

# **Green synthesis of *Blumea balsamifera* oil nanoemulsions stabilized by natural emulsifiers and its effect on wound healing**

**Lingfeng Du<sup>1</sup>, Chunfang Ma<sup>1</sup>, Bingnan Liu<sup>1</sup>, Wei Liu<sup>1</sup>, Yue Zhu<sup>2,3</sup>, Zuhua**

**Wang<sup>2,3</sup>, Teng Chen<sup>2,3\*</sup>, Luqi Huang<sup>4\*</sup>, Yuxin Pang<sup>1,2,5\*</sup>**

<sup>1</sup>College of Chinese Medicine Resources, Guangdong Pharmaceutical University, Yunfu 527325, China

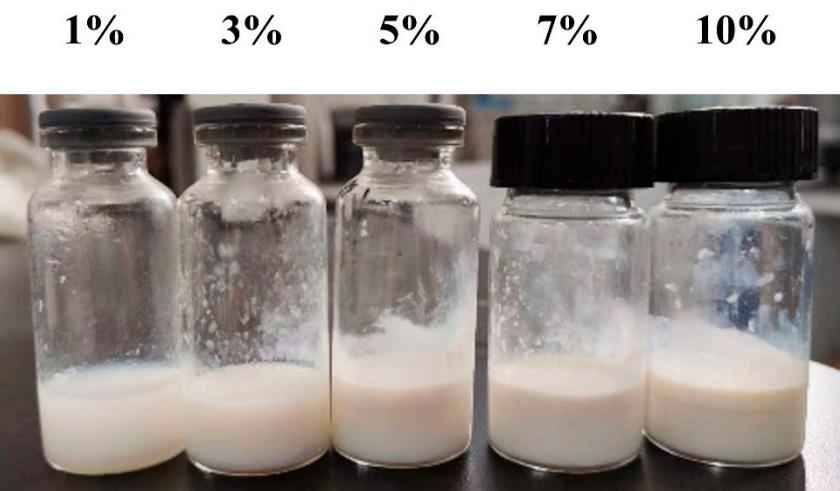
<sup>2</sup>College of Pharmaceutical Sciences, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, China;

<sup>3</sup> Nano-Drug Technology Research Center, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, China

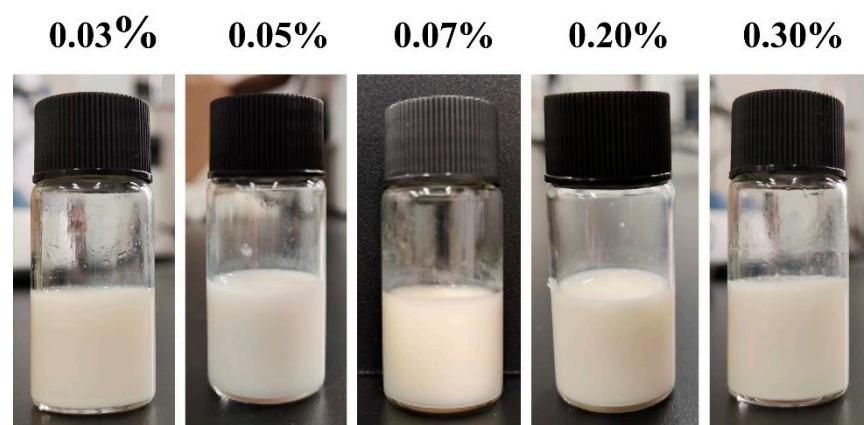
<sup>4</sup> China Academy of Chinese Medical Sciences, Beijing 100700, China

<sup>5</sup> Yunfu Traditional Chinese Medicine Resources and Germplasm Resources Database Management Center, Yunfu 527325, China

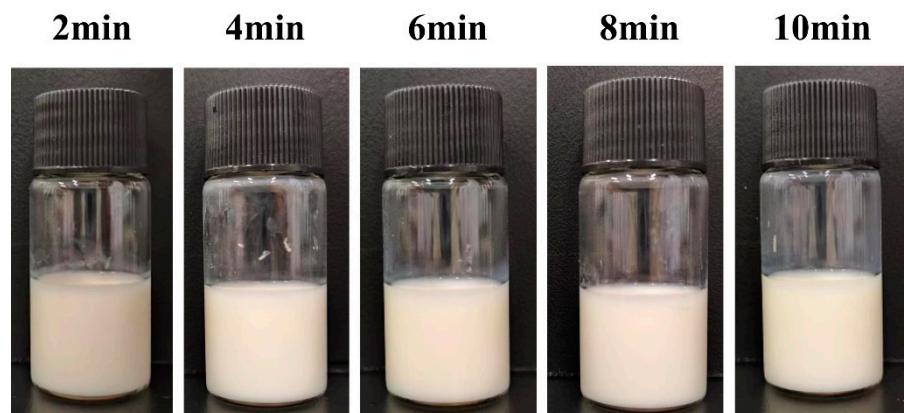
\*Correspondence: **Teng Chen**, chenteng0907@163.com; **Luqi Huang**, huangluqi01@126.com; **Yuxin Pang**, pyxmarx@126.com



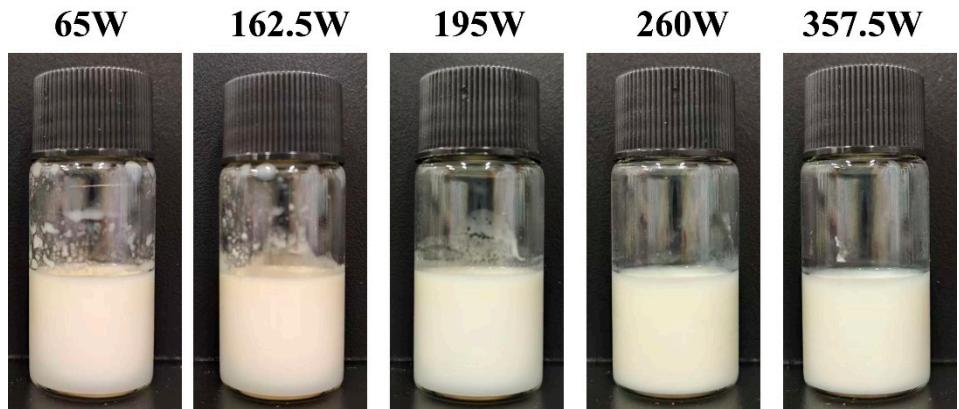
**Figure S1.** Effect of oil phase content on BBG-NEs.



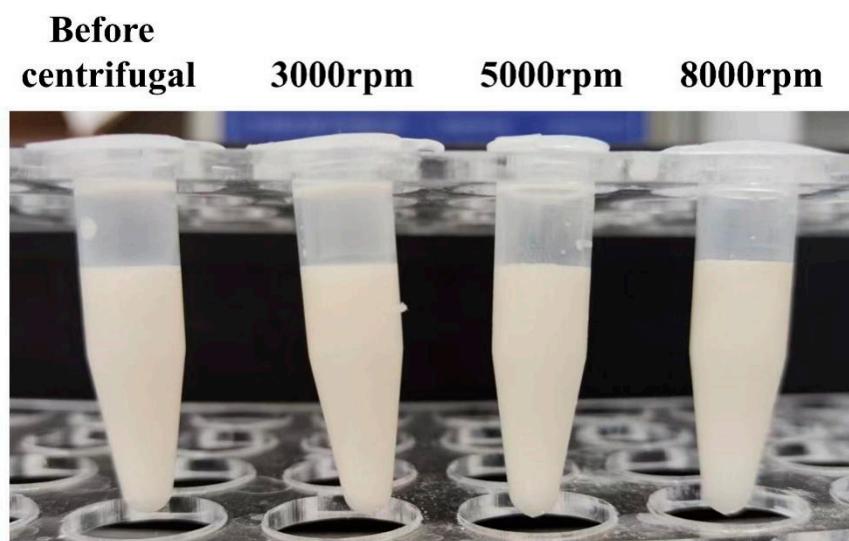
**Figure S2.** Effect of *Bletilla striata* polysaccharide content on BBG-NEs.



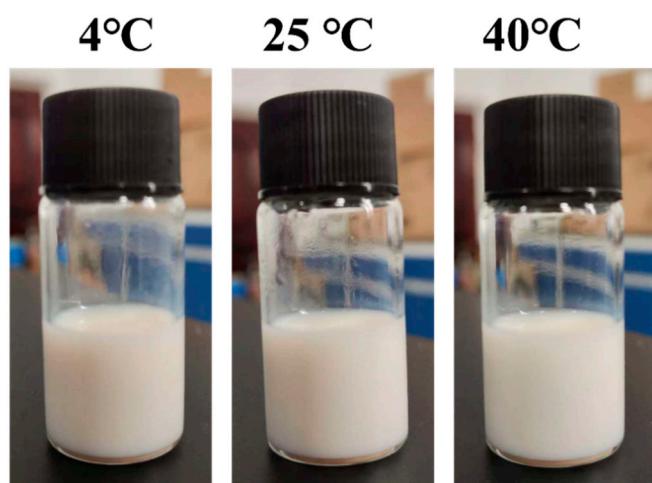
**Figure S3.** Effect of ultrasonic time on BBG-NEs.



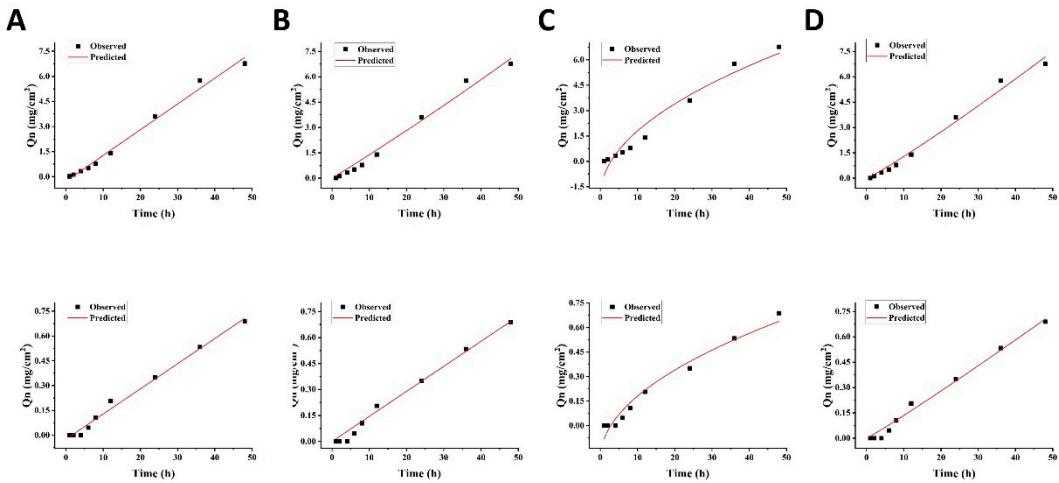
**Figure S4.** Effect of ultrasonic power on BBG-NEs.



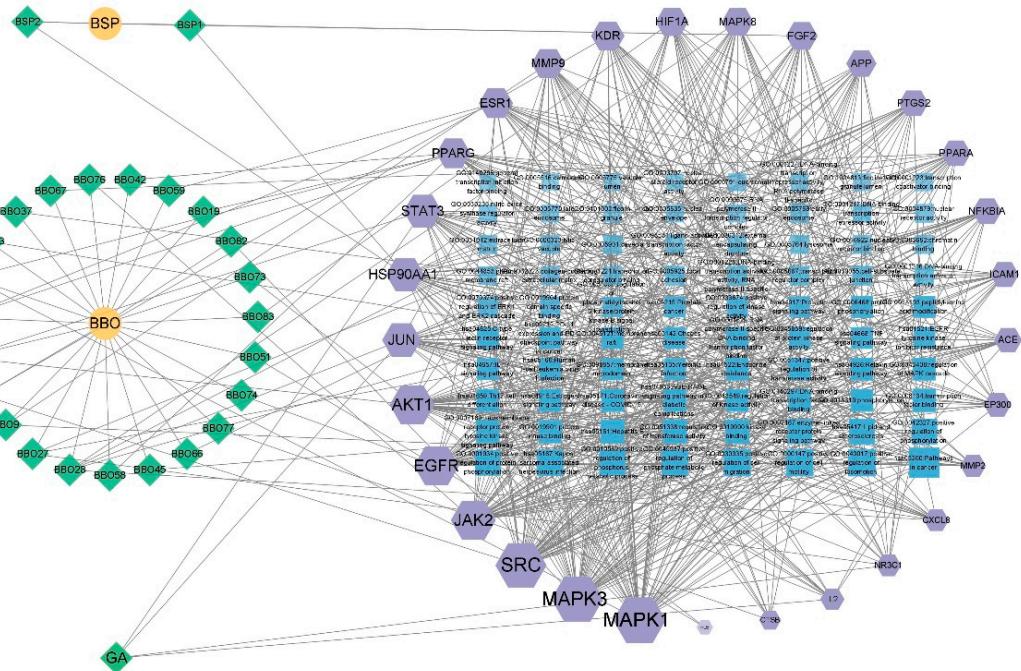
**Figure S5.** Centrifugal stability of BBG-NEs.



**Figure S6.** Temperature stability of BBG-NEs.



**Figure S7.** Transdermal comparison of BBG-NEs (upper) and BBO (lower):  
 (A) Zero-order; (B) First-order; (C) Higuchi; (D) Ritger-peppas.



**Figure S8.** Ingredient-target-pathway network, with purple hexagonal nodes representing the targets, the blue quadrilateral nodes represent pathways, and green diamond-shaped nodes represent ingredients in the formulation. The larger the area of the node graph and the darker the color

represent the larger the DEGREE value of the component.

**Table S1.** Particle size, PDI and potential of BBG-NEs prepared by ultrasonication with different BBO content.

No.	Oil phase (BBO), w/w	Appearance	Zeta potential (mV)	Particle size(nm)	PDI
1	1%	Homogeneous milky white liquid, opaque	-25.48±1.69	222.2±7.06	0.243±0.013
2	3%	Homogeneous milky white liquid, opaque	-55.98±13.58	229.71±6.82	0.238±0.001
3	5%	Milky white liquid, layered	--	--	--
4	7%	Milky white liquid, layered	--	--	--
5	10%	Milky white liquid, layered	--	--	--

**Table S2.** Particle size, PDI and potential of BBG-NEs prepared by ultrasonication with different content of BSP.

No.	Emulsifie r (BSP), w/w	Appearance	Zeta potential (mV)	Particle size(nm)	PDI
1	0.03%	Homogeneous milky white liquid, opaque	-41.70±1.52	200.39±2.17	0.238±0.001
2	0.05%	Homogeneous milky white liquid, opaque	-30.17±0.72	213.95±5.89	0.238±0.002
3	0.07%	Homogeneous milky white liquid, opaque	-38.37±0.94	209.74±3.61	0.241±0.005
4	0.2%	Homogeneous milky white liquid, opaque	-48.28±10.35	221.59±2.22	0.268±0.039
5	0.3%	Homogeneous milky white liquid, opaque	-32.94±0.53	212.66±3.51	0.306±0.044

**Table S3.** Particle size, PDI and potential of BBG-NEs prepared by ultrasonication with different ultrasonic time.

No.	Ultrasonic time, min	Appearance		Zeta potential	Particle size(nm)	PDI
1	2min	Homogeneous white liquid,	milky opaque	-38.37±0.94	209.74±3.61	0.241±0.005
2	4min	Homogeneous white liquid,	milky opaque	-35.23±0.81	177.04±3.05	0.231±0.007
3	6min	Homogeneous white liquid,	milky opaque	-32.86±0.71	170.2±2.39	0.212±0.008
4	8min	Homogeneous white liquid,	milky opaque	-27.51±0.99	194.11±8.34	0.281±0.056
5	10min	Homogeneous white liquid,	milky opaque	-32.23±3.13	162.24±3.99	0.189±0.018

**Table S4.** Particle size, PDI and potential of BBG-NEs prepared by ultrasonication with different ultrasonic power.

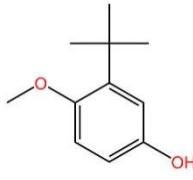
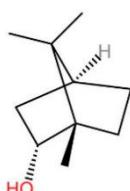
No.	Ultrasonic power , W	Appearance		Zeta potential	Particle size(nm)	PDI
1	65	Homogeneous white liquid,	milky opaque, with oil droplets on the surface	-22.18±0.57	268.06±6.95	--
2	162.5	Homogeneous white liquid,	milky opaque	-32.86±0.71	170.2±2.39	0.212±0.008
3	195	Homogeneous white liquid,	milky opaque	-34.63±1.63	177.85±5.96	0.209±0.015
4	260	Homogeneous white liquid,	milky opaque	-32.22±1.99	173±2.97	0.214±0.012
5	357.5	Homogeneous white liquid,	milky opaque	-29.69±2.05	143.16±3.463	0.207±0.036

**Table S5.** Parameters of genes associated with BBG-NEs for wound healing (top 5).

Protein names	Gene names	Betweenness unDir	Closeness unDir	Degree unDir
Serine/Threonine Kinase 1	AKT1	601.5349403	0.005617978	98
C-X-C Motif Chemokine Ligand 8	CXCL8	529.9941482	0.004901961	73
Epidermal Growth Factor Receptor	EGFR	206.7625745	0.005263158	86
Hypoxia Inducible Factor 1 Subunit Alpha	HIF1A	399.3425545	0.005154639	82
Jun Proto-Oncogene, AP-1 Transcription Factor Subunit	JUN	262.9061061	0.005050505	78

**Table S6.** Compounds in the drug-ingredient-target network (top 5).

Compound name	Molecular function	2D structure	Degree
Tetrahydrofuran-2-carboxylic acid	C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>		6
1,7,7-Trimethylbicyclo[2.2.1]heptan-2-yl-3-methylenecyclopentene-carboxylate	C <sub>17</sub> H <sub>26</sub> O <sub>2</sub>		4
Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>		3

4-Methoxy-3-tert-butylphenol	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>		3
Geranyl acetone	C <sub>13</sub> H <sub>22</sub> O		3
L-borneol	C <sub>10</sub> H <sub>18</sub> O		/

**Table S7.** The molecular docking results (top 5).

Protein	AKT (PDB code 5YVN)	CXCL8 (PDB code 6N2U)	EGFR (PDB code 8A27)	HIF1A (PDB code 8HE3)	JUN (PDB code 7NYO)
Affinity kcal/mol					
Compound					
1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl-3-methylenecyclopentane-carboxylate	-5.42	-5.20	-5.11	-3.83	-4.35
Tetrahydrofuran-2-carboxylic acid	-4.12	-3.80	-3.5	-3.23	-2.41
Eugenol	-3.61	-4.26	-4.35	-2.45	-2.19
4-Methoxy-3-tert-butylphenol	-3.86	-4.07	-3.41	-3.07	-2.62
Geranyl acetone	-4.21	-4.50	-3.22	-2.73	-2.68
L-borneol	-5.14	-5.11	-5.29	-4.2	-3.7

**Table S8.** Nanoemulsion formulation with different BBO content.

No.	Oil phase (BBO), w/w	Emulsifier (polysaccharide), w/w	Co-emulsifier (glycyrrhizic acid), w/w	Ultrasonic time, min	Ultrasonic power, W
1	1%	1%	0.3%	2	162.5
2	3%	1%	0.3%	2	162.5
3	5%	1%	0.3%	2	162.5
4	7%	1%	0.3%	2	162.5
5	10%	1%	0.3%	2	162.5

**Table S9.** Nanoemulsion formulation with different content of BSP.

No.	Oil phase (BBO), w/w	Emulsifier (polysaccharide), w/w	Co-emulsifier (glycyrrhizic acid), w/w	Ultrasonic time, min	Ultrasonic power, W
1	3%	0.03%	0.3%	2	162.5
2	3%	0.05%	0.3%	2	162.5
3	3%	0.07%	0.3%	2	162.5
4	3%	0.2%	0.3%	2	162.5
5	3%	0.3%	0.3%	2	162.5

**Table S10.** Nanoemulsion formulation with different ultrasonic time.

No.	Oil phase (BBO), w/w	Emulsifier (polysaccharide), w/w	Co-emulsifier (glycyrrhizic acid), w/w	Ultrasonic time, min	Ultrasonic power, W
1	3%	0.07%	0.3%	2	162.5
2	3%	0.07%	0.3%	4	162.5
3	3%	0.07%	0.3%	6	162.5
4	3%	0.07%	0.3%	8	162.5
5	3%	0.07%	0.3%	10	162.5

**Table S11.** Nanoemulsion formulation with different ultrasonic power.

No.	Oil phase (BBO), w/w	Emulsifier (polysaccharide), w/w	Co-emulsifier (glycyrrhizic acid), w/w	Ultrasonic time, min	Ultrasonic power, W
1	3%	0.07%	0.3%	6	65
2	3%	0.07%	0.3%	6	162.5
3	3%	0.07%	0.3%	6	195
4	3%	0.07%	0.3%	6	260
5	3%	0.07%	0.3%	6	357 . 5