**Balanites aegyptiaca** Extract to Treat Risk Factors of Alzheimer’s Disease: an *in vitro* Study

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**Abstract**

Alzheimer’s disease is a neurological disorder causing memory loss and cognitive decline. The purpose of this study was investigated the ability of *B. aegyptiaca* crude extract in treating and/or controlling Alzheimer’s disease risk factors based on its inhibitory effect on neurotransmitter hydrolysing enzymes (AChE and butyrylcholine esterase BuChE)), amyloid accumulation-related factor (tyrosinase), antioxidant and anti-inflammatory characteristics. The inhibitory effect of the crude extract of *Balanites aegyptiaca* dates was tested *in vitro* against three Alzheimer’s disease biomarkers—i.e., acetylcholine esterase, butyrylcholine esterase, and tyrosinase. In addition, *B. aegyptiaca* extract was examined for its antioxidant activity by several methods and for its anti-inflammatory effect by its inhibition of cyclooxygenase (COX)-1 and COX-2. Results demonstrated that *B. aegyptiaca* extract successfully inhibited acetylcholine esterase (IC₅₀; 193.78 ± 10.50 µg/mL), butyrylcholine esterase (IC₅₀; 490.91 ± 15.45 µg/mL), and tyrosinase (IC₅₀; 1.97±0.08, 9.61 ± 0.11, and 12.03 ± 0.90 μg/mL at incubation times of 10, 20, and 40 min, respectively); it also showed selective anti-inflammatory effect against COX-2 not COX-1. Additionally, *B. aegyptiaca* extract recorded potent antioxidant properties, including free radicals and oxygen reactive species (ROS) scavenging, metal chelation, reducing capability, and lipid peroxidation inhibition. These activities may be attributed to its unique chemical composition containing alkaloids, phenols, flavonoids, and coumarins. The results of this study suggest that *B. aegyptiaca* extract showed promise anti-Alzheimer’s disease activity *in vitro*, qualified it to incorporate in advanced preclinical trials for discovering alternative herbal medications.

**Keywords:** Alzheimer’s disease; *Balanites aegyptiaca* crude extract; tyrosinase activity; choline esterase ability; antioxidant characters; selective inti-inflammatory effect.

**Introduction**

Alzheimer’s disease is a neurological deficit wherein neuronal death causes memory loss and cognitive decline. About 60%–70% cases of dementia are attributed to Alzheimer’s disease.[1] Alzheimer’s disease has many risk factors such as age, family history, genetic factors, head injury, heart–head connection, and senility. Alzheimer’s disease is not considered to be a phase in normal aging; however, age is a known risk factor of Alzheimer’s disease. [2] Alzheimer’s disease symptoms begin slowly and worsen over time until they deteriorate and impede even daily tasks. Alzheimer’s disease has four stages: pre-dementia, early, moderate, and advanced stages. The pre-dementia stage is correlated with conditions of aging or stress, and it is described by moderate cognitive problems, which can disrupt most of the complex daily human activities. [3] In the early stage, learning skills and memory are more impaired, and Alzheimer’s disease can be diagnosed. In the moderate stage, symptoms become progressively worse, and the patient is unable to practice daily activities without help. In addition, some skills including speech, reading, and writing become fragmented and are progressively lost. Long-term memory impairments eventually lead to the inability to recognize relatives. In the advanced stage of the...
disease, Alzheimer’s patients lose self-dependence and speech and can become aggressive, apathetic, exhausted, bedridden, and unable to feed themselves. Nevertheless, Alzheimer’s disease itself does not cause death. [4] Current drugs for Alzheimer’s disease focus on symptoms and are concentrated on treating cognitive disorders that affect memory, language, judgment, and cognitive processes without treating other disease problems. In addition, the side effects of these drugs pose an additional concern. [5] Tyrosinase (EC 1.14.18.1) is the enzyme catalyzes the conversion of amino acid tyrosine to 3,4-dihydroxyphenylalanine (DOPA) that is an important and rate-limiting enzyme in melanin production. Additionally, tyrosinase can oxidize the catechol ring of dopamine to the highly reactive species dopamine-quinone. Dopamine-quinone can react with the sulfhydryl group of cysteine residues in the cytosol and form protein adducts, thus leading to irreversible changes or suppression of protein roles. Dopamine oxidation suppress dopamine movement, glutamate movement and mitochondrial respiration. Cytosolic quinones can invade the nucleus and make DNA alterations. Therefore, tyrosinase has the potential to damage neurons by producing dopamine-quinones. [6-7] Tyrosinase inhibitors are groups of drugs that can inhibit the tyrosinase activity. Despite the existence of a large number of tyrosinase inhibitors, to date only a few are marketed as safe. Thus, the search for new tyrosinase inhibitors is important. [8] Tyrosinase inhibitors produced from natural origins have attracted broad attention from investigators because of their lower toxicity and better bioavailability. Natural materials as bacteria, plants and fungi have gradually become a research hotspot to extract tyrosinase inhibitors. Some plant extracts and fungi have gradually become a research hotspot to investigate the ability of plant extracts and fungi to inhibit tyrosinase. [9] Many studies have investigated the biological activities of B aegyptiaca fruit extracts and demonstrated its antioxidant, hepatoprotective, and hypoglycaemic activities. [12] The current study is an in vitro study, aimed to investigate the ability of B. aegyptiaca crude extract in treating and/or controlling Alzheimer’s disease risk factors based on its inhibitory effect on neurotransmitter hydrolysing enzymes (AChE and butyrylcholine esterase BuChE), amyloid accumulation-related factor (tyrosinase), antioxidant and anti-inflammatory characteristics.

**TABLE 1. Phenolic compounds occur in Balanites crude extract.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Area</th>
<th>µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic Acid</td>
<td>1030.78</td>
<td>2573.25</td>
</tr>
<tr>
<td>Chlorgenic acid</td>
<td>110.08</td>
<td>251.23</td>
</tr>
<tr>
<td>Caffeine</td>
<td>85.17</td>
<td>92.03</td>
</tr>
<tr>
<td>Syringic Acid</td>
<td>27.73</td>
<td>39.65</td>
</tr>
<tr>
<td>Rutin</td>
<td>37.26</td>
<td>212.31</td>
</tr>
<tr>
<td>Propyl Gallate</td>
<td>60.93</td>
<td>55.53</td>
</tr>
<tr>
<td>4’,7-Dihydroxy</td>
<td>66.90</td>
<td>64.32</td>
</tr>
<tr>
<td>Quercetin</td>
<td>95.17</td>
<td>287.79</td>
</tr>
<tr>
<td>Cinnamic Acid</td>
<td>104.57</td>
<td>32.67</td>
</tr>
</tbody>
</table>

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TABLE 2. NO scavenging and Lipid peroxidation inhibition activity of *Balanites aegyptiaca* crude extract compared to Ascorbic Acid and BHT.

<table>
<thead>
<tr>
<th>Concentrations (ug/ml)</th>
<th>NO scavenging activity</th>
<th>Lipid peroxidation inhibition activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>B. aegyptiaca</em></td>
<td>Ascorbic Acid</td>
</tr>
<tr>
<td>100</td>
<td>18.63±0.70</td>
<td>64.39±1.61</td>
</tr>
<tr>
<td>250</td>
<td>26.58±0.60</td>
<td>78.68±1.70</td>
</tr>
<tr>
<td>500</td>
<td>31.59±0.70</td>
<td>86.15±1.95</td>
</tr>
<tr>
<td>750</td>
<td>35.04±1.13</td>
<td>90.25±2.00</td>
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<tr>
<td>1000</td>
<td>37.64±1.41</td>
<td>93.27±2.73</td>
</tr>
<tr>
<td>IC50</td>
<td>2350.243</td>
<td>19.96</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE. Data were analyzed by t-test one-way (n = 3 replicates). A p-value < 0.05 was considered to be statistically significant. Data are followed with small letter; a means significant difference with Ascorbic acid, b means significant difference with BHT. (BHT: butylated hydroxytoluene).

**Figure. 1** Tyrosinase inhibition activity of different concentrations (100-1000 μg/mL) of *B. aegyptiaca* crude extract compared to reference materials Kojic acid. Data presented as mean ± SE. t-test was used for data analysis (n=3, p<0.05). Data are followed with small letter; a means significant difference with Kojic acid.

**Figure. 2** Choline esterase inhibition activity of different concentrations (100-1000 μg/mL) of *B. aegyptiaca* crude extract; Acetylcholine esterase (part A) and Butyrylcholine esterase (part B). Data presented as mean ± SE.

**Figure. 3** COX-1 (part 1) and COX-2 (part 2) suppression activity of different concentrations (100-1000μg/ml) of *B. aegyptiaca* crude extract, compared to reference drug Celecoxib. Data are presented as mean ± SE. t-test was used for data analysis (n=3, P<0.05). Data are followed with small letter; a means significant difference with celecoxibe.

**Figure. 4** Radical scavenging activities, DPPH• (part A) and ABTS+ (part B) of different concentrations (100-1000 μg/mL) of *B. aegyptiaca* crude extract, compared to Ascorbic Acid and BHT. (BHT: butylated hydroxytoluene; DPPH: 1,1diphenyl-2-picryl-hydrazyl free radical). Data presented as mean ± SE. t-test was used for data analysis.
analysis (n=3, P<0.05). Data are followed with small letter; a means significant difference with Ascorbic acid, b means significant difference with BHT.

Figure 5. ROS scavenging activity of B. aegyptiaca crude extract, O_2^- (part A) and H_2O_2 (part B) compared to Ascorbic Acid and BHT at different concentrations (100-1000 μg/mL). (BHT: butylated hydroxytoluene).
Data presented as mean ± SE. t-test was used for data analysis (n=3, P<0.05). ANOVA one-way was used for data analysis (n=3, p<0.05). Data are followed with small letter; a means significant difference with Ascorbic acid, b means significant difference with BHT.

Figure 6 Reduction capability effect (part A) and Metal chelation capacity (part B) of different concentrations (100-1000 μg/mL) of B. aegyptiaca crude extract compared to Ascorbic Acid and BHT. (BHT: butylated hydroxytoluene).
Data presented as mean ± SE. t-test was used for data analysis (n=3, P<0.05). Data are followed with small letter; a means significant difference with Ascorbic Acid, b means significant difference with BHT.

Experimental: Materials and Methods

Chemicals
Kojic acid, tyrosinase enzyme, L-3,4-dihydroxyphenylalanine (L-DOPA), AChE enzyme, acetyltiiothioline iodide, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), butyrylthiocholine iodide, BuChE enzyme, leuco-2,7-dichlorofluorescien diacetate, hematin, arachidonic acid, cyclooxygenase enzymes (COX-1 from sheep, EC. 1.14.99.1, or COX-2), polyoxyethylene sorbitan monolaurate (Tween-20), ascorbic acid (vitamin C), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), peroxidase, hydrogen peroxide (H_2O_2), 2,2-azino- bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), sodium nitroprusside (SNP), sulfanilamide, ortho-H_2PO_4, naphthylethylene diamine dihydrochloride, diammonium salt, ferrous chloride, trichloroacetic acid (TCA), potassium ferricyanide, and 3-(2-pyridyl)-5,6-bis (4-phenyl-
sulfonic acid)-1,2,4-triazine-p,p'-disulfonic acid (FerroZine) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium thiocyanate was purchased from Merck (Frankfurter Str. 250 Darmstadt, Germany). All chemicals and solvents were analytical grades.

Plant collection and extraction
Fruits of B. aegyptiaca were collected from Aswan at a mature stage. Fruits were authenticated by Prof. Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen (voucher no. 201) was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Pulp (fruit without seeds) was dried, and the dried powder (1 kg) was exhaustively extracted with 70% ethanol by soaked soaking at room temperature. The filtrate was collected and was evaporated under reduced pressure until dry. The

extract was then lyophilized, and the remaining powder was kept at ~20°C until use.

Chemical composition of B. aegyptiaca crude extract

Total phenolic content was determined by the Folin–Ciocalteau method. [15] Total phenol values are expressed in terms of gallic acid (mg gallic/100 g dry weight). The gravimetric determination procedure of alkaloids was followed as described previously Onwuka. [16]

High-performance liquid chromatography analysis of phenolic compounds of B. aegyptiaca crude extract

Phenols of B. aegyptiaca crude extract were identified by high-performance liquid chromatography (HPLC) analysis, which was performed as described Kim et al. [17] using an Agilent Technologies 1260 series. Separation was performed with a C18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and acetonitrile (B) at a flow rate of 1 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5–8 min (40% A); 8–12 min (50% A); 12–14 min (80% A), and 14–16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μL for each of the sample solutions. The column temperature was maintained at 35°C.

Assessments of B. aegyptiaca crude extract to inhibit Alzheimer’s disease biomarkers

Inhibition tyrosinase (neurofibrillary tangle formation) activity

Tyrosinase inhibition assay was carried out according to the methods of Liu et al. [18]. Briefly, crude extract and kojic acid were dissolved in DMSO prepared as 0.1 mg/mL. The reaction was carried out and the spectrophotometer (Jasco, serial No. C317961148, Japan) was used to measure the absorbance at 475 nm. The extract (40 μL each) was dissolved in methanol with 80 μL of phosphate buffer (pH 6.8), and 40 μL of tyrosinase enzyme and 40 μL of L-DOPA were placed in each well. Each sample was accompanied by a blank that had all the components except for L-DOPA. Kojic acid was used as the standard inhibitor. The percentage of tyrosinase inhibition was calculated as follows:

\[
\text{Tyrosinase inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A means absorbance. The IC₅₀ was calculated by log-probit analysis.

Acetylcholinesterase inhibition (Anti-neurotransmitter degradation)

AChE enzymatic activity was analyzed using an adaptation of the method of Ingkaninan et al. [19] Different concentrations from B. aegyptiaca crude extract (2.5, 5, 10, 20, or 40 μg/mL) were prepared in ethyl alcohol (HPLC grade). Briefly, 500 μL of DTNB [3 mM], 100 μL of acetylythiocholine iodide [15 mM], 275 μL of Tris-HCl buffer [50 mM, pH 8], and 100 μL of each sample at different concentrations were added to a 1 mL cuvette, to which 25 μL AChE enzyme solution containing 0.28 U/mL was then added. The same procedure was performed with a blank reaction without enzyme. The color was monitored for 5 min at 405 nm, and the inhibition % was measured as follows:

\[
\text{Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A means absorbance. The IC₅₀ was calculated by log-probit analysis.

Butyrylcholinesterase inhibition (Anti-neurotransmitter degradation)

Butyrylcholinesterase enzymatic activity was analyzed using an adaptation of the method of Ingkaninan et al. [19] DTNB (500 μL of [3 mM]), 100 μL of butrylythiocholine iodide [15 mM], 275 μL of Tris-HCl buffer [50 mM, pH 8], and 100 μL of each sample at different concentrations (2.5, 5, 10, 20, or 40 μg/mL) were dissolved in ethyl alcohol (HPLC grade). This was added to a 1 mL cuvette and was used as a blank. In the reaction cuvette, 25 μL of Tris-HCl buffer was replaced by the same volume of BuChE enzyme solution containing 0.28 U/mL. The color development was monitored for 5 min at 405 nm.

\[
\text{Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

where A means absorbance. The IC₅₀ was calculated by log-probit analysis.

Anti-inflammatory activity assay of B. aegyptiaca crude extract

The ability of B. aegyptiaca crude extract and celecoxib, a reference drug, to inhibit COX-1 and COX-2 was assessed according to method of Larsen et al. [20]

Antioxidant characteristics of B. aegyptiaca crude extract

Ability to scavenge free radicals

DPPH radical scavenging activity

The ability of the extract to scavenge DPPH was determined according to the method of Yamaguchi et al. [21]
ABTS radical cation scavenging activity

Per the method described by Miller and Rice-Evans [22] and modified by Arnao et al. [23], the ability of the extract to scavenge ABTS radical cations was determined.

Reactive oxygen species (ROS) scavenging ability

Superoxide anion scavenging activity

According to the method described by Liu et al. [24], the ability of the extract to scavenge superoxide anion (O2-) was assessed.

Scavenging of hydrogen peroxide

The ability of the extract or standard controls to scavenge H2O2 was estimated per the method of Ruch et al. [25].

Nitric oxide radical scavenging ability

Nitric oxide (NO) generated from SNP in aqueous solution at physiological pH to produce nitrite ions were measured by Greiss reagent. [26]

Reduction of ferric ion power

The ferric ion (Fe3+) reducing power of the extract and controls was assayed as previously described Oyaizu. [27]

Ferrous ion (Fe2+) chelating capacity

Based on the method of Dinis et al. [28], the ferrous ion (Fe2+) chelating action of the extract or reference materials was determined.

Lipid Peroxidation-Ammonium Thiocyanate

The ability of the extract to inhibit lipid peroxidation was assayed according to the method of Gülçin et al. [29]

Statistical analysis

Data are presented as mean ± SD. Data were analyzed by t-test one-way (n = 3 replicates). A p-value < 0.05 was considered to be statistically significant.

Results and Discussion

Results

Chemical composition of B. aegyptiaca crude extract

B. aegyptiaca extract contained alkaloids (101.26 ± 10.42 mg/g extract) and polyphenols (12.46 ± 1.22 mg gallic acid/g extract) as shown in Table 1. The extract contains phenolic acid, including gallic acid (2573.25 μg/g), Chlorgenic acid (251.23 μg/g), Syringic Acid (39.03μg/g), and Cinnamic Acid (32.67μg/g). Also, it contains flavonoids, including rutin (212.31 μg/g), 4'-7-Dihydroxy isoflavones (64.32 μg/g) and queretin (287.79 μg/g). 92.03 μg/g caffeine is present in the extract as an alkaloids compound.

Assessment of B. aegyptiaca crude extract to inhibit Alzheimer’s disease biomarkers

The anti-Alzheimer’s disease properties of B. aegyptiaca extract was assessed by determining its participation in four mechanisms that are used in Alzheimer’s disease treatment strategies: anti-neurotransmitter degradation, anti-neurofibrillary tangle formation, anti-inflammation, and antioxidant.

Inhibition tyrosinase activity (Anti-neurofibrillary tangle formation)

Anti-neurofibrillary tangle formation was evaluated by determining the inhibitory effect of the crude extract on tyrosinase as compared with kojic acid as a positive control. Evaluation of anti-tyrosinase activity in the assay using L-DOPA as the substrate was carried out with five consecutive concentrations at two different times. Results demonstrated that B. aegyptiaca crude extract had a potent anti-tyrosinase capacity that increased with increasing concentrations and incubation time, compared with reference material (Figure 1). The anti-tyrosinase activity of the extract started from 3.24% ± 0.24% after incubation for 10 min with a concentration of 100 μg/mL and increased to 12.35% ± 0.65% and 19.58% ± 0.58% after 20 and 40 min, respectively at the same concentration. By contrast the values for kojic acid under the same conditions were: 9.34% ± 0.66%, 37.55% ± 1.45%, and 70.32% ± 1.68%, respectively.

The ability of B. aegyptiaca extract to inhibit tyrosinase continuously increased with increasing concentration and time of incubation, and it reached a maximum inhibition percentage at the highest concentration (1000 μg/mL) and longest incubation period (40 min). Incubation of tyrosinase with extract for 10 min showed tyrosinase inhibition by approximately 23.55% ± 0.56%, and the inhibition percentage was increased to 43.11% ± 0.89% and 66.45% ± 1.55% after 20 and 40 min, respectively. By contrast, kojic acid under the same parameters showed 40.35% ± 1.65%, 70.23% ± 1.77% and 100.00% ± 0.0% inhibition, respectively.

IC50 of the extract was more effective than kojic acid at all incubation periods. The extract’s IC50 values were 1.97, 9.61, and 12.03 μg/mL at
incubation times of 10, 20, and 40 min compared with those of kojic acid at 6.00, 29.62, and 62.97 μg/mL at the same respective incubation times.

Acetylcholinesterase inhibition (Anti-neurotransmitter degradation)

B. aegyptiaca crude extract had a high AChE inhibitory ability in a manner dependent on concentration (Figure 2). AChE inhibition caused by the extract ranged from 40.14% ± 1.85% to 84.16% ± 1.68% at concentrations ranging 100–1000 μg/mL. To inhibit 50% of AChE activity, 193.78 μg/mL of extract was required.

Butyrylcholinesterase inhibition (Anti-neurotransmitter degradation)

B. aegyptiaca crude extract displayed good BuChE inhibitory effect in a concentration-dependent manner (Figure 2). The extract at the lowest concentration 100 μg/mL decreased the activity of BuChE by 36.24% ± 0.76%; meanwhile, increasing extract concentration to 1000 μg/mL increased BuChE inhibition percentage to 64.08% ± 1.92%. Inhibition of BuChE activity by 50% required 490.91 μg/mL of the extract.

The anti-inflammatory ability of B. aegyptiaca crude extract

B. aegyptiaca crude extract showed selective anti-inflammatory effect against COX-2 not COX-1. Extract at all concentrations suppressed COX-2 activity similarly to celecoxib (Figure 3). The extract caused COX-2 suppression from 44.79% ± 1.80% to 76.27% ± 1.73% for concentrations of 100 to 1000 μg/mL, respectively, compared with the effect of celecoxib (41.23% ± 0.77% and 72.49% ± 1.51% at the same concentrations, respectively). No significant difference was noticed in COX-2 suppression percentage between each extract concentration and each corresponding celecoxib concentration. In addition, no significant difference was observed between IC50 of the extract and celecoxib: 219.26 and 221.72 μg/mL, respectively.

B. aegyptiaca crude extract did not exhibit considerable COX-1 suppression activity. COX-1 inhibition with the lowest concentration (100 μg/mL) reached 4.29% ± 0.19% and elevated to 10.99% ± 0.65% at the highest concentration (1000 μg/mL) compared with the inhibition percentage recorded by celecoxib (52.50% ± 2.50% and 67.82% ± 2.20%, at 100 and 1000 μg/mL, respectively).

Antioxidant characteristics of B. aegyptiaca crude extract

Free radical scavenging activity

DPPH• scavenging effect

B. aegyptiaca crude extract exhibited potent DPPH• scavenging activity compared with reference materials (Figure 4). It scavenged DPPH• at 85.72% ± 0.78% to 88.90% ± 1.10% at concentrations of 100 to 1000 μg/mL, respectively, compared with that of ascorbic acid (75.17% ± 1.30% and 100% ± 0.00%) or BHT (80.46% ± 2.52% and 98.32% ± 1.69%) at the same concentrations.

ABST+ cation scavenging effect

B. aegyptiaca crude extract showed a strong ability to scavenge ABTS•+ radicals, as compared with that of reference materials, in a concentration-dependent manner (Figure 4). The extract at 100 μg/mL neutralized 47.98% ± 2.00% of ABTS•+ in the medium, and this percentage was increased to 96.92% ± 3.04% at 1000 μg/mL in comparison with ascorbic acid (66.32% ± 2.68% to 100% ± 0.0%) or BHT (62.18% ± 1.81% to 100% ± 0.0%) at the same concentrations. To neutralize 50% of ABTS•+ cations in the reaction medium, 112.27 μg/mL was needed from B. aegyptiaca crude extract compared with that of ascorbic acid (18.61 μg/mL) or BHT (33.44 μg/mL).

Reactive oxygen species scavenging ability

Superoxide anion scavenging activity

B. aegyptiaca crude extract had high superoxide anion (O2•−) scavenging activity in a concentration-dependent manner in comparison with reference materials (Figure 5). The extract inhibited generation of O2•− in NBT–NADH-PMS medium by approximately 33.16% ± 1.84% at 100 μg/mL, and the inhibition percentage significantly magnified to 91.04% ± 3.00% at 1000 μg/mL compared with that of ascorbic acid (66.21 ± 1.79 to 98.11% ± 2.25%) or BHT (73.22% ± 2.78% to 98.11% ± 1.89%). The IC50 of the extract was 226.98 μg/mL, compared with that of ascorbic acid and BHT: 22.35 and 20.551 μg/mL, respectively.

Hydrogen peroxide scavenging activity

B. aegyptiaca extract captured most of the H2O2 molecules in the reaction medium of the in vitro assessment. At the lowest concentration (100 μg/mL), the extract scavenged a high amount of H2O2 (73.78% ± 2.22%); higher than the amount scavenged by ascorbic acid (75.17% ± 1.30%) or BHT (62.18% ± 1.81%) (Figure 5). Increasing the extract concentration increased the scavenged H2O2 amount, which was close to that of ascorbic acid and was

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higher than that of BHT. \( \text{H}_2\text{O}_2 \) scavenging activity of \( \textit{B. aegyptiaca} \) extract reached maximum percentage, 97.21% ± 1.79% at the highest concentration (1000 \( \mu \text{g/mL} \)) compared with that of ascorbic acid (98.21% ± 1.79%) and BHT (94.31% ± 2.69%). \( \textit{B. aegyptiaca} \) extract demonstrated a low \( \text{IC}_{50} \) (4.13 \( \mu \text{g/mL} \)) that was relatively close to that of ascorbic acid (7.19 \( \mu \text{g/mL} \)) and was lower than that of BHT (27.44 \( \mu \text{g/mL} \)).

**Nitric oxide scavenging action**

\( \textit{B. aegyptiaca} \) crude extract exhibited a weak ability to scavenge NO\(^*\), although it was increased in a concentration-dependent manner, compared with reference materials (Table 2). The extract scavenged NO\(^*\) radicals by approximately 18.63% ± 0.70% to 37.64% ± 1.17% at concentrations 100 to 1000 \( \mu \text{g/mL} \), compared with the scavenging percentage of ascorbic acid (64.39% ± 1.61% and 93.27% ± 2.73%) or BHT (65.99% ± 2.00% and 95.88% ± 1.89%) at the same concentrations. The \( \text{IC}_{50} \) value of the extract was greater than that of the ascorbic acid or BHT (2,350.24, 19.96, and 16.93 \( \mu \text{g/mL} \), respectively).

**Ferric ion reducing antioxidant power (FRAP)**

\( \textit{B. aegyptiaca} \) crude extract exhibited a moderate \( \text{Fe}^{3+}\text{Fe}^{2+}\)-transformation capacity in comparison with reference materials (Figure 6). \( \text{Fe}^{3+}\) reductive capacity of the extract, represented as absorbance, ranged between 0.153 ± 0.02 to 0.464 ± 0.03 at 100 and 1000 \( \mu \text{g/mL} \), respectively, compared with those of ascorbic acid (0.392 ± 0.004 to 0.780 ± 0.003) or BHT (0.287 ± 0.013 to 0.603 ± 0.03) at the same concentrations. Transforming of 50% of \( \text{Fe}^{3+} \) ions to \( \text{Fe}^{2+} \), the reduced form, occurred at a concentration of 1134.48 \( \mu \text{g/mL} \) of the extract, while that of ascorbic acid or BHT were 237.13 and 518.05 \( \mu \text{g/mL} \), respectively.

**Ferrous ion chelation ability**

\( \textit{B. aegyptiaca} \) crude extract showed ferrous ion (\( \text{Fe}^{2+} \)) chelating ability in a concentration-dependent manner (Figure 6). The extract captured \( \text{Fe}^{2+} \) with a range from 25.97% ± 1.03% at the lowest concentration (100 \( \mu \text{g/mL} \)) and 83.69% ± 2.07% at the highest concentration (1000 \( \mu \text{g/mL} \)) compared with that of ascorbic acid (62.99% ± 1.01% to 98.19% ± 1.82%) or BHT (52.41% ± 1.59% to 99.09% ± 0.90%) at the same concentrations. The \( \text{IC}_{50} \) value of the extract was larger than that of ascorbic acid or BHT; 553.07, 26.36, and 68.88 \( \mu \text{g/mL} \), respectively.

**Lipid peroxidation suppression activity**

\( \textit{B. aegyptiaca} \) crude extract has a moderate ability to suppress lipid peroxidation, and it prevented inoleic acid oxidation by about 28.94% ± 1.90% to 59.83% ± 1.89% at concentrations of 100 to 1000 \( \mu \text{g/mL} \), compared to ascorbic acid (64.73% ± 2.20% to 100% ± 0.0%) or BHT (60.31% ± 1.69% to 100% ± 0.0%) at the same concentrations, respectively (Table 2). The \( \text{IC}_{50} \) of the extract was larger than that of ascorbic acid or BHT reference materials at 666.92, 28.64, and 39.30 \( \mu \text{g/mL} \), respectively.

**Discussion**

The current study aimed to discover a new natural product for the treatment of Alzheimer’s disease to avoid side effects of synthetic drugs. Our study demonstrated that \( \textit{B. aegyptiaca} \) crude extract had anti-Alzheimer’s disease activity via inhibition of tyrosinase and cholinesterase as well as antioxidant and anti-inflammatory abilities. \( \textit{B. aegyptiaca} \) crude extract played a role in suppression of Alzheimer’s disease biomarkers through its antioxidant (metal chelation, ROS scavenging, and radical activities) and anti-inflammatory characteristics. In addition, the anti-Alzheimer’s disease activities of \( \textit{B. aegyptiaca} \) may be attributed to its unique chemical composition of alkaloids, polyphenols, flavonoids, tannins, and coumarins.

L-DOPA, also known as Levodopa and L-3, 4- dihydroxyphenylalanine, is best known to the world as a treatment for the neurological disorder. L-DOPA is the precursor for the neurotransmitter dopamine, and is produced by the action of the enzyme tyrosinase on the amino acid tyrosine. In Alzheimer’s disease, aggregation of the A peptide leads to the development of amyloid plaque formation followed by neurodegenerative changes. L-DOPA and dopamine can dissolve fibrils of a peptide and can inhibit the formation of protein tangles [30]. Unfortunately, tyrosinase can oxidize the catechol ring of dopamine to the highly reactive species dopamine-quinone. Dopamine oxidation also inhibits dopamine transporter, glutamate transport and mitochondrial respiration. Thus, inhibition tyrosinase can control and treat Alzheimer’s disease. [6-7]

Hasegawa et al. [31-32] established catecholaminergic neuronal cell lines that express tyrosinase in response to doxycycline, an exogenous inducer. In these cell lines, the overexpressed tyrosinase protein increased intracellular dopamine and ROS, followed by the formation of melanin pigments in neuronal somata, which eventually causes apoptotic cell death. Juneja et al. [33] used

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blastula protease-10 peptide as a tyrosinase-like mimic to characterize both intermediates and mechanisms for Type II-Copper proteins such as tyrosinase. They found that H$_2$O$_2$ activated this model and serves as the second substrate in a bisubstrate reaction. Tyrosinase is a copper-containing enzyme that catalyzes the oxidized tyrosine into dopa and subsequently dopaquinone. Because of the nature of the copper structure in tyrosinase, several investigations have been focused on the potential role of metal chelating compounds such as kojic acid, catechol, gentisic acid, flavonol, and hydroxamic acid on controlling tyrosinase activity. [34] Generally, flavonoids with a hydroxyl group at A and B rings are very important tyrosinase inhibitors by Cu$^{2+}$ chelation. [35]

The anti-tyrosinase activity that appeared by extract may be due to its H$_2$O$_2$ scavenging activity, which was demonstrated in this study. B. aegyptiaca crude extract has a potent H$_2$O$_2$ scavenging activity with low IC$_{50}$ of 4.13 μg/mL. And its metal chelating capacity. And its phenolic content that estimated as 12.46 ±1.22 mg gallic acid/g extract, where the hydroxyl groups of the phenolic compounds can bind hydrogen bonding with the active site of the enzyme, causing inhibition of tyrosinase [34]. Quercetin was reported for its strong ability to inhibit tyrosinase. Benzoic, cinnamic, and methoxy-cinnamic acids as well as hydroxyl-cinnamoyl derivatives are also well known tyrosinase inhibitors. [36] B. aegyptiaca crude extract contains 32.67 μg of cinnamic acid/g extract and 287.79 μg quercetin/g extract.

In the Alzheimer’s disease patients, a depletion in the acetylcholine (ACh), a neurotransmitter, considered to be crucial element in the development of dementia. Therefore, Alzheimer’s disease patients need to restore the level of acetylcholine via the suppression of both major form of cholinesterase: AChE and BChE. Where, suppression of AChE has an important role on enhancing cholinergic transmission in the brain and reducing the aggregation of amyloid beta peptide (Aβ) and the formation of the neurotoxic fibrils in Alzheimer’s disease [37]. AChE protein consists of 531 amino acid residues with an ellipsoid shape. AChE has two binding sites; the first is involved in the interaction of positively charged nitrogen in alkaloid derivatives, and the second can interact with other non-alkaloid components as phenols. [38]

The AChE inhibitor activity that appeared by extract may be due to its antioxidant properties, its content of phenols, flavonoids, and alkaloids that demonstrated as AChE inhibitors. Additionally, the active constituents of the extract, including gallic acid, Chlorgenic acid, Cinnamic Acid, and quercetin.

Polyphenolics have been demonstrated to be antioxidant agents that are important for the treatment of Alzheimer’s disease. Polyphenols are able to reduce the incidence of diseases related to aging and neurological disorders. B. aegyptiaca extract contains polyphenols of 12.46 ±1.22 mg gallic acid/g, which may play a key role in exerting its effect as an AChE inhibitor. Orhan et al. [39] found that gallic acid and quercetin showed anti-AChE and anti-BuChE activities, and chlorogenic acid exhibited anti-BuChE activity. In addition, B. aegyptiaca crude extract contains gallic acid, quercetin, chlorogenic acid in the amounts of 2573.25, 287.79, and 251.23 μg/g extract, respectively. Szwajgier [40] demonstrated the anti-AChE and anti-BuChE activities of gallic acid compared with those of eserine. Lan et al. [41] reported that cinnamic acid inhibited AChE and BuChE activity. B. aegyptiaca extract contains 32.67 μg/g of cinnamic acid, which may be the cause of higher selectivity to AChE than BuChE. Flavonoids can bind with AChE peripheral anionic site via −OH groups at the aromatic ring (B-ring), resulting in blockage of the entrance to the active site. Quercetin reacts with the amino acid residues at the anionic subsite and conjugates through hydrogen bonds. [42] The existence of an −OH group in the C3 position of the C-ring appears to be crucial in increasing inhibition activity. B. aegyptiaca crude extract contains many flavonoids that are documented as AChE inhibitors such as quercetin (287.79 μg/g extract). Alkaloids are the most promising natural products in the treatment of Alzheimer’s disease. This ability is based on their complex nitrogen-containing structures. Therefore, most of the AChE inhibitors contain nitrogen. Galantamine drug is an alkaloid that was first isolated from Galanthus nivalis L (Amaryllidaceae family), and recently it has been obtained from daffodil bulbs for industrial purposes. This drug ameliorates cholinergic transport via allosteric modulation of nicotinic receptors. [43] Physostigmine (eserine) is an alkaloid isolated from the seeds of Physostigma venenosum Balf. (Fabaceae), which have shown a reversible and selective inhibitory effect against AChE. [44] Many alkaloids that are active cholinesterase inhibitors have already been described in different families including the isoquinoline-type alkaloids from Amaryllidaceae [45], piperine alkaloid of Piper nigrum [34], and pyrrolidine alkaloids from Holarrhena antidysenterica. [46] Each gram of B. aegyptiaca extract contains 101.26 ±10.42 mg alkaloids.

It is believed that Alzheimer risk factors including environmental, biological and genetic factors associated with elevating level of inflammatory biomarkers that can promote advanced cognitive deterioration. Also, it was demonstrated that the development of different central nervous

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system (CNS) diseases, e.g. Alzheimer’s disease, is related to neuroinflammation. Inflammatory process development implicates a widely spread range of molecular interactions which represent important CNS changes outcomes. These changes contribute in CNS regulation and function impairment that increased level of inflammatory marker [47]. When the inflammatory cascade was initiated the neuroinflammatory process becomes highly activated to make further cellular damage to lose their function which is accompanied with neurofibrillary tangles and amyloid plaques formation, Alzheimer’s hallmarks [48]. Therefore, we determined the anti-inflammatory effect of the extract. The current study demonstrated the selective anti-inflammatory of the extract against proinflammatory enzyme COX-2 not anti-inflammatory one COX-1.

Conclusions

Alzheimer’s disease contains insufficient acetylcholine in the brain and myasthenia gravis due to its decomposition into choline and acetate by acetylcholinesterase enzymes (AChE), resulting in muscle weakness. In addition, tyrosinase has a role in neuromelanin production and damage to the neurons. Thus, suppression of cholinesterase and tyrosinase enzymes is considered a perfect strategy for treating Alzheimer’s disease. In the current study, B. aegyptiaca crude extract exhibited anti-Alzheimer’s disease activity via suppression of these enzymes involved in Alzheimer’s disease including AChE and tyrosinase. The anti-Alzheimer’s disease activity of B. aegyptiaca extract may also be attributed to its antioxidant and anti-inflammatory characteristics as well as its rich chemical composition including polyphenols, alkaloids, flavonoids, and coumarins that have been demonstrated as anti-Alzheimer’s disease materials. The results of this study have guided us to the possibility of using B. aegyptiaca extract in advanced pre-clinical and clinical trials to assess its anti-Alzheimer’s disease activity in in vivo models as an alternative drug for Alzheimer’s disease from a natural source.

Conflicts of interest

We declare that we have no conflict of interest.

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