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β-Amyrin Rich *Bombax ceiba* Leaf Extract with Potential Neuroprotective Activity against Scopolamine-Induced Memory Impairment in Rats

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Abstract: The neuroprotective potential of *Bombax ceiba* L. (Malvaceae) leaves extract (BCLE) was evaluated herein for the first time. Memory impairment was induced in rats by scopolamine (SC) administration for seven days (1.5 mg/kg/day, i.p.). BCLE (200 mg/kg) or donepezil (1mg/kg) was administered orally a week before and then concomitantly with SC for another week. BCLE improved the rats' behaviors estimated by Morris water maze and passive avoidance tests, where the rats showed prolongation in time spent in platform quadrant and step-through latency, respectively, compared to administration of SC alone. BCLE also ameliorated the antioxidant parameters (Catalase with malondialdehyde (MDA), and the SC-induced elevation of acetylcholinesterase (AChE) in rats' brain tissues. Histopathological studies supported the above results. BCLE phytochemical study resulted in isolation and structural elucidation of β-sitosterol linoleate (first to be isolated from the genus) and β-amyrin. Twenty-seven compounds (93.35%) were identified in BCLE by gas chromatography/mass spectrometry (GC/MS). The major components were triterpenoids (69.82%), composing mainly of β-amyrin (45.28%), lupeol (15.03%) and olean-12-en-3-one (6.27%), in addition to fatty acid esters (13.37%) and steroids (7.75%), which were rich in methyl palmitate (9.87%) and stigmasterol acetate (4.91%), respectively. These compounds may be responsible for ameliorating the SC-induced oxidative stress and cholinergic dysfunction.

Keywords: *Bombax ceiba*; GC/MS; β-amyrin; neuroprotective; scopolamine.

1. Introduction

Bombax ceiba L. (Malvaceae) has been used traditionally for blood purification, lightening of skin in acne and pigmentation, treatment of wounds, leucorrhoea, weakness, cold and coughs [1]. In addition, it provides nutritional values as it has some edible parts like many other Malvales plants [2, 3]. *B. ceiba* is characterized phytochemically by the presence of flavonoids, xanthones, quinines, naphthoquinones, triterpenes, sterols, hydrocarbons, fatty acids and their esters [4, 5]; and

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pharmacologically by various activities such as antimicrobial [6], hypotensive, hypoglycemic [7], antioxidant [8-11], cytotoxic [12], antiproliferative [10], and antitumor [11] activities.

Alzheimer disease is a progressive disorder that involves neurodegeneration of the brain tissues, decrease in acetylcholine levels, and impairment of memory and cognition abilities [13]. Scopolamine (SC) is an anti-muscarinic agent that causes Alzheimer-type dementia by producing a status of oxidative stress and increasing the levels of AChE causing a decline in the cholinergic activity [14]. However, Donepezil is a selective AChE inhibitor that has been approved as a drug for treating Alzheimer by elevating the brain cholinergic transmission and decreasing the amyloid β-fibrils toxic forms [15,16].

Thus, the present study was conducted to evaluate BCLE antioxidant and neuroprotective activities, as compared to donepezil, against SC-induced memory impairment and learning deficit in rats. This was supported by behavioral, neurochemical and histopathological studies. In addition, BCLE was investigated phytochemically and for the first time by GC/MS analysis, in order to identify possible components that may be responsible for the observed effects.

2. Materials and Methods

2.1. Plant Material

Bombax ceiba L. *(*Malvaceae) leaves were collected from El-Orman Botanical Garden, Egypt and authenticated by Mrs. Trease Labib, Plant Taxonomy Consultant at the Egyptian Ministry of Agriculture. A voucher specimen (PHG-P-BC-2) was kept at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

2.2. Chemicals

Scopolamine (SC) was obtained from Sigma Aldrich, MO, USA. Donepezil® tablets were purchased from a local pharmacy. For measuring the neurochemical parameters; catalase, Malondialdehyde (MDA), and acetylcholinesterase (AChE) kits were purchased from Bio-Diagnostics Company, Dokki, Giza, Egypt. All chemicals used in this study were of analytical grade.

2.3. Extract Preparation

The fresh leaves of *B. ceiba* (1 kg) were extracted with neat methanol till complete exhaustion. The combined methanol extracts were distilled off at 45°C in a rotary evaporator till complete dryness, and then lyophilized to obtain a powdered residue that was stored in refrigerator till biological evaluation. For GC/MS analysis, BCLE was dissolved in the least amount of distilled water and fractionated with hexane. The hexane fraction was kept in sealed vials in the refrigerator for subsequent evaluation.

2.4. Compounds Isolation and Identification

The concentrated total methanol extract was mixed with sufficient amount of activated charcoal with continuous stirring for an hour to remove most of the chlorophyll. The extract was then filtered and distilled off at 45°C in rotary evaporator till complete dryness. The obtained residue (10 g) was dissolved in the least amount of methanol, where a heavy yellow precipitate was formed that was isolated and dissolved in dichloromethane, then subjected to repetitive preparative silica TLC using solvent system Hexane: Dichloromethane (7: 3) to yield compound **1** (β-sitosterol linoleate, 7 mg) that gave a pinkish violet color upon spraying with vanillin-sulphuric reagent and heating at 100°C.

BCLE methanol-soluble fraction was further fractionated by a Diaion HP-20 column chromatography (100 L, 4.5 i.d. cm). The elution process started with distilled water, then 25%, 50%

and 75% methanol in water, followed by neat methanol and finally acetone to ensure complete elution. Similar fractions were pooled together according to the results obtained from TLC analysis of the eluted fractions, then dried *in vacuo* at 45°C to yield six main fractions. The fraction eluted with neat methanol was reduced in volume using rotary evaporator at 45°C, where needle crystals were precipitated and then washed with acetone to yield compound **2** (β-amyrin, 20 mg). Structure elucidation was done by comparing the compounds ^{1}H-NMR and APT-spectral data with literature.

2.5. NMR Spectroscopy

^{1}H-NMR and APT-spectral data were measured in $CDCl_3$ on Bruker Ascend 400 spectrometer, Avance BioSpin Inc., at 400 and 100 MHz, respectively. Chemical shifts, in ppm, were recorded using tetramethylsilane (TMS) as an internal standard.

2.6. GC/MS Analysis

Shimadzu GC/MS-QP2010 (Tokyo, Japan) was used to record the mass spectrum. It was equipped with a 30 m Rtx-5MS (Restek, USA) fused-bonded column (0.25 mm i.d., 0.25 µm film thickness) coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany) and having a split-splitless injector. The carrier gas (helium) flow rate was 1.41 mL/ min. The column temperature was kept initially at 45 °C for 2 minutes isothermal, programmed to 300°C (5 °C/min) then kept at 300°C (isothermal) for 5 minutes. The injector temperature was 250°C. A filament emission current of 60 mA, an ionization voltage of 70 eV, and 200°C ion source were applied. The sample was diluted (1% v/v) and injected with a split mode of a ratio 1: 15. Compound identification was based on mass spectral data (MS), comparing with published retention indices (RI) in NIST Mass Spectral Library, Wiley Registry of Mass Spectral Data, other published data [17-22] as well as co-chromatography with authentic samples.

2.7. Animals

Male Sprague–Dawley rats (150–200 g) were purchased from National Research Center (Dokki, Giza, Egypt) and acclimatized for at least 1 week in the animal house facility, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt prior to the experiment. The rats were kept at 23 ± 2°C and supplied with standard food pellets and access to water *ad libitum*. The rat treatment protocol was approved by the Ethical Committee for Animal Use, Faculty of Pharmacy, Ain Shams University.

2.8. Experimental Design

Animals were divided into four groups of six rats each. Group 1: Control group received distilled water orally for 14 days. Group 2: received distilled water orally 1 week before scopolamine administration and then concomitantly with scopolamine (1.5 mg/kg/day, i.p.) for another week. Group 3 and 4: received donepezil (1mg/kg/day) or BCLE (200 mg/kg/day), respectively orally 1 week before scopolamine administration and then concomitantly with scopolamine (1.5 mg/kg/day, i.p.) for another week.

2.9. Passive Avoidance Test

It was done in two identical chambers, according to the method of Yang et al. (2013) [23]. One chamber was illuminated by a bulb, while the other was non-illuminated with stainless steel rods in the floor. A guillotine door was located between the two chambers. In the first trial (acquisition), the rats were placed in the lighted chamber and the door in-between was opened for 20 s. As soon as the rat entered to the dark area, the door was automatically closed and a 0.5 mA electrical foot shock was

delivered to the rat through the stainless steel rod for the duration of 3 seconds. Twenty-four hours later after this trial, the rats repeated the test for the retention trials by placing in the illuminated chamber and recording the time taken for each rat to enter the dark chamber as the latency times in both the acquisition and the retention trials, with a maximum of 300 seconds.

2.10. Morris Water Maze

The maze is a circular pool of 150 cm diameter, a height of 60 cm and filled with water at room temperature with a featureless inner surface. The tank was mounted in a dimly lit and in a sound-proof room with numerous visual cues. The pool was divided conceptually into four quadrants, and a platform of 12 cm diameter and height of 40 cm was placed in one of the water pool quadrants and was submerged 2 cm below the water surface so that it becomes invisible at the water level. The rats underwent a training period of four subsequent days, where each rat was put in water a different quadrant randomly each time and allowed to swim for 90 seconds till reaching the platform and in case it failed in finding the platform, the rat was put onto the platform for 30 seconds. These trials were repeated for each rat three times a day. After each trial, the animals were dried under an infrared lamp. The test was repeated on the fifth day (test trial) with the platform removed. The time of swimming in the quadrant of the removed platform and the number of crossing times to that quadrant were recorded using a video camera for three times for each rat.

2.11. Preparation of Rats' Brain Tissues

At the end of the study (14 days), the rats were anesthetized and sacrificed by decapitation. Their skulls were open onto iced isotonic saline. Sample brain tissues were fixed in 10% formalin in saline for histopathological examination. Other brain tissues were dissected out into striata, cortices, and hippocampi then 10% (w/v) homogenates were prepared in isotonic saline for neurochemical analysis.

2.12. Neurochemical Parameters

Determination of catalase, lipid peroxidation expressed as MDA equivalents and acetylcholinesterase (AChE) activities in the rats' brain tissues were according to the kit manufacturer instructions (Bio-Diagnostics Company, Dokki, Giza, Egypt). Catalase was determined according to the method of Aebi (1984) [24] and was expressed as U/g tissue. MDA was determined according to the method of Ohkawa et al. (1979) [25] and was expressed as μmol/ g tissue, while AChE activity expressed as nM/min/mg tissue was determined according to Ellman et al. (1961) method [26]. The samples were run in triplicates and expressed as mean ± SEM.

2.13. Histopathological Examination

Brain autopsy samples taken from different groups were fixed in 10% formalin in saline for 24 hours, washed with tap water then serial alcohol dilutions for dehydration. Specimens cleared in xylene were embedded in paraffin at 56 degrees for 24 h. in a hot air oven. The tissue blocks were then sectioned, put onto glass slides, deparaffinized, and stained with hematoxylin and eosin stain. Light electric microscopy is used for examination [27].

2.14. Statistical Results

Data were expressed as mean ± SEM, and then analyzed by one-way ANOVA followed by Tukey *post hoc* tests. Analyses and graphs were performed and sketched by GraphPad Prism version 5.01 software, California, USA. Results were considered statistically significant at $P < 0.05$.

3. Results and Discussion

Dementia was induced in rats by SC injection (1.5 mg/kg/day, i.p.) for 7 consecutive days. The neuroprotective activity of BCLE (200 mg/kg, orally) was evaluated, for the first time, as compared to a reference drug (donepezil, 1 mg/kg orally) by administration a week before and then concomitantly with SC for another week.

3.1. Morris Water Maze Test

To test BCLE amelioration effect on SC-induced memory impairment, the rats went into Morris water maze test (Figure 1A-C). The SC-treated group demonstrated prolonged escape latency, spent the least amount of time (-84%) in the platform quadrant and showed a significant decrease (45.45%) in crossing times of the platform quadrant when compared to the control group. However, BCLE-treated rats were efficient in finding the platform quadrant, with increased crossing times and prolonged time spent in the platform quadrant (305.85%, compared to the SC-group). BCLE effects were close to those of donepezil.

3.2. Passive Avoidance Test

The passive avoidance test (Figure 1D, E) was carried out to evaluate more of the rats' behavioral parameters. The SC-treated group showed a significant shortening in the step-through latency (-91.14%, comparing to the control group). Both donepezil and BCLE treatments prolonged the SC-shortened latency with only -10.34 and -13.76%, respectively, as compared to the control group. In the acquisition trial, no differences in the latency times were observed among the tested groups. These results proved that BCLE ameliorated the rats' behavioral parameters.

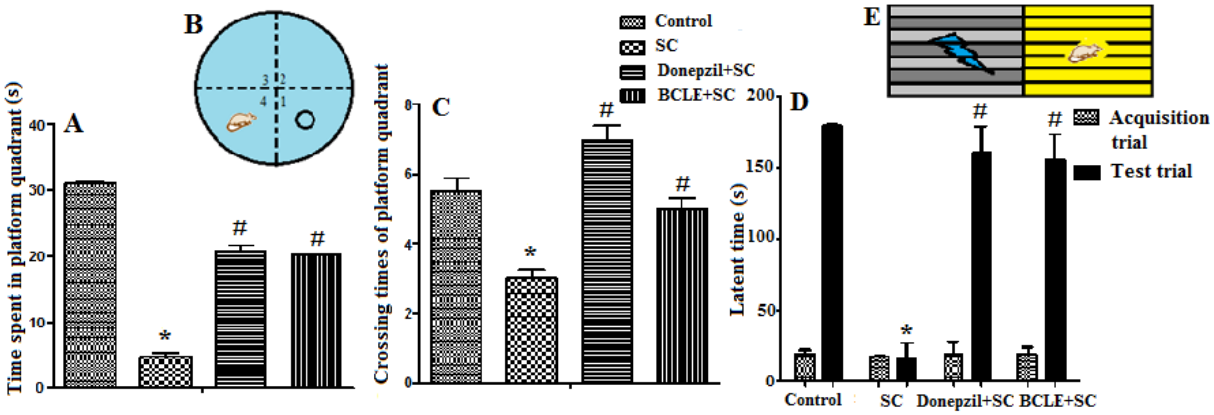

Figure 1. Effect of BCLE on Morris water maze (A, C) and passive avoidance tests (D), which were represented graphically in B and E, respectively. Data were expressed as mean ± SEM, n= 6. $^{*}P <$ 0.05, significant difference from the control group. $^{\#}P < 0.05$, significant difference from the SC-treated group.

3.3. Antioxidant Parameters

The effect on the antioxidant parameters was evaluated in rats' brain tissues through measuring the catalase activity and the malondialdehyde (MDA) equivalents (Figure 2A, B). SC treatment decreased the catalase activity and increased MDA in rats' hippocampus by 18.25 and 55.27%, respectively, when compared to the control group. While, both donepezil and BCLE increased the SC-

induced reduction in catalase activity by 46.04 and 9.25%, respectively, and decreased the MDA equivalents by 31.11 and 75.10%, respectively, as compared to SC treatment alone. Worth of mentioning that MDA equivalent of BCLE-treated group was significantly reduced compared to control group by 61.34%, which indicates a powerful reduction in lipid peroxidation and potentially significant antioxidant effect of BCLE. The MDA equivalent of the donepezil-treated group was close to the control group with a percent change of 6.95%.

3.4. Acetylcholinesterase (AChE) Activity

The acetylcholine content in rats' brain hippocampus tissue was evaluated through measuring the AChE activity (Figure 2C). SC treatment significantly increased the AChE by 87.5% compared to control group. Both donepezil and BCLE significantly decreased the SC-induced elevation in AChE levels by 36.67 and 48%, respectively. BCLE administration recovered the AChE levels back to normal values (-2.5%, compared to the control group).

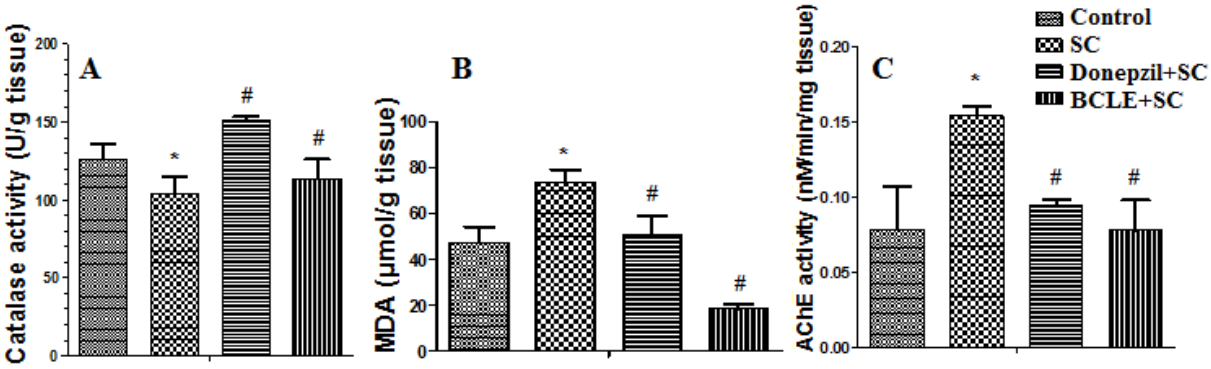

Figure 2. Effect of BCLE on antioxidant parameters (catalase (A), MDA(B)) and AChE activity (C) in SC-induced memory impairment. Data were expressed as mean ± SEM, n= 6. $^{*}P < 0.05$, significant difference from the control group. $^{\#}P < 0.05$, significant difference from the SC-treated group.

3.5. Histopathological Studies

Further confirmation was done by histopathological studies of the rats' brains (Figure 3). In the control and BCLE-treated groups, no histopathological alterations were observed and normal histological structure of the neurons in the cerebral cortex, subiculum, fascia dentate and hilus of the hippocampus, as well as the striatum, were recorded. Similar results were shown in the donepezil-treated group except for some nuclear pyknosis and degeneration in the striatum neuronal cells.

Intraperitoneal administration of 1.5 mg/kg of SC daily to rats for seven consecutive days has altered the normal features of the brain tissues, where both nuclear pyknosis and degeneration were detected in the neurons of the cerebral cortex, fascia dentate, hilus of the hippocampus. There was no histopathological alteration in the neurons of the subiculum of the hippocampus, while the striatum showed small-sized eosinophilic plagues formation with nuclear pyknosis and degeneration in the neuronal cells.

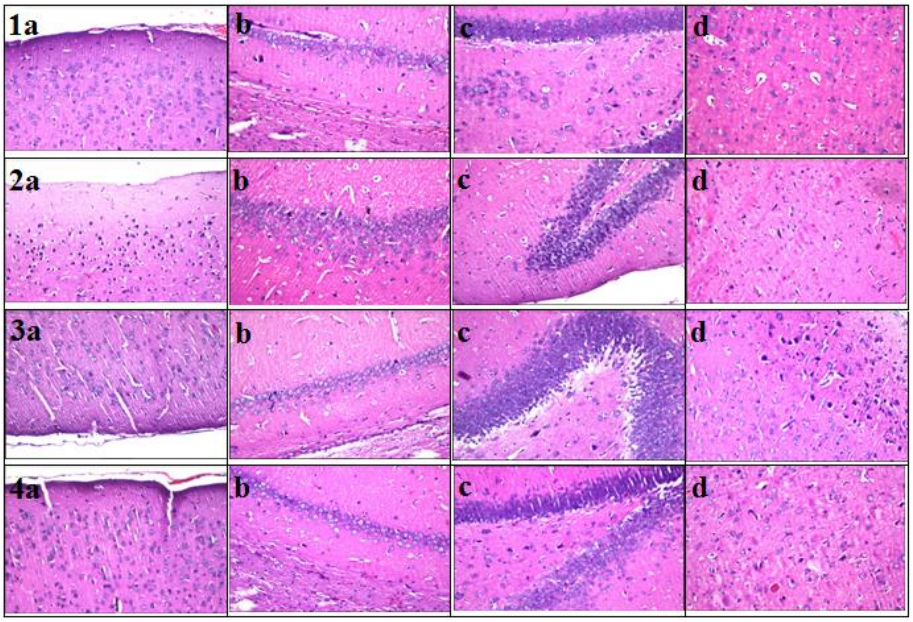

Figure 3. Hematoxylin and Eosin (H & E) stained microphotographs of (a) cerebral cortex, (b) subiculum, (c) fascia dentate, hilus of the hippocampus, and (d) striatum of rats' brains. Control group (1) showed no histopathological alteration, SC group (2) showed nuclear pyknosis, degeneration (2a, c, d), and small size eosinophilic plagues formation (2d). Donepezil (3) or BCLE groups (4) recovered the normal tissue architecture.

3.6. Phytochemical Analysis

Phytochemical investigation of BCLE resulted in isolation and structural elucidation of β-sitosterol linoleate and β-amyrin. The former was isolated for the first time from the genus. Their structures (Figure 4) were elucidated using spectroscopic techniques and comparing with literature data.

*β-Sitosterol linoleate (**1**):* Colorless oil (7 mg), ^{1}H-NMR (400 MHZ, $CDCl_3$) δ ppm: 5.3-5.4 (*m*, H-5, 9′, 10′, 12′, 13′), 3.51 (1H, *m*, H-3), 2.79 (*t*, *J*= 6 Hz, H-11′), 2.32 (*m*, H-2′), 2.02-2.08 (*m*, H-8′, 14′), 1.63 (*m*, H-3′), 1.28-1.33 (*m*, H-4′, 5′, 6′, 7′, 15′, 16′, 17′), 1.04 (3H, *s*, H-29), 0.94 (3H, *d*, *J*= 6 Hz, H-19), 0.83-0.92 (*m*, H-18′, 24, 26, 27), 0.7 (3H, *s*, H-28). APT-NMR (100 MHz, $CDCl_3$) δ ppm: 173.25 (C-1′), 139.74 (C-5), 130.23 (C-9′), 130.08 (C-13′), 127.99 (C-10′), 127.86 (C-12′), 122.56 (C-6), 73.73 (C-3), 56.64 (C-14), 56.05 (C-17), 50.05 (C-9), 45.76 (C-22), 42.26 (C-13), 42.26 (C-4), 39.63 (C-12), 38.12 (C-1), 36.59 (C-10), 36.16 (C-18), 34.72 (C-2′), 33.96 (C-20), 31.93 (C-2), 31.92 (C-16′), 31.88 (C-7), 31.88 (C-8), 29.71-29.10 (C-4′, 5′, 6′, 7′, 15′), 29.60 (C-25), 28.25 (C-16), 27.83 (C-8′), 27.20 (C-14′), 26.10 (C-21), 25.64 (C-3′), 25.06 (C-11′), 24.30 (C-15), 23.08 (C-23), 22.70 (C-17′), 21.04 (C-11), 19.82 (C-26), 19.33 (C-27), 19.04 (C-19), 18.79 (C-28), 14.12 (C-18′), 11.99 (C-24), 11.86 (C-29). These data were in accordance with those reported in literature by Chaturvedula and Prakash, 2012 [28] and Yang et al., 2003 [29] for sitosterol and linoleate moieties, respectively.

*β-Amyrin (**2**):* Colorless needle crystals (20 mg), ^{1}H-NMR (400 MHZ, $CDCl_3$) δ ppm: 5.2 (1H, *t*, *J*= 3.5, H-12), 3.25 (1H, *dd*, *J*= 4.4, 11, H-3), 1.92 (*m*, H_β-19), 1.89 (*m*, H_β -15), 1.79 (*m*, H-22), 1.71 (*m*, H_β-16), 1.16 (3H, *s*, H-27), 1.02 (3H, *s*, H-26), 0.99 (3H, *s*, H-24), 0.96 (3H, *s*, H-26), 0.89 (6H, *s*, H-29, 30), 0.86 (3H, *s*, H-25), 0.81 (3H, *s*, H-23), 0.69 (1H, *d*, *J*= 11, H-5). APT-NMR (100 MHz, $CDCl_3$) δ ppm: 145.18 (C-13), 121.64 (C-12), 79.03 (C-3), 55.17 (C-5), 47.63 (C-9), 47.24 (C-18), 46.83 (C-19), 41.73 (C-14), 39.81 (C-8), 38.60 (C-1), 38.76 (C-4), 37.10 (C-22), 36.94 (C-10), 34.73 (C-21), 33.35 (C-29), 32.65 (C-17), 32.49 (C-7), 31.09 (C-20), 28.40 (C-28), 28.08 (C-23), 27.23 (C-

2), 26.95 (C-15), 26.12 (C-16), 26.01 (C-27), 23.69 (C-30), 23.54 (C-11), 18.38 (C-6), 16.80 (C-26), 15.58 (C-25), 15.48 (C-24). These data were in accordance with those reported in literature [30].

3.7. GC-MS Analysis

The GC-MS analysis of BCLE was carried out for the first time. It revealed the presence of triterpenoids and their acetate esters (69.82%) which were composed mainly of β-amyrin (45.28%), lupeol (15.03%) and olean-12-en-3-one (6.27%). Fatty acid esters (13.37%) were rich in methyl palmitate (9.87%), while steroidal compounds (7.75%) were rich in stigmasterol acetate (4.91%), as shown in Table 1. The structures of the major compounds identified in the GC-MS analysis are demonstrated in Figure 4.

Table 1. Chemical composition of *B. ceiba* leaves extract (BCLE)

No.	Compound [a]	RI calculated	RI reported	% Composition [b]	Identification
1.	*n*-Octane	799	800	0.06	RI, MS
2.	*n*-Hexenal	801	806	0.28	RI, MS
3.	2,7-Dimethyloctane	888	887	0.06	RI, MS
4.	(*E*)-2-Heptenal	952	947	0.13	RI, MS
5.	Methyl tetradecanoate	1727	1722	0.1	RI, MS
6.	1-Octadecene	1791	1789	0.09	RI, MS
7.	Hexahydrofarnesyl acetone	1846	1844	1.34	RI, MS
8.	**Methyl palmitate**	**1928**	**1921**	**9.87**	**RI, MS**
9.	Methyl heptadecanoate	2027	2028	0.28	RI, MS
10.	Methyl linoleate	2102	2101	0.31	RI, MS
11.	Linolenic acid	2110	2107	0.24	RI, MS
12.	Phytol	2120	2114	0.09	RI, MS, AU
13.	Methyl stearate	2133	2130	1.28	RI, MS
14.	Stearyl acetate	2216	2211	0.1	RI, MS
15.	4-hexadecyl hexanoate	2362	2362	0.91	RI, MS
16.	2-Monopalmitin	2521	2519	0.52	RI, MS
17.	**Stigmasterol acetate**	**3072**		**4.91**	MS
18.	Stigmastan-3,5-diene	3090	3040	0.18	RI, MS
19.	α-Tocopherol	3114	3111	0.12	RI, MS
20.	α-Amyrin acetate	3175		0.24	MS
21.	β-Stigmasterol	3278	3275	0.94	RI, MS, AU
22.	β-Sitosterol	3327	3335-3355 [20,21]	1.65	RI, MS, AU
23.	**Olean-12-en-3-one**	**3351**	**3370**	**6.27**	**RI, MS**
24.	**β-Amyrin**	**3386**	**3368-3424** [20, 22]	**45.28**	**RI, MS, AU**
25.	Lupeyl acetate	3412	3414	3	RI, MS
26.	**Lupeol**	**3444**	**3486**	**15.03**	**RI, MS**
27.	3,5-Stigmastadien-7-one	3452	3462	0.07	RI, MS
	Total			93.35%	

[a] Numbering according to elution order on Rtx-5MS fused bonded column, [b] Percentages are the mean of three analyses. Identification was based on comparing RI: published retention indices in Wiley Registry of Mass Spectral Data (8th edition), NIST Mass Spectral Library and other published data [17-22], MS: mass spectral data and AU: co-chromatography with authentic samples. Major components were highlighted in bold.

The presence of a wide range of data reported for the retention indices of some sterols and/ or triterpenoids can be attributed to the differences in experimental conditions, column properties and size, in addition to measurement errors [31].

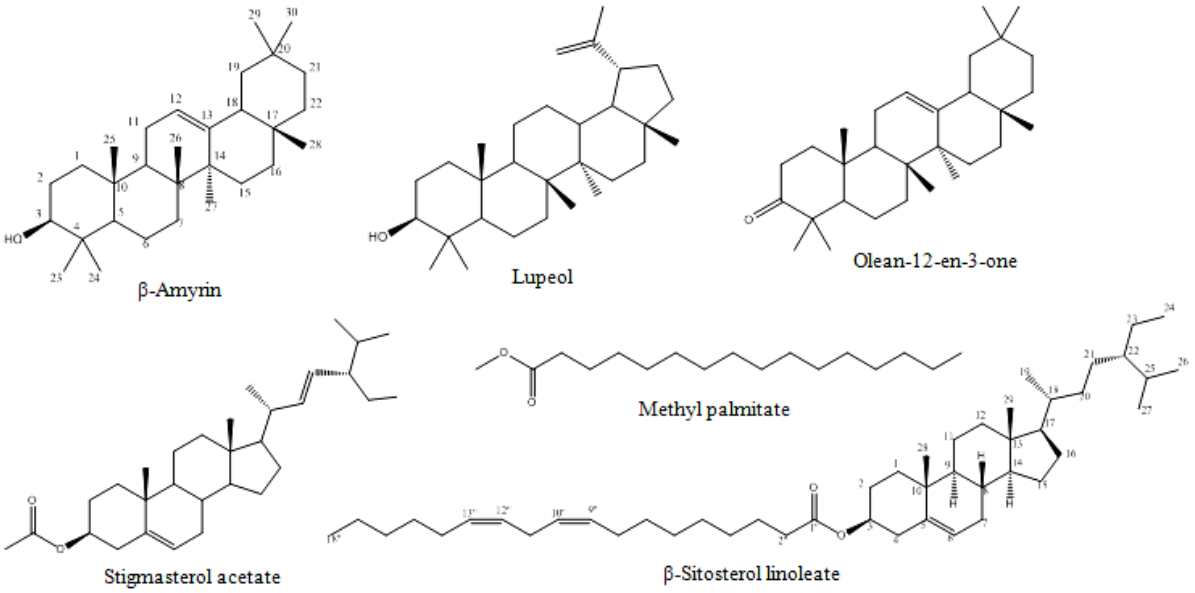

Figure 4. Major compounds identified and/or isolated from BCLE.

Alzheimer is a neurodegenerative disease with progressive disorders in the brain leading to dementia and characterized by a functional decline in memory, disturbance in behavior and decrease in the level of brain acetylcholine [32, 33]. SC is an antagonist for the cholinergic receptors. It is frequently used in experimental models of memory impairment and dementia of Alzheimer type. It acts by blocking the cholinergic neurotransmission leading to impaired cognition and dysfunction [34]. Different classes of acetylcholinesterase (AChE) inhibitors, such as donepezil, have been used in the symptomatic treatment of Alzheimer. Yet, their non-selectivity, cholinergic adverse effects, and hepatotoxicity limit their use and lead our concern to phytochemicals and herbal supplements for a safer management of Alzheimer [35].

The neuroprotective and cognition-enhancing abilities of BCLE were investigated against SC-induced memory deficit in rats through estimating the behavioral, biochemical parameters and histological studies. Morris water maze was experimented on rats to test the amelioration effect on spatial memory and learning abilities [16]. BCLE-treated group showed a significant improvement in spatial learning through efficiency in finding the platform quadrant, as evident from increased crossing times and prolonged period of time spent in the platform quadrant. The memory enhancement effects demonstrated by the extract-treated group were closely similar to those of the drug donepezil. This proves the potential amelioration effects of BCLE on SC-induced impairment of spatial memory.

The passive avoidance test was carried out to study the effect on long-term memory and to evaluate the processes of acquisition, retention, and retrieval of the learned abilities [36]. BCLE showed prolongation in the SC-shortened step-through latency. These results proved that BCLE ameliorated the rats' behavioral parameters.

To elucidate BCLE biochemical mechanism of anti-amnesia, the antioxidant parameters (catalase and MDA) were evaluated along with the AChE activities in rats' brain tissues. Catalase is one of the antioxidant defense enzymes capable of scavenging free radicals and reducing oxidative stress in brain tissues by breaking down hydrogen peroxide and protecting our bodies against reactive hydroxyl radicals [37]. MDA is the end product in the process of peroxidation of lipids [38], thus it is a potential marker of oxidative stress. SC produces a status of oxidative damage in the brain tissues which leads to cholinergic dysfunction as the acetylcholine levels show an inverse correlation with MDA equivalents [39]. BCLE elevated the SC-induced reduction in catalase activity, while it significantly reduced the MDA levels, indicating a powerful antioxidant effect of its phytoconstituents.

BCLE effects on the cholinergic neurotransmission were evaluated by measuring the AChE levels. Acetylcholine is the neurotransmitter that plays an important role in memory regulations and learning abilities, its level is regulated by cholinergic enzymes in the neurons [39]. AChE is the enzyme that hydrolyzes acetylcholine, thus by inhibiting AChE a sufficient amount of acetylcholine will be retained in the brain tissue for proper cognitive function [40]. Raised levels of AChE as those induced by SC lead to mitochondrial dysfunction and thus elevated oxidative stress and memory decline [39]. Administration of BCLE concomitantly with SC recovered the normal AChE levels by decreasing the SC-induced elevation in its levels. Our results gave an indication that BCLE may exert its memory enhancing effect through modification of the cholinergic neurotransmission and restoration of the levels of brain antioxidants.

These results were further evidenced by the absence of histopathological alterations such as nuclear pyknosis, degeneration and eosinophilic plagues formation in the BCLE-group rats' neuronal tissue and restoration of the normal histological architecture of the rats' brain tissues that were altered by SC administration.

Phytochemical investigation of BCLE resulted in isolation and structural elucidation of β-sitosterol linoleate and β-amyrin. The former was isolated for the first time from the genus. GC-MS analysis of the leaves extract was carried out for the first time as well and it revealed the presence of the triterpenoids: β-amyrin, lupeol and olean-12-en-3-one; fatty acid esters such as methyl palmitate, sterols as stigmasterol acetate, diterpenes and hydrocarbons.

Several identified compounds were reported to have neuroprotective properties. Park et al. (2014) reported that β-amyrin can recover the SC-induced mice memory impairment through enhancement of the ERK (extracellular signal regulated kinase) and the GSK-3β (glycogen synthase kinase-3β) signaling in the hippocampus, in addition to inhibiting AChE activity *in vitro* [41]. Stigmasterol was reported to possess neuroprotective activities in mice, in addition to protective effects against glutamate-induced toxicity in hippocampal HT-22 cell line by inhibiting both reactive oxygen species and calcium ion production [42, 43]. Kumar and Khanum (2012) reported the neuroprotective potential of α-tocopherol [44]. Moreover, Kaundal et al. (2017) stated that lupeol ameliorates amyloid beta-induced neuronal damage in rats' brain tissues by improving the rats' behavior, restoring the levels of neurotransmitters, reducing oxidative stress and inflammation [45]. These results were confirmed by Badshah et al. (2016) who reported the neuroprotective effects of lupeol against lipopolysaccharide (LPS)-induced neural inflammation in mice brains through phosphorylation of p38 protein kinase and c-Jun N-terminal kinase [46]. On the other hand, the long-term effects of linoleate-rich diet on increasing the aged rats learning abilities were reported [47]. Lin et al. (2014) reported that methyl palmitate and methyl stearate are released simultaneously from the sympathetic ganglion and that methyl palmitate has potential vasodilatory properties, especially in cerebral ischemia. In addition, both fatty acid esters can provide neuroprotection in focal and global cerebral ischemia in rats [48]. These results were further confirmed by Lee et al. (2016), who also stated that methyl esterification of fatty acids is related to neuroprotective abilities and vasodilation [49].

4. Conclusion

We conclude that, BCLE is rich in valuable phytoconstituents and has potential antioxidant and neuroprotective effects against Alzheimer-type dementia and memory impairment induced by SC. BCLE ameliorated both the behavioral and neurochemical parameters of the rats' brains, in addition to restoration of the normal histological architecture of the brain tissues. The leaves triterpenoids, fatty acid esters, and steroidal content may be responsible for ameliorating the oxidative stress and cholinergic dysfunction in the Alzheimer-type dementia induced by SC.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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