

Supplementary Information

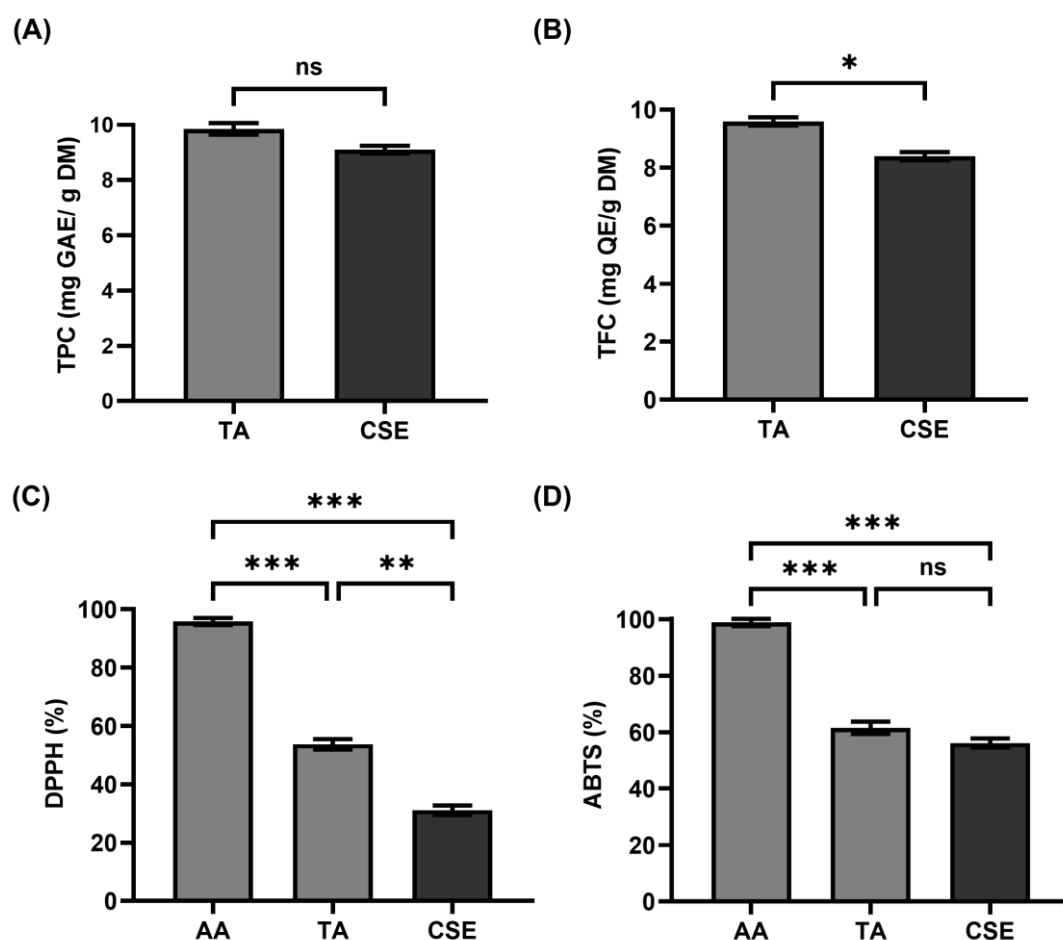
***Coelastrella* sp. extract attenuates inflammatory responses and NF- κ B p65 nuclear accumulation
in LPS-stimulated RAW 264.7 macrophages**

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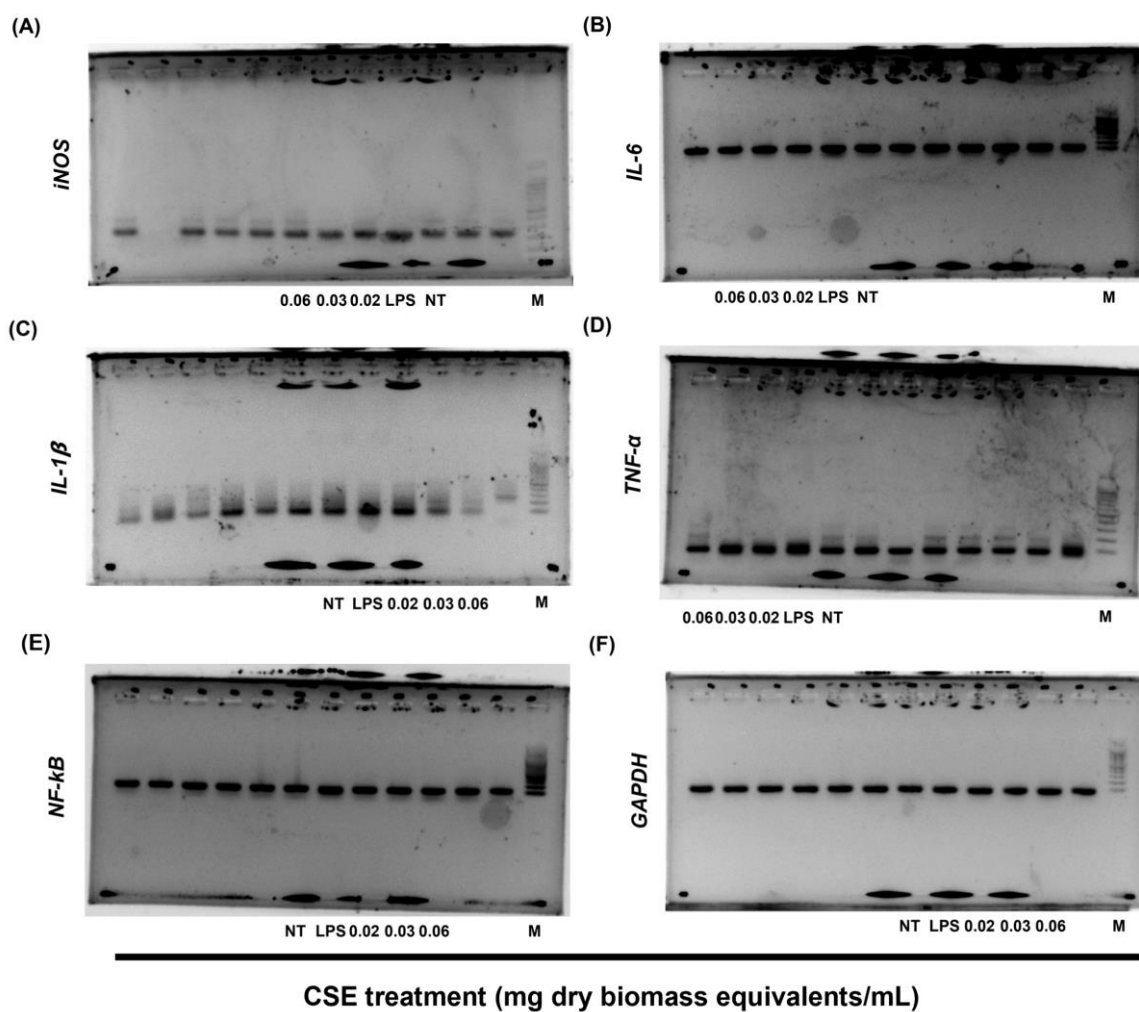
Supplementary Table S1. Primer sequences used for semi-quantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis of inflammation-related genes in RAW 264.7 macrophages.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Amplicon size (bp)
<i>GAPDH</i>	TGCCCCCATGTTTGTGATG	TGTGGTCATGAGCCCTTCC	151
<i>IL-1β</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT	89
<i>IL-6</i>	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC	76
<i>iNOS</i>	CCGAAACGCTTCACTTCCAA	ACAATCCACAACCTCGCTCCAA	300
<i>NF-κB</i>	GCTCAGCTTGTGAGGGATCTG	CCCAACCCTCAGCAAATCCT	150
<i>TNF-α</i>	GCAACCCCAGGGTGGATGAA	ACACACGGGCCAGAAAGGT	86

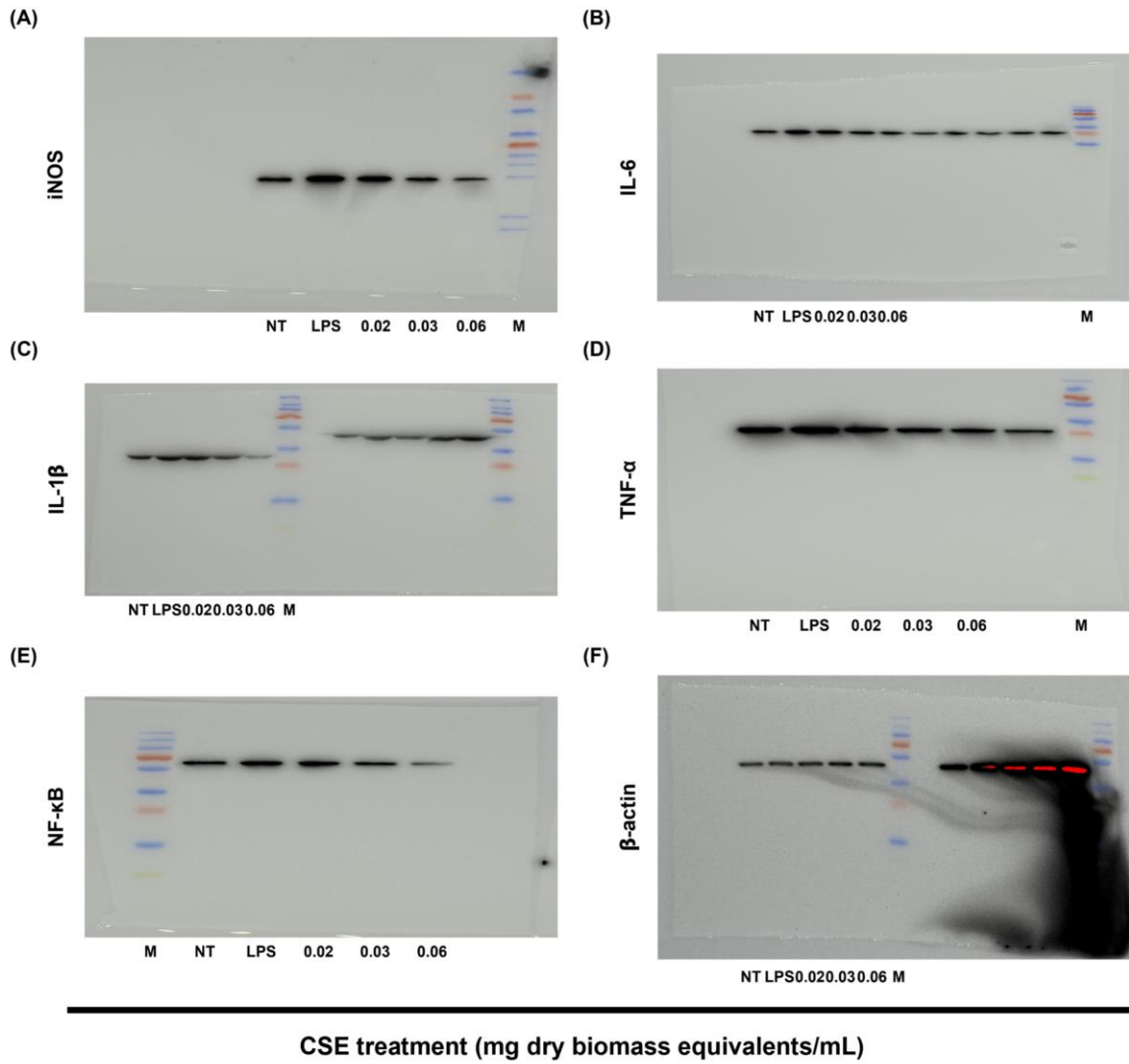
GAPDH was used as an internal control for normalization. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *IL-1 β* , interleukin-1 beta; *IL-6*, interleukin-6; *iNOS*, inducible nitric oxide synthase; *NF- κ B*, nuclear factor kappa B; *TNF- α* , tumor necrosis factor- α .



Supplementary Fig. S1. Secondary metabolite content and antioxidant activity of *Coelastrella* sp. extract (CSE). (A) Total polyphenol content (TPC). (B) Total flavonoid content (TFC). (C) DPPH radical-scavenging activity. (D) ABTS radical-scavenging activity. Tannic acid (TA) and ascorbic acid (AA) were used as reference compounds. Data are presented as the mean $\pm$ SD of three independent experiments ($n = 3$). Statistical analysis was performed using an unpaired two-tailed Student's *t*-test for panels A and B. For panels C and D, one-way ANOVA followed by Tukey's multiple comparisons test was used. ns, not significant; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for the indicated comparisons.



Supplementary Fig. S2. Full-length RT-PCR gel images corresponding to the representative bands shown in Fig. 4A. (A) *iNOS*, (B) *IL-6*, (C) *IL-1 β* , (D) *TNF- α* , (E) *NF- κ B*, and (F) *GAPDH*. Lane labels indicate the non-treated group (NT), LPS-treated control group (LPS), and CSE-treated groups at 0.02, 0.03, and 0.06 mg dry biomass equivalents/mL. M indicates the DNA molecular size marker. The labeled lanes correspond to the representative bands shown in Fig. 4A; the remaining lanes are retained as part of the full-length gel images.



Supplementary Fig. S3. Full-length Western blot images corresponding to the representative bands shown in Fig. 5A. (A) iNOS, (B) IL-6, (C) IL-1 β , (D) TNF- α , (E) NF- κ B p65, and (F) β -actin. The expected molecular masses of the quantified proteins were approximately 130 kDa for iNOS, 24 kDa for IL-6, 31 kDa for IL-1 β , 26 kDa for TNF- α , 65 kDa for NF- κ B p65, and 43 kDa for β -actin. Lane labels indicate the non-treated group (NT), the LPS-treated control group (LPS), and the CSE-treated groups at 0.02, 0.03, and 0.06 mg dry biomass equivalents/mL. M indicates the protein molecular weight marker. The β -actin blot shown in (F) was obtained from a separate membrane using the same set of protein samples loaded in the same group order and was used as the loading control for normalization of the target blots shown in (A–E). For β -actin, only the non-saturated lanes (NT, LPS, 0.02, 0.03, and 0.06) were used for densitometric quantification. The full original membrane is shown without trimming the edges, so that all bands are retained and the unprocessed original image is presented; the localized

high-background area and the colorimetric molecular-weight marker positions visible toward the right-hand edge lie outside the quantified region and were not modified. In all panels, the labeled lanes correspond to the representative bands shown in Fig. 5A, and the remaining lanes are retained as part of the full-length blot images.