

Supplementary Materials

Neurobehavioral Effects of Fermented Rice Bran Extract in Zebrafish Larvae Model

Jin Sil Chae ^{1,2,†}, Seong Soon Kim ^{1,†}, Kyu-Seok Hwang ^{1,†}, Hyemin Kan ¹, Jung Yoon Yang ¹,
Byunghoi Lee ¹, Dae-Seop Shin ¹, Byounghee Park ³ and Myung Ae Bae ^{1,2,*}

¹ Bio Platform Technology Research Center, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea; tlf7556@naver.com (J.S.C.); kimss@krikt.re.kr (S.S.K.); kshwang@krikt.re.kr (K.-S.H.); rgpalsl@gmail.com (H.K.); yjy1608@krikt.re.kr (J.Y.Y.); bnhlee@krikt.re.kr (B.L.); dsshin@krikt.re.kr (D.-S.S.)

² Department of Medicinal Chemistry and Pharmacology, University of Science and Technology, Daejeon 34113, Republic of Korea

³ R&D Center, Raphagen Inc., Seoul 07286, Republic of Korea; park@raphagen.com

* Correspondence: mbae@krikt.re.kr

† These authors contributed equally on this work.

Figure S1. Schematic illustrating the purification of RBF30.

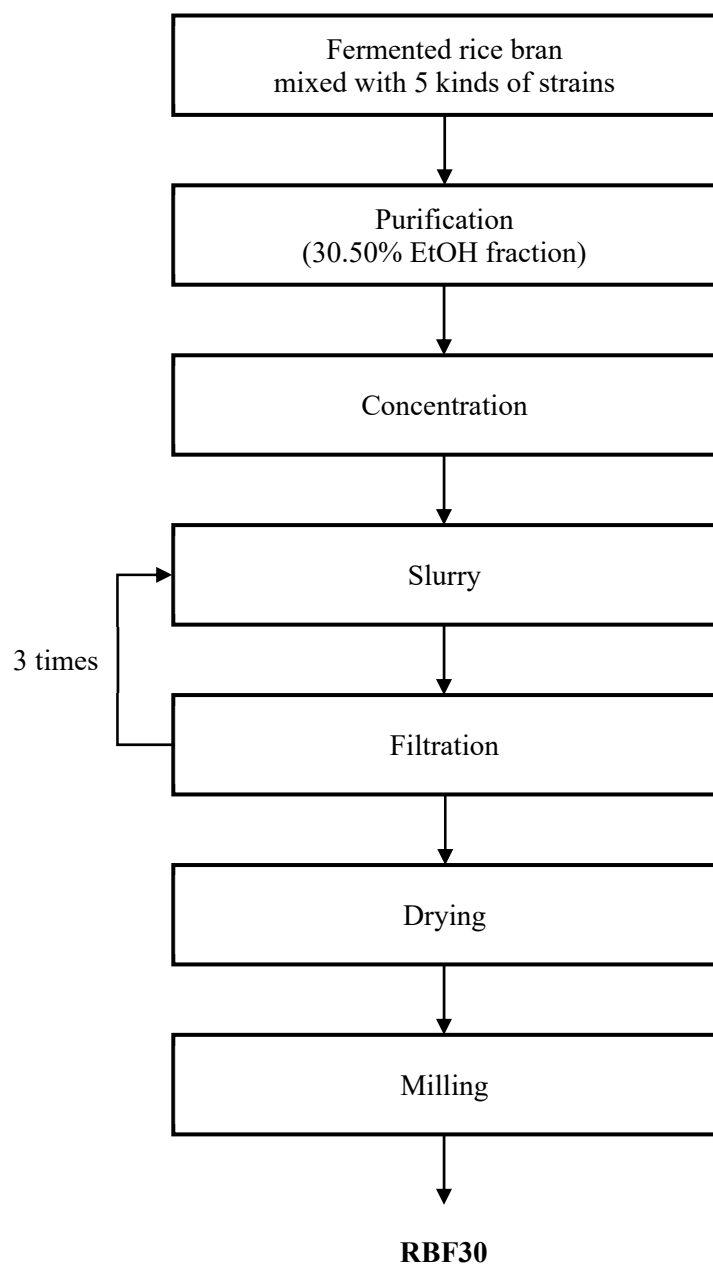


Figure S2. Behavioral effects of ethanol (EtOH) fractions 0 to 100 % (v/v) of various concentrations of RBF30 in 5 dpf zebrafish larvae. Data was represented as means \pm standard error of the mean (n=18). Significance was set at $*p \leq 0.05$, $p \leq 0.01$, and $***p \leq 0.001$ versus control.**

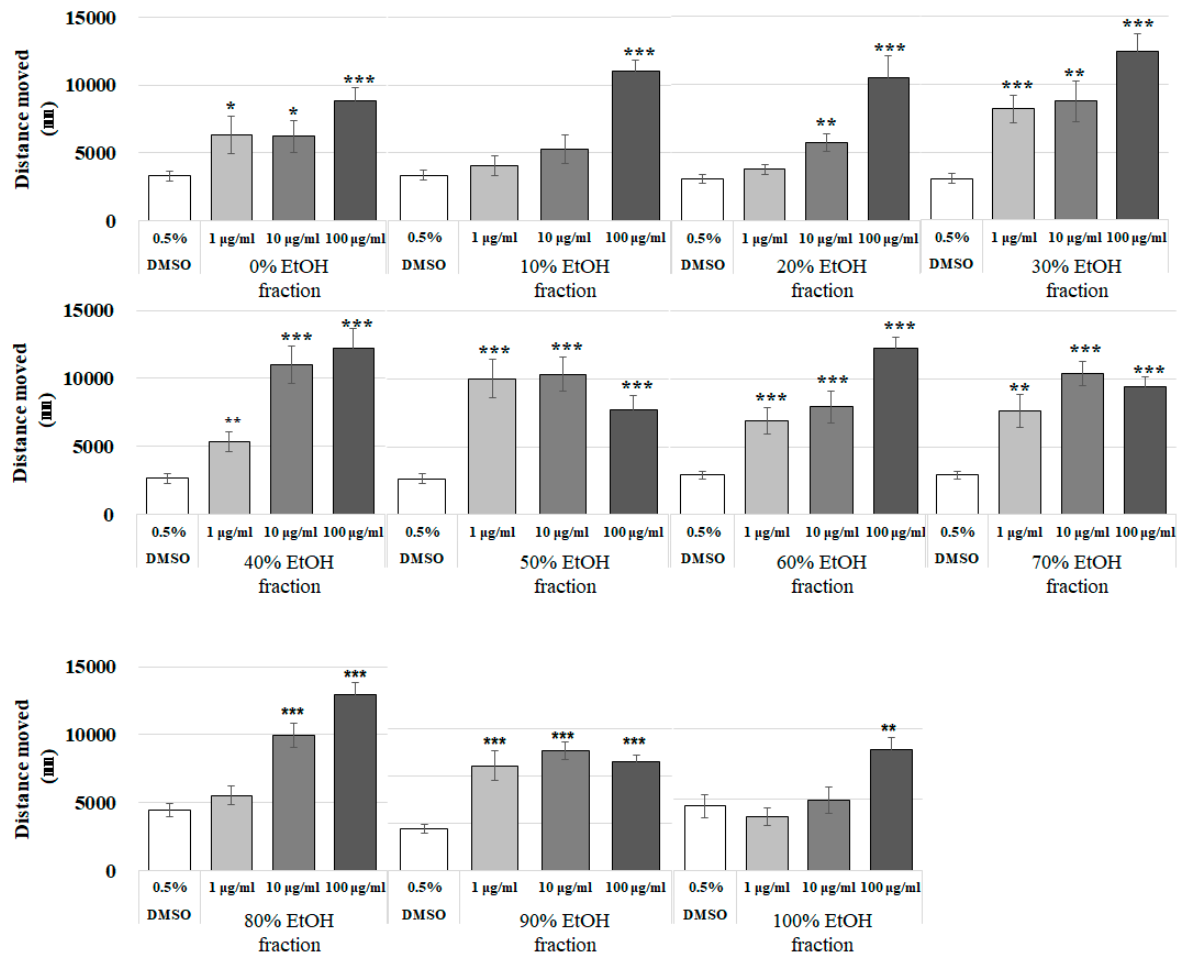


Figure S3. Larval locomotor activity in 5 dpf zebrafish larvae. Distance moved (percentage of control) of control, pentylenetetrazole (PTZ), modafinil (MDF), and RBF30-treated larvae. Data was represented as means \pm standard error of the mean (n=18). Significance was set at $*p \leq 0.05$, $p \leq 0.01$, and $***p \leq 0.001$ versus control.**

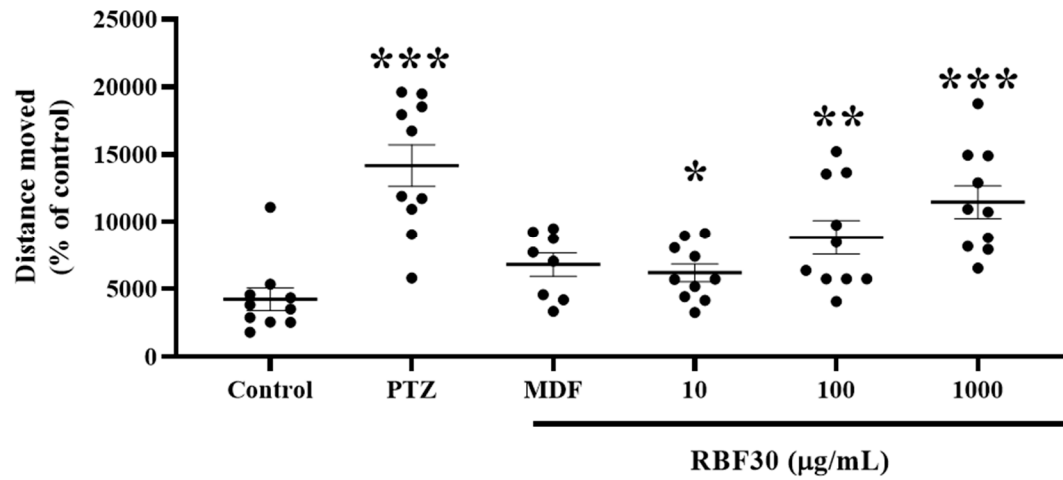


Figure S4. Larval locomotor activity in alternating periods of light and dark after administration of MDF and PTZ. (A and B) Total distance moved under alternating light/dark cycles in each 2-minute period. (C) Distance moved (percentage of control) of control and, MDF or PTZ-treated larvae in each 10-minute light–dark period. Data was represented as means \pm standard error of the mean (n=8). Significance was set at $*p \leq 0.05$, $**p \leq 0.01$, and $***p \leq 0.001$ versus control.

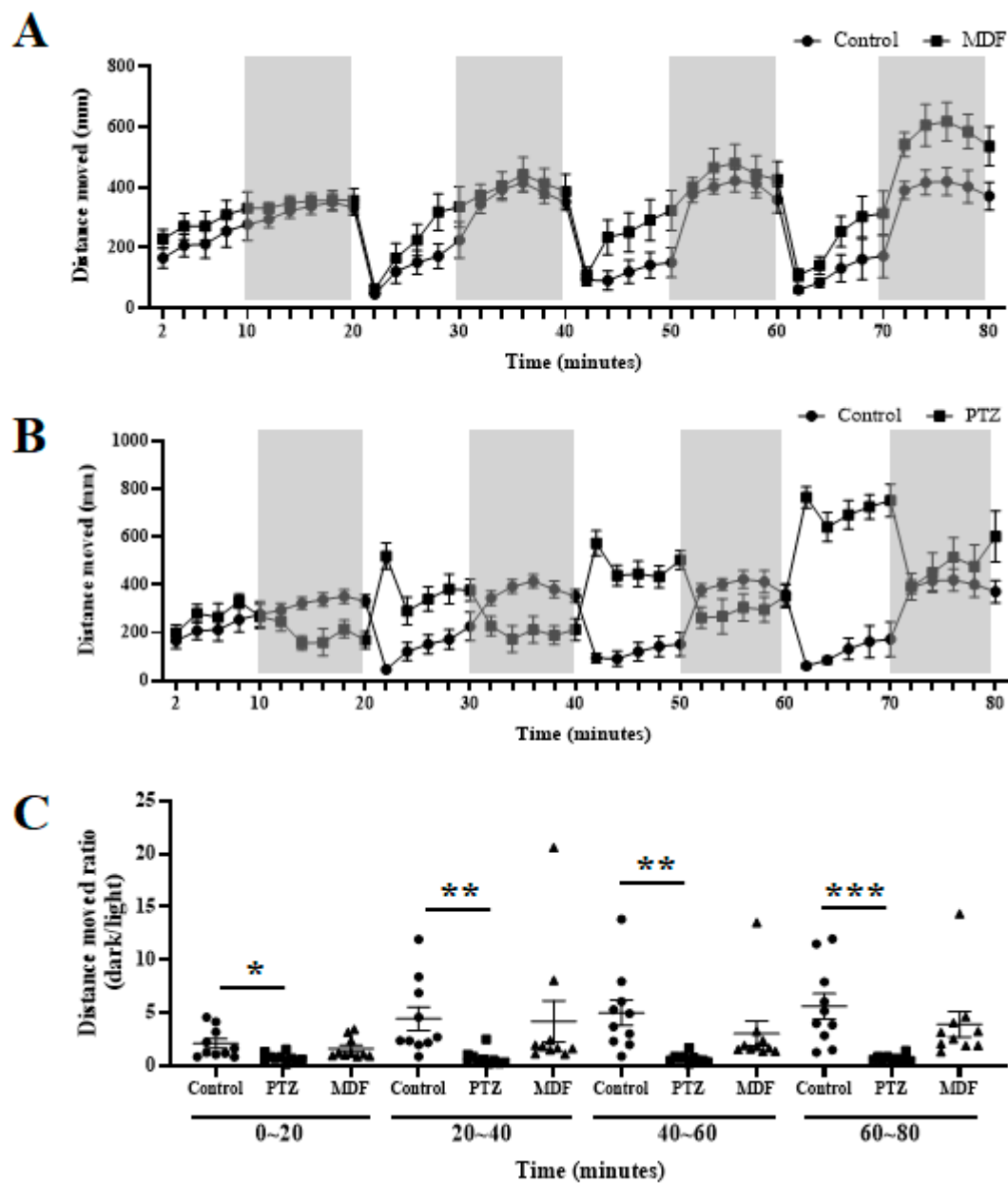


Figure S5. (A) UV spectrums scan of RB30 and RBF30 at wavelengths from 210 nm to 500 nm. (B and C) Full MS Scan (ESI⁺) of RB30 and RBF30 at m/z 50 to 500. The integral peak was assumed as a niacin ([M+H]⁺ 124 ion).

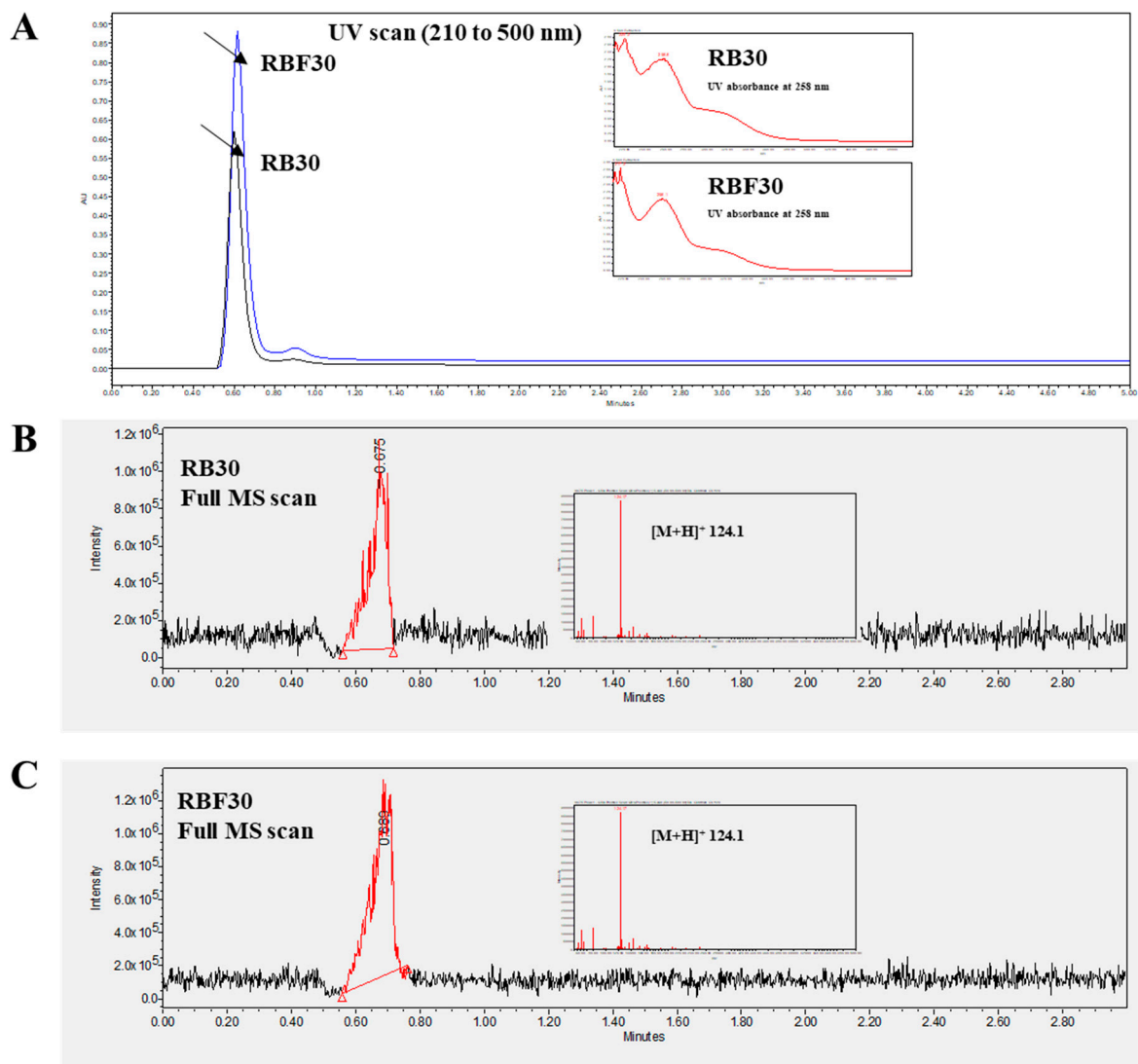


Table S1. Statistical analysis of color preference using two-way Anova (treatment versus color)

Color	Treatment	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Blue	Control vs. PTZ	49.83	42.90 to 56.76	****	<0.0001
	Control vs. MDF	-2.167	-7.561 to 3.228	ns ^a	0.656
	Control vs. RBF30	16.33	9.878 to 22.79	****	<0.0001
	PTZ vs. MDF	-52	-58.39 to -45.61	****	<0.0001
	PTZ vs. RBF30	-33.5	-43.32 to -23.68	****	<0.0001
	MDF vs. RBF30	18.5	10.48 to 26.52	****	<0.0001
Red	Control vs. PTZ	-23.5	-30.11 to -16.89	****	<0.0001
	Control vs. MDF	3	-1.440 to 7.440	ns	0.2473
	Control vs. RBF30	0.3333	-5.153 to 5.820	ns	0.9979
	PTZ vs. MDF	26.5	19.82 to 33.18	****	<0.0001
	PTZ vs. RBF30	23.83	16.17 to 31.50	****	<0.0001
	MDF vs. RBF30	-2.667	-7.501 to 2.167	ns	0.408
White	Control vs. PTZ	10.83	6.481 to 15.19	****	<0.0001
	Control vs. MDF	1.667	-3.671 to 7.005	ns	0.8012
	Control vs. RBF30	0.8333	-5.536 to 7.202	ns	0.9805
	PTZ vs. MDF	-9.167	-13.69 to -4.645	***	0.0002
	PTZ vs. RBF30	-10	-15.45 to -4.553	***	0.0005
	MDF vs. RBF30	-0.8333	-6.961 to 5.294	ns	0.9782
Yellow	Control vs. PTZ	-28.5	-31.89 to -25.11	****	<0.0001
	Control vs. MDF	-1.667	-4.890 to 1.557	ns	0.4618
	Control vs. RBF30	-7.333	-11.05 to -3.617	***	0.0003
	PTZ vs. MDF	26.83	21.95 to 31.72	****	<0.0001
	PTZ vs. RBF30	21.17	15.77 to 26.56	****	<0.0001
	MDF vs. RBF30	-5.667	-9.102 to -2.231	**	0.0014

^aNS, not significant

Significance was set at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$