

***Boswellia sacra* Fleuka. Oleo-gum-resin Nanophytosome: Development, Standardization, and Cholinesterase Inhibitory Potential**

Table S1. Molar ratios of ingredients, including *Boswellia* extract, cholesterol, and soy lecithin, in different Phytosome formulations (F1–F10)

Formulation	<i>Boswellia</i> extract (mg)	Cholesterol (mg)	Soy lecithin (mg)
F1	1	1	1
F2	1	1	2
F3	1	1.5	2
F4	1	1.5	1.5
F5	1	2	2
F6	1.5	2	1
F7	1.5	1	2
F8	2	1	1
F9	2	1	2
F10	2	1.5	1

Material S1. *In vitro* AChE and BChE Inhibitory Activity Assay

Cholinesterase inhibitory activities of all derivatives were assessed using the modified Ellman method. Briefly, 20 μ L of AChE (0.18 U/mL) or BChE (0.162 U/mL), 20 μ L of DTNB (301 μ M), and 200 μ L of sodium phosphate buffer (0.1 M, pH 7.4) were added to separate wells of a 96-well microplate and gently mixed. Subsequently, 10 μ L of different concentration of samples were added, and the mixtures were incubated at 37 $^{\circ}$ C for 15 min. The enzymatic reaction was initiated by adding 20 μ L of acetylthiocholine iodide (ATCh) or butyrylthiocholine iodide (BTCh), resulting in a final substrate concentration of 452 μ M. Absorbance was measured at 415 nm using a microplate reader. Wells containing test compounds and buffer were used as compound interference controls to evaluate background color and possible precipitation effects. Wells containing enzyme and vehicle without inhibitor were used as negative controls. Donepezil was used as the positive control. IC₅₀ values were calculated from concentration–response curves using CurveExpert software fitted with the Modified Morgan–Mercer–Flodin (MMF) or Weibull model. All experiments were performed independently in triplicate, and the results are expressed as mean $\pm$ SD

The percentage of enzyme inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}$$

A_{control} is the absorbance of the control reaction (enzyme without inhibitor) and A_{sample} is the absorbance in the presence of the test sample.

IC₅₀ values were determined from dose–response curves using different concentrations of each sample and calculated by nonlinear regression analysis using GraphPad Prism software. Donepezil was used as the positive control under the same experimental conditions as the tested sample.

Table S2. ¹H and ¹³C NMR spectral data of compounds **1** (AKBA) and **2** (KBA) in CDCl₃

No.	Compound 1		Compound 2	
	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)
1	34.19	1.54 (m, H _α), 3.00 (m, H _β)	32.9	2.55 (d, J = 12.6)
2	21.29	1.84 (m, H _α), 2.72 (m, H _β)	24.3	1.60-1.42 (m)
3	72.83	5.94 (br s)	70.58	4.10 (br s)
4	47.22	-	47.1	
5	51.50	1.94 (d, J = 12.0)	57.4	1.60-1.42 (m)
6	18.80	2.55 (m, H _α), 2.70 (m, H _β)	18.89	1.95-1.85 (m)
7	30.98	4.47 (dd, J = 4.2, 11.4)	31.9	1.75-1.65 (m)
8	45.51	-	45.3	-
9	60.63	2.77 (s)	59.02	2.44 (s)
10	34.75	-	33.95	-
11	200.1	-	199	-
12	129.5	5.87 (s)	130.57	5.54 (s)
13	165.1	-	165.2	-
14	44.12	-	41.8	-
15	24.10	2.47 (m, H _α), 2.12 (m, H _β)	26.35	1.95–1.85 (m)
16	27.4	0.94 (m, H _α), 2.09 (m, H _β)	27.13	2.01 (dd, J = 12.0, 4.7, H _β)
17	32.91	-	33.85	-
18	59.98	1.54 (m)	59.1	1.60-1.42 (m)
19	39.47	1.47 (m)	37.45	1.60-1.42 (m)
20	37.48	0.81 (m)	34.3	0.97 (bs)
21	29.09	1.24 (m, H _α), 1.32 (m, H _β)	28.9	1.32 (s)
22	40.29	1.24 (m, H _α), 1.37 (m, H _β)	39.29	1.60-1.42 (m)
23	23.8	1.50 (s)	21.17	1.37 (s)
24	182.0	-	182.8	1.32 (s)
25	13.21	1.71 (s)	13.16	-
26	18.4	1.56 (s)	18.24	1.15 (s)
27	20.74	1.52 (s)	20.48	1.21 (s)
28	27.8	0.84 (s)	27.6	0.85 (s)
29	17.37	0.80 (d, J = 6.0)	17.48	0.80 (d, J = 6.42)
30	20.66	0.87 (d, J = 6.0)	20.61	0.97 (bs, 4H)

31 (CH ₃ CO)	21.21	2.08 (s)	-	-
Acetyl C=O	170.7	-	-	-

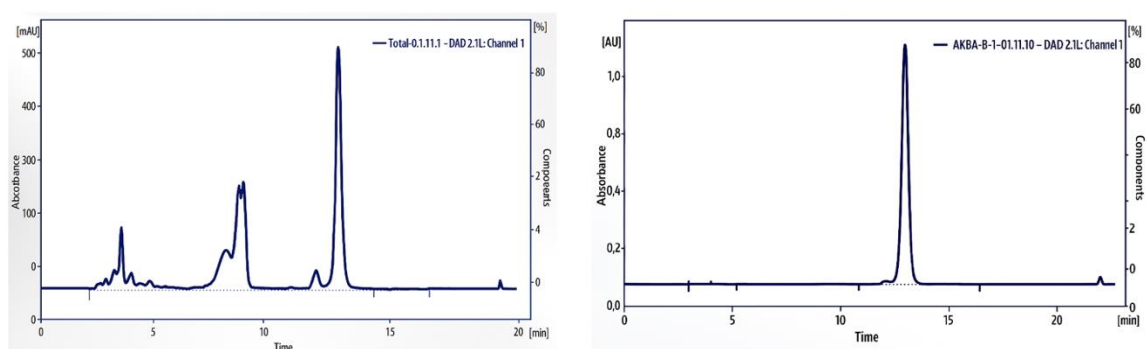


Figure S1a. HPLC chromatograms of *B. sacra* extract (left) and purified AKBA (Right).

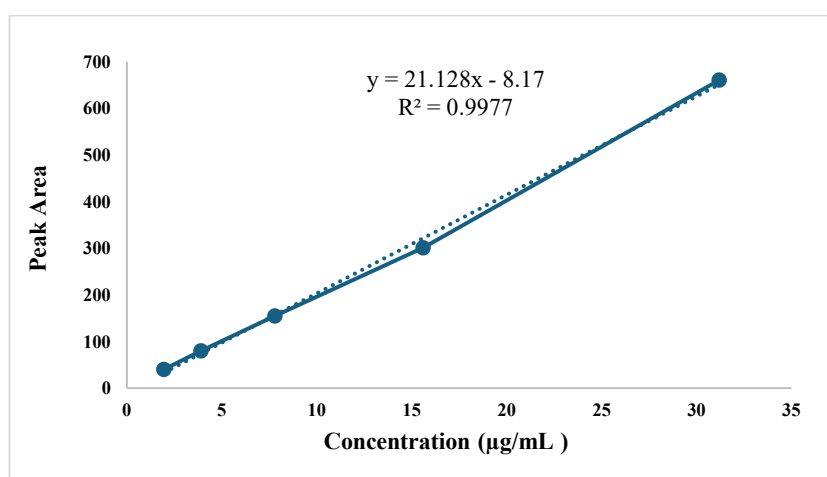


Figure S1b. Calibration curve of standardized AKBA of *B. sacra* extract

Table S3. Particle Size, PDI, and Zeta Potential of the Optimized Phytosome Over 6 Months of Storage at 4 ± 3 °C*

Time	Particle size (nm)	PDI	Zeta potential (mV)
First week	100.5	0.14	-53.8
Second week	112.3	0.26	-46.0
Third week	166.1	0.3	-45.0
Fourth week	169.2	0.35	-43.0
Third month	183.5	0.37	-32.8
Sixth month	202	0.43	-23

*All stability measurements were performed on the same batch of phytosomes used for initial characterization (F3). Values represent single measurements per time point (no SD shown for clarity). The initial particle size of the freshly prepared batch was 112 ± 12.28 nm (mean $\pm$ SD, $n = 3$; Table 1). The week 1 value (100.5 nm) falls within the standard deviation range of the initial measurement (99.72-124.28 nm), confirming batch consistency. Minor variations in particle size over time reflect gradual colloidal evolution of the formulation during storage.

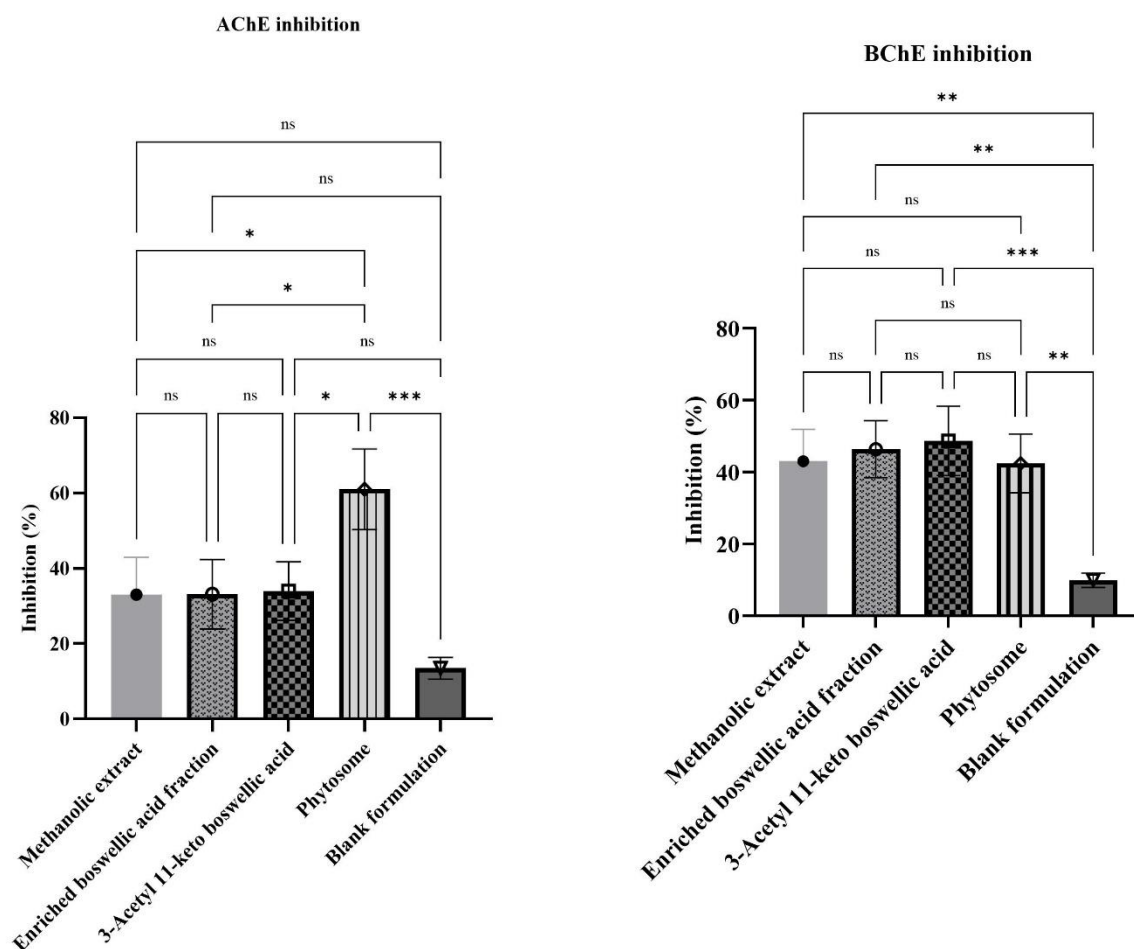


Figure S2. Acetylcholinesterase inhibitory activity (Left) and Butyrylcholinesterase inhibitory activity (Right) of the Methanolic extract, Enriched-boswellic acid fraction, AKBA, the Phytosomal formulation, and Blank formulation. Data are expressed as mean $\pm$ SD ($n = 3$). Statistical significance is indicated as $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)