrillary tangles and some neurons appeared pyknotic with pericellular space. Also, neuropil vacuolation was observed (Figure 7b). Hippocampus of *B. papyrifera* treated rats revealed pyramidal neurons with nearly normal triangular shapes but few neurons are still pyknotic with pericellular space. There was neuroglia pericellular space in both molecular and polymorphic layers. Perivascular space was also noticed (Figure 7c). While rat's hippocampus of *S. aromaticum* treated rats revealed degenerated pyramidal neurons with pericellular space, pyknotic neuroglia with pericellular space, and few pyramidal neurons appeared normal (Figure 7d). The hippocampus of rat's brains treated by a combination of *B. papyrifera* and *S. aromaticum* showed nearly normal-shaped pyramidal neurons with diminished neuropil vacuolation. Some neuroglia appeared pyknotic with pericellular space (Figure 7e)

4. Discussion

Herbal medicines have traditionally been used to treat AD-related illnesses and enhance cognitive performance. Numerous possible uses for plant antioxidant properties in human healthcare exist (Shudo et al., 2009; Chen et al., 2022). In our study, the ameliorative effects of a combination of B. *papyrifera* and *S. aromaticum* were investigated against AD rats. The chemical profile of these plants was identified using GC-MS analysis. It could be noticed that *B. papyrifera* has eight major compounds. Investigators have reported more than 300 bioactive molecules from alkaloids, flavonoids, and other organic compounds derived from different parts of *Broussonetia* genus (Chen et al., 2022). Shudo et al. reported the role of flavanol derivatives in the suppression of cholinesterase enzymes linked to AD (Shudo et al., 2009). Moreover, Ryu et al. explained the role of retinoids in the maintenance of neural cells and their promising roles in neurodegenerative disorders (Ryu et al., 2012). Furthermore, analysis of *S. aromaticum* methanol extract revealed the presence of eight major compounds. On the other hand, eugenol and caryophyllene were detected as major compounds of *S. aromaticum* extract with antioxidant potential (Teles et al., 2021). AD was induced in our study using $AlCl_3$. Al for both people and animals is primarily sourced from manufactured meals and drinking water. It can enter the body through a variety of medical uses, such as dental resin composites or vaccination adjuvants, skin contact, and the breathing

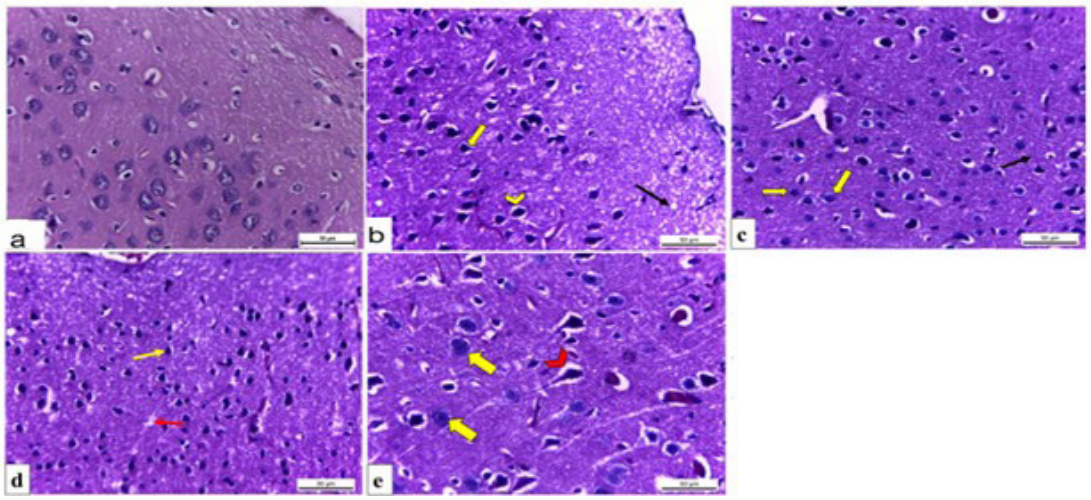

Figure 6. Cerebral cortex sections of albino rats (H&E; X400) (a) control rats had normal neurons, neuroglia, and neuropil (b) Group II rats showed pyknotic pyramidal cells with neurofibrillary tangles (yellow arrow), perineuronal space (chevron) and neuropil vacuolation (black arrow); (c) Group III rats had nearly normal pyramidal neurons (yellow arrow) and some pyknotic neurons with neurofibrillary tangles (black arrow); (d) Group IV rats showed pyknotic neurons (yellow arrow) with neuropil vacuolation (red arrow); (e) Group V rats showed nearly normal pyramidal neurons (yellow arrow) and few pyknotic neurons with pericellular space (chevron).

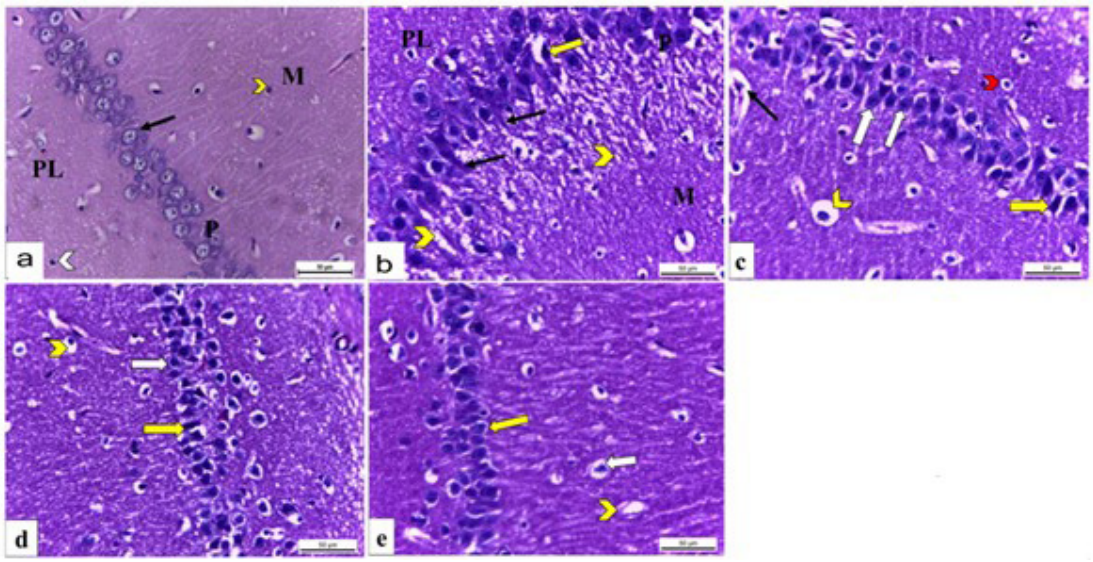

Figure 7. Hippocampus sections of albino rats (H&E X400) (a) Control rats (Group I) had normal structure of molecular (M), pyramidal (P), and polymorphic (PL) layers. normal pyramidal cells (arrow) and neuroglia (chevron) were observed; (b) Group II rats showed pyramidal neurons with neurofibrillary tangles (black arrow), pyknotic neurons with pericellular space (yellow arrow), and neuropil vacuolation (chevron); (c) Group III rats revealed nearly normal pyramidal neurons (white arrows), few pyknotic neurons (yellow arrow), and neuroglia with pericellular space (yellow chevron) in the molecular layer and the polymorphic layer (red chevron); (d) Group IV rats had few nearly normal pyramidal neurons (white arrow), pyknotic degenerated neurons with perivascular space (yellow arrow), and pyknotic neuroglia with pericellular space (chevron); (e) Group V rats showed nearly normal-shaped pyramidal neurons (yellow arrow), and diminished neuropil vacuolation. Some neuroglia appeared pyknotic with pericellular space (white arrow).

of dust (Ausiello et al., 2013; Newairy et al., 2009). In our investigation, the spatial working memory of the AD group was severely diminished as reflected by the decrease in the number of arm entries and the decrease in the SAP%. The SAP depends on the natural tendency of the rats to alternate between different three arms. However, treatment with B. *papyrifera* and *S. aromaticum* and/or a combined group showed a substantial improvement in

spatial working memory as visualized by an increment in the SAP%. These results were in agreement with other previous studies that reported the roles of natural products such as *Pinus halepensis* and coconut oil in neuroprotection due to their antioxidant capabilities (Postu et al., 2019; Khalil et al., 2020).

The role of $AlCl_3$ could be explained through an allosteric association between Al and the peripheral anionic position of the enzyme molecule, exposure to $AlCl_3$ boosted cholinesterase activity (Abd-Elhady et al., 2013). Although the pathways causing neuronal loss are not fully understood, the idea of apoptosis has been put forth (Sargholi Nootarki et al., 2015). However, the administration of B. *papyrifera* and *S. aromaticum* singly or in combination enhances AChE activity compared to the AD group. The bioactive components in *X. parviflora* extract may be able to prevent the death of neurons by acting as antioxidants and anti-apoptotic agents (Shaba, 2017).

Oxidative stress is one of the main hypotheses that was responsible for declining brain performance in AD rats (Yuan et al., 2012). Chronic Al intake alters SOD, CAT, and GSH levels, and their activities drastically dropped with rising MDA levels (Breijyeh and Karaman, 2020). However, administration of B. *papyrifera* and *S. aromaticum* singly or in combination reduces MDA levels and enhances SOD and GSH levels compared to the AD group. *S. aromaticum* was discovered to be capable of regulating scavenging reactive oxygen species (ROS), while also raising the fraction of anti-oxidant mechanisms, as demonstrated by a previous study (Shekhar et al., 2018). In accordance with Rajabian et al., reported the molecules derived from Boswellia species to modulate mechanisms regulating neurodegenerative disorders (Rajabian et al., 2020). Furthermore, Miran et al., reported that *B. sacra* is an essential origin of terpenoids with biomedical applications for many diseases (Miran et al., 2022). Besides, Amir Rawa et al. identified several molecular mechanisms used by Syzygium species in neuroprotection, including suppression of pro-inflammatory mediators, prevention of microglial invasion, and modulation of ß-cell insulin release (Amir Rawa et al., 2022). Also, some studies proposed that the bioactive molecules in herbal extracts protect sensitive neurons, increase memory and blood flow, stimulate neural performance, and stimulate neurogenesis by decreasing ROS accumulation (Botton et al., 2010; Spencer, 2010; Yadang et al., 2020). For instance, Botton and his colleagues investigate the memory enhancer role of caffeine against scopolamine in adult mice (Botton et al., 2010). Also, Spencer demonstrates the mechanisms of flavonoids in the brain (Spencer, 2010). While Yadang and his colleagues found a beneficial role for Carissa edulis (Forssk.) against AD induced by scopolamine in mice (Yadang et al., 2020).

These changes were associated with neurofibrillary tangles, perineuronal gaps, and neuropil vacuolation in both the cortex and the hippocampus of AD rats. Alzheimer's patients' brains comprise amyloid "plaques" and neurofibrillary tau protein "tangles," as well as significant loss of neurons in different areas of the brain (Walton, 2007). Neurons and their connections in brain regions, including entorhinal cortex and hippocampus are frequently destroyed by AD. Whereas the hippocampus rapidly loses structure, which is related to the functional separation from other brain regions (Carmona and Pereira, 2013; Rao et al., 2022). However, the administration of B. *papyrifera* and *S. aromaticum* singly or in combination was able to reverse these changes and maintain the brain architecture. These findings were in agreement with previous studies (Khalil et al., 2020; Postu et al., 2019), who reported the roles of natural products such as *Pinus halepensis* and coconut oil in maintaining the brain architecture due to their antioxidant capabilities (Postu et al., 2019; Khalil et al., 2020).

5. Conclusion

Herbs have a long history of usefulness and safety, which is probably because of their variety of constituent parts and how these parts engage with the body's various physiological goals. The present study highlighted some of the proposed functions of *B. papyrifera* and S. *aromaticum* extracts to enhance the status of AD which is a growing disease in many communities. These combinations improve cognitive dysfunction, enhance AChE activity, reduce the cortical and hippocampal MDA levels, and increase the SOD and GSH levels. These changes were associated with restoring the cortical and hippocampal normal structure. The limitation of this study is that we did not use LC-MS analysis to verify the neuroprotective activities of the extracts. Therefore, future research could be done separately on these extracts to include LC-MS analysis and to explore their underlying mechanisms and different molecular pathways.

Acknowledgements

This work was funded by the University of Jeddah, Jeddah, Saudi Arabia, under grant No. (UJ-22-DR-29). The author, therefore, acknowledges with thanks the University of Jeddah for its technical and financial support.

Ethical approval

The experimental design was in strict accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Veterinary Institutional Animal Care and Use Committee (VET-IACUC).

References

ABD-ELHADY, R.M., ELSHEIKH, A.M. and KHALIFA, A.E., 2013. Anti-amnestic properties of Ginkgo biloba extract on impaired memory function induced by aluminum in rats. *International Journal of Developmental Neuroscience*, vol. 31, no. 7, pp. 598-607. http://dx.doi.org/10.1016/j.ijdevneu.2013.07.006. PMid:23933390.

AGBOOLA, J.B., EHIGIE, A.F., EHIGIE, L.O., OJENIYI, F.D. and OLAYEMI, A.A., 2022. Ameliorative role of *Syzygium aromaticum* aqueous extract on synaptosomal tyrosine hydroxylase activity, oxidative stress parameters, and behavioral changes in lead-induced

neurotoxicity in mice. *Journal of Food Biochemistry*, vol. 46, no. 7, e14115. http://dx.doi.org/10.1111/jfbc.14115. PMid:35246863.

AMIR RAWA, M.S., MAZLAN, M.K.N., AHMAD, R., NOGAWA, T. and WAHAB, H.A., 2022. Roles of Syzygium in anti-cholinesterase, anti-diabetic, anti-inflammatory, and antioxidant: from Alzheimer's perspective. *Plants*, vol. 11, no. 11, pp. 1476. http://dx.doi.org/10.3390/plants11111476. PMid:35684249.

AUSIELLO, P., CASSESE, A., MIELE, C., BEGUINOT, F., GARCIA-GODOY, F., DI JESO, B. and ULIANICH, L., 2013. Cytotoxicity of dental resin composites: an in vitro evaluation. *Journal of Applied Toxicology: JAT*, vol. 33, no. 6, pp. 451-457. http://dx.doi.org/10.1002/jat.1765. PMid:22120598.

BATIHA, G.E.-S., ALKAZMI, L.M., WASEF, L.G., BESHBISHY, A.M., NADWA, E.H. and RASHWAN, E.K., 2020. *Syzygium aromaticum* L. (Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, vol. 10, no. 2, pp. 202. http://dx.doi.org/10.3390/biom10020202. PMid:32019140.

BOTTON, P.H., COSTA, M.S., ARDAIS, A.P., MIORANZZA, S., SOUZA, D.O., DA ROCHA, J.B.T. and PORCIÚNCULA, L.O., 2010. Caffeine prevents disruption of memory consolidation in the inhibitory avoidance and novel object recognition tasks by scopolamine in adult mice. *Behavioural Brain Research*, vol. 214, no. 2, pp. 254-259. http://dx.doi.org/10.1016/j.bbr.2010.05.034. PMid:20553765.

BREIJYEH, Z. and KARAMAN, R., 2020. Comprehensive review on Alzheimer's disease: causes and treatment. *Molecules*, vol. 25, no. 24, pp. 5789. http://dx.doi.org/10.3390/molecules25245789. PMid:33302541.

CAMPBELL, A., 2002. The potential role of aluminium in Alzheimer's disease. *Nephrology, Dialysis, Transplantation*, vol. 17, suppl. 2, pp. 17-20. http://dx.doi.org/10.1093/ndt/17.suppl_2.17. PMid:11904353.

CARMONA, F. and PEREIRA, A.M.S., 2013. Herbal medicines: old and new concepts, truths and misunderstandings. *Revista Brasileira de Farmacognosia*, vol. 23, no. 2, pp. 379-385. http://dx.doi.org/10.1590/S0102-695X2013005000018.

CHANG, D., ZHANG, X., RONG, S., SHA, Q., LIU, P., HAN, T. and PAN, H., 2013. Serum antioxidative enzymes levels and oxidative stress products in age-related cataract patients. *Oxidative Medicine and Cellular Longevity*, vol. 2013, pp. 587826. http://dx.doi.org/10.1155/2013/587826. PMid:23781296.

CHEN, Y., WANG, L., LIU, X., WANG, F., AN, Y., ZHAO, W., TIAN, J., KONG, D., ZHANG, W., XU, Y., BA, Y. and ZHOU, H., 2022. The genus *Broussonetia*: an updated review of phytochemistry, pharmacology and applications. *Molecules*, vol. 27, no. 16, pp. 5344. http://dx.doi.org/10.3390/molecules27165344. PMid:36014582.

CUMMINGS, J., LEE, G., RITTER, A., SABBAGH, M. and ZHONG, K., 2019. Alzheimer's disease drug development pipeline: 2019. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, vol. 5, no. 1, pp. 272-293. http://dx.doi.org/10.1016/j.trci.2019.05.008. PMid:31334330.

DEYAB, M.A., EL-SHEEKH, M.M., HASAN, R.S.A., ELSADANY, A.Y. and ABU AHMED, S.E.-S., 2021. Phytochemical components of two cyanobacterial local strains. *Scientific Journal for Damietta Faculty of Science*, vol. 11, no. 1, pp. 67-75. http://dx.doi.org/10.21608/sjdfs.2021.195593.

FARSHCHI, A., GHIASI, G., FARSHCHI, S. and MALEK KHATABI, P., 2010. Effects of *Boswellia papyrifera* gum extract on learning and memory in mice and rats. *Iranian Journal of Basic Medical Sciences.*, vol. 13, no. 2, pp. 9-15. http://dx.doi.org/10.22038/ijbms.2010.5075.

GUPTA, Y.K., MEENU, M. and PESHIN, S.S., 2019. Aluminium utensils: is it a concern. *The National Medical Journal of India*, vol. 32, no. 1, pp. 38-40. http://dx.doi.org/10.4103/0970-258X.272116. PMid:31823940.

HEO, H.J., KIM, M.-J., LEE, J.-M., CHOI, S.J., CHO, H.-Y., HONG, B., KIM, H.-K., KIM, E. and SHIN, D.-H., 2004. Naringenin from Citrus junos has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia. *Dementia and Geriatric Cognitive Disorders*, vol. 17, no. 3, pp. 151-157. http://dx.doi.org/10.1159/000076349. PMid:14739537.

HOUGHTON, P.J., REN, Y. and HOWES, M.-J., 2006. Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Reports*, vol. 23, no. 2, pp. 181-199. http://dx.doi.org/10.1039/b508966m. PMid:16572227.

HUKINS, D., MACLEOD, U. and BOLAND, J.W., 2019. Identifying potentially inappropriate prescribing in older people with dementia: a systematic review. *European Journal of Clinical Pharmacology*, vol. 75, no. 4, pp. 467-481. http://dx.doi.org/10.1007/s00228-018-02612-x. PMid:30610274.

KAWAHARA, M., 2005. Effects of aluminum on the nervous system and its possible link with neurodegenerative diseases. *Journal of Alzheimer's Disease*, vol. 8, no. 2, pp. 171-182. http://dx.doi.org/10.3233/JAD-2005-8210. PMid:16308486.

KHAJEHDEHI, M., KHALAJ-KONDORI, M. and BARADARAN, B., 2022. Molecular evidences on anti-inflammatory, anticancer, and memory-boosting effects of frankincense. *Phytotherapy Research*, vol. 36, no. 3, pp. 1194-1215. http://dx.doi.org/10.1002/ptr.7399. PMid:35142408.

KHALIL, H.M.A., SALAMA, H.H., AL-MOKADDEM, A.K., ALJUAYDI, S.H. and EDRIS, A.E., 2020. Edible dairy formula fortified with coconut oil for neuroprotection against aluminium chloride-induced Alzheimer's disease in rats. *Journal of Functional Foods*, vol. 75, pp. 104296. http://dx.doi.org/10.1016/j.jff.2020.104296.

KIM, J.K., BAE, H., KIM, M.-J., CHOI, S.J., CHO, H.Y., HWANG, H.-J., KIM, Y.J., LIM, S.T., KIM, E.K., KIM, H.K., KIM, B.Y. and SHIN, D.-H., 2009. Inhibitory effect of *Poncirus trifoliate* on acetylcholinesterase and attenuating activity against trimethyltin-induced learning and memory impairment. *Bioscience, Biotechnology, and Biochemistry*, vol. 73, no. 5, pp. 1105-1112. http://dx.doi.org/10.1271/bbb.80859. PMid:19420715.

KIM, W., KIM, D.W., YOO, D.Y., JUNG, H.Y., NAM, S.M., KIM, J.W., HONG, S.-M., KIM, D.-W., CHOI, J.H., MOON, S.M., YOON, Y.S. and HWANG, I.K., 2014. *Dendropanax morbifera* Léveille extract facilitates cadmium excretion and prevents oxidative damage in the hippocampus by increasing antioxidant levels in cadmium-exposed rats. *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, pp. 428. http://dx.doi.org/10.1186/1472-6882-14-428. PMid:25362479.

KITANAKA, J., KITANAKA, N., HALL, F.S., FUJII, M., GOTO, A., KANDA, Y., KOIZUMI, A., KUROIWA, H., MIBAYASHI, S., MURANISHI, Y., OTAKI, S., SUMIKAWA, M., TANAKA, K.-I., NISHIYAMA, N., UHL, G.R. and TAKEMURA, M., 2015. Memory impairment and reduced exploratory behavior in mice after administration of systemic morphine. *Journal of Experimental Neuroscience*, vol. 9, pp. 27-35. http://dx.doi.org/10.4137/JEN.S25057. PMid:25987850.

LOIZZO, M.R., TUNDIS, R., MENICHINI, F. and MENICHINI, F., 2008. Natural products and their derivatives as cholinesterase inhibitors in the treatment of neurodegenerative disorders: an update. *Current Medicinal Chemistry*, vol. 15, no. 12, pp. 1209-1228. http://dx.doi.org/10.2174/092986708784310422. PMid:18473814.

MAHMOUDI, A., HOSSEINI-SHARIFABAD, A., MONSEF-ESFAHANI, H.R., YAZDINEJAD, A.R., KHANAVI, M., ROGHANI, A., BEYER, C. and SHARIFZADEH, M., 2011. Evaluation of systemic administration

of *Boswellia papyrifera* extracts on spatial memory retention in male rats. *Journal of Natural Medicines*, vol. 65, no. 3-4, pp. 519-525. http://dx.doi.org/10.1007/s11418-011-0533-y. PMid:21479965.

MELESIE TAYE, G., BULE, M., ALEMAYEHU GADISA, D., TEKA, F. and ABULA, T., 2020. In vivo antidiabetic activity evaluation of aqueous and 80% methanolic extracts of leaves of *Thymus schimperi* (Lamiaceae) in alloxan-induced diabetic mice. *Diabetes, Metabolic Syndrome and Obesity*, vol. 13, pp. 3205-3212. http://dx.doi.org/10.2147/DMSO.S268689. PMid:32982351.

MIRAN, M., AMIRSHAHROKHI, K., AJANII, Y., ZADALI, R., RUTTER, M.W., ENAYATI, A. and MOVAHEDZADEH, F., 2022. Taxonomical investigation, chemical composition, traditional use in medicine, and pharmacological activities of *Boswellia sacra* Flueck. *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, pp. 8779676. http://dx.doi.org/10.1155/2022/8779676. PMid:35222678.

NEWAIRY, A.-S.A., SALAMA, A.F., HUSSIEN, H.M. and YOUSEF, M.I., 2009. Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, vol. 47, no. 6, pp. 1093-1098. http://dx.doi.org/10.1016/j.fct.2009.01.032. PMid:19425229.

NIU, Q., YANG, Y., ZHANG, Q., NIU, P., HE, S., DI GIOACCHINO, M., CONTI, P. and BOSCOLO, P., 2007. The relationship between Bcl-gene expression and learning and memory impairment in chronic aluminum-exposed rats. *Neurotoxicity Research*, vol. 12, no. 3, pp. 163-169. http://dx.doi.org/10.1007/BF03033913. PMid:17967740.

OJO, A.B., GYEBI, G.A., ALABI, O., IYOBHEBHE, M., KAYODE, A.B., NWONUMA, C.O. and OJO, O.A., 2022. *Syzygium aromaticum* (L.) Merr. & L.M.Perry mitigates iron-mediated oxidative brain injury via *in vitro*, *ex vivo*, and *in silico* approaches. *Journal of Molecular Structure*, vol. 1268, pp. 133675. http://dx.doi.org/10.1016/j.molstruc.2022.133675.

PANAHZADEH, F., MIRNASURI, R. and RAHMATI, M., 2022. Exercise and Syzygium aromaticum reverse memory deficits, apoptosis and mitochondrial dysfunction of the hippocampus in Alzheimer's disease. *Journal of Ethnopharmacology*, vol. 286, pp. 114871. http://dx.doi.org/10.1016/j.jep.2021.114871. PMid:34856360.

PLATT, B., FIDDLER, G., RIEDEL, G. and HENDERSON, Z., 2001. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain Research Bulletin*, vol. 55, no. 2, pp. 257-267. http://dx.doi.org/10.1016/S0361-9230(01)00511-1. PMid:11470325.

POSTU, P.A., SADIKI, F.Z., EL IDRISSI, M., CIOANCA, O., TRIFAN, A., HANCIANU, M. and HRITCU, L., 2019. Pinus halepensis essential oil attenuates the toxic Alzheimer's amyloid beta (1-42)-induced memory impairment and oxidative stress in the rat hippocampus. *Biomedicine and Pharmacotherapy*, vol. 112, pp. 108673. http://dx.doi.org/10.1016/j.biopha.2019.108673. PMid:30784941.

PRATICÒ, D., 2002. Lipid peroxidation and the aging process. *Science of Aging Knowledge Environment*, vol. 2002, no. 50, pp. re5. http://dx.doi.org/10.1126/sageke.2002.50.re5. PMid:14603026.

RAJABIAN, A., SADEGHNIA, H., FANOUDI, S. and HOSSEINI, A., 2020. Genus Boswellia as a new candidate for neurodegenerative disorders. *Iranian Journal of Basic Medical Sciences.*, vol. 23, no. 3, pp. 277-286. http://dx.doi.org/10.22038/IJBMS.2020.35288.8419. PMid:32440312.

RAO, Y.L., GANARAJA, B., MURLIMANJU, B.V., JOY, T., KRISHNAMURTHY, A. and AGRAWAL, A., 2022. Hippocampus and its involvement in Alzheimer's disease: a review. *3 Biotech*, vol. 12, no. 2, pp. 55. http://dx.doi.org/10.1007/s13205-022-03123-4. PMid:35116217.

RIFAT, S.L., EASTWOOD, M.R., MCLACHLAN, D.R. and COREY, P.N., 1990. Effect of exposure of miners to aluminium powder. *Lancet*, vol. 336, no. 8724, pp. 1162-1165. http://dx.doi.org/10.1016/0140-6736(90)92775-D. PMid:1978033.

RYU, H.W., CURTIS-LONG, M.J., JUNG, S., JEONG, I.Y., KIM, D.S., KANG, K.Y. and PARK, K.H., 2012. Anticholinesterase potential of flavonols from paper mulberry (*Broussonetia papyrifera*) and their kinetic studies. *Food Chemistry*, vol. 132, no. 3, pp. 1244-1250. http://dx.doi.org/10.1016/j.foodchem.2011.11.093. PMid:29243607.

SANTOS, T.C., GOMES, T.M., PINTO, B.A.S., CAMARA, A.L. and PAES, A.M.A., 2018. Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. *Frontiers in Pharmacology*, vol. 9, pp. 1192. http://dx.doi.org/10.3389/fphar.2018.01192. PMid:30405413.

SARGHOLI NOOTARKI, Z., KESMATI, M. and POORMEHDI BORUJENI, M., 2015. Effect of magnesium oxide nanoparticles on atropine-induced memory impairment in adult male mice. *Avicenna Journal of Neuropsychophysiology*, vol. 2, no. 4, pp. 106-112. http://dx.doi.org/10.17795/ajnpp-36924.

SCHELTENS, P., BLENNOW, K., BRETELER, M.M.B., DE STROOPER, B., FRISONI, G.B., SALLOWAY, S. and VAN DER FLIER, W.M., 2016. Alzheimer's disease. *Lancet*, vol. 388, no. 10043, pp. 505-517. http://dx.doi.org/10.1016/S0140-6736(15)01124-1. PMid:26921134.

SCHMIECH, M., ULRICH, J., LANG, S.J., BÜCHELE, B., PAETZ, C., ST-GELAIS, A., SYROVETS, T. and SIMMET, T., 2021. 11-keto-α-boswellic acid, a novel triterpenoid from *Boswellia* spp. with chemotaxonomic potential and antitumor activity against triple-negative breast cancer cells. *Molecules*, vol. 26, no. 2, pp. 366. http://dx.doi.org/10.3390/molecules26020366. PMid:33445710.

SEVASTRE-BERGHIAN, A.C., FĂGĂRĂSAN, V., TOMA, V.A., BÂLDEA, I., OLTEANU, D., MOLDOVAN, R., DECEA, N., FILIP, G.A. and CLICHICI, S.V., 2017. Curcumin reverses the diazepam-induced cognitive impairment by modulation of oxidative stress and ERK 1/2/NF-κB pathway in brain. *Oxidative Medicine and Cellular Longevity*, vol. 2017, pp. 3037876. http://dx.doi.org/10.1155/2017/3037876. PMid:29098059.

SHABA, E., 2017. Nutritive and anti-nutritive composition of *Xylopia parviflora* seed obtained from Pati Shabakolo in Lavun Local Government Area, Niger State, Nigeria. *Cell Biology*, vol. 5, no. 5, pp. 53-56.

SHEKHAR, S., YADAV, Y., SINGH, A.P., PRADHAN, R., DESAI, G.R., DEY, A.B. and DEY, S., 2018. Neuroprotection by ethanolic extract of Syzygium aromaticum in Alzheimer's disease like pathology via maintaining oxidative balance through SIRT1 pathway. *Experimental Gerontology*, vol. 110, pp. 277-283. http://dx.doi.org/10.1016/j.exger.2018.06.026. PMid:29959974.

SHUDO, K., FUKASAWA, H., NAKAGOMI, M. and YAMAGATA, N., 2009. Towards retinoid therapy for Alzheimer's disease. *Current Alzheimer Research*, vol. 6, no. 3, pp. 302-311. http://dx.doi.org/10.2174/156720509788486581. PMid:19519313.

SINGH, N.A., BHARDWAJ, V., RAVI, C., RAMESH, N., MANDAL, A.K.A. and KHAN, Z.A., 2018. EGCG nanoparticles attenuate aluminum chloride induced neurobehavioral deficits, beta amyloid and tau pathology in a rat model of Alzheimer's disease. *Frontiers in Aging Neuroscience*, vol. 10, pp. 244. http://dx.doi.org/10.3389/fnagi.2018.00244. PMid:30150930.

SINGH, N.A., MANDAL, A.K.A. and KHAN, Z.A., 2016. Potential neuroprotective properties of epigallocatechin-3-gallate

(EGCG). *Nutrition Journal*, vol. 15, no. 1, pp. 60. http://dx.doi.org/10.1186/s12937-016-0179-4. PMid:27268025.

SPENCER, J.P.E., 2010. Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. *The Proceedings of the Nutrition Society*, vol. 69, no. 2, pp. 244-260. http://dx.doi.org/10.1017/S0029665110000054. PMid:20158941.

TELES, A.M., SILVA-SILVA, J.V., FERNANDES, J.M.P., ABREU-SILVA, A.L., CALABRESE, K.S., MENDES FILHO, N.E., MOUCHREK, A.N. and ALMEIDA-SOUZA, F., 2021. GC-MS characterization of antibacterial, antioxidant, and antitrypanosomal activity of syzygium aromaticum essential oil and eugenol. *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, pp. 6663255. http://dx.doi.org/10.1155/2021/6663255. PMid:33688364.

UDDIN, M.S., AL MAMUN, A., KABIR, M.T., JAKARIA, M., MATHEW, B., BARRETO, G.E. and ASHRAF, G.M., 2019. Nootropic and anti-Alzheimer's actions of medicinal plants: molecular insight into therapeutic potential to alleviate Alzheimer's neuropathology. *Molecular Neurobiology*, vol. 56, no. 7, pp. 4925-4944. http://dx.doi.org/10.1007/s12035-018-1420-2. PMid:30414087.

WALTON, J.R., 2007. An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration. *Journal of Inorganic Biochemistry*, vol. 101, no. 9, pp. 1275-1284. http://dx.doi.org/10.1016/j.jinorgbio.2007.06.001. PMid:17662457.

YADANG, F.S.A., NGUEZEYE, Y., KOM, C.W., BETOTE, P.H.D., MAMAT, A., TCHOKOUAHA, L.R.Y., TAIWÉ, G.S., AGBOR, G.A. and BUM, E.N., 2020. Scopolamine-Induced memory impairment in mice: neuroprotective effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) aqueous extract. *International Journal of Alzheimer's Disease*, vol. 2020, pp. 6372059. http://dx.doi.org/10.1155/2020/6372059. PMid:32934845.

YIANNOPOULOU, K.G. and PAPAGEORGIOU, S.G., 2020. Current and future treatments in Alzheimer disease: an update. *Journal of Central Nervous System Disease*, vol. 12, pp. 1179573520907397. http://dx.doi.org/10.1177/1179573520907397. PMid:32165850.

YUAN, C.-Y., LEE, Y.-J. and HSU, G.-S.W., 2012. Aluminum overload increases oxidative stress in four functional brain areas of neonatal rats. *Journal of Biomedical Science*, vol. 19, no. 1, pp. 51. http://dx.doi.org/10.1186/1423-0127-19-51. PMid:22613782.

ZATTA, P., LUCCHINI, R., VAN RENSBURG, S.J. and TAYLOR, A., 2003. The role of metals in neurodegenerative processes: aluminum, manganese, and zinc. *Brain Research Bulletin*, vol. 62, no. 1, pp. 15-28. http://dx.doi.org/10.1016/S0361-9230(03)00182-5. PMid:14596888.