

# Amentoflavone-Enriched *Selaginella rossii* Protects Against Ultraviolet- and Oxidative Stress-Induced Aging in Skin Cells

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## 1. Amentoflavone (AMF) content and phytochemical analysis in *Selaginella rossii* (SR) extracts

### *High-performance liquid chromatography (HPLC) Analysis of SR70E and SR95E*

The components of 70% and 95% ethanol extracts of SR aerial (SR70E and SR95E) were analyzed using a HPLC-diode array detector (HPLC-DAD) system (Shimadzu Corp., Tokyo, Japan). Extracts were separated on a Brownlee SPP C18 column (4.6 × 50 mm, 2.7 μm; Perkin Elmer, Inc, Waltham, MA, USA). The compositions of mobile phase are 0.1% acetic acid in water (mobile phase A) and acetonitrile (mobile phase B). The linear gradient elution program was as follows: 5–50% B at 0–15 min, 50–100% B at 15–20 min, 100% B at 20–25 min, 100–5% B at 25–27 min, and 5% B at 27–30 min. The absorbance of the HPLC profile was 225 nm, and the flow rate was 1.8 mL/min. Amentoflavone (AMF) (Biopurify Phytochemicals Ltd., Chengdu, China) was used as an external standard. The purity of AMF was 98.0%.

The AMF contents of SR70E and SR95E were 46.8 mg AMF/g extract and 66.6 mg AMF/g extract, respectively (Figure S1).

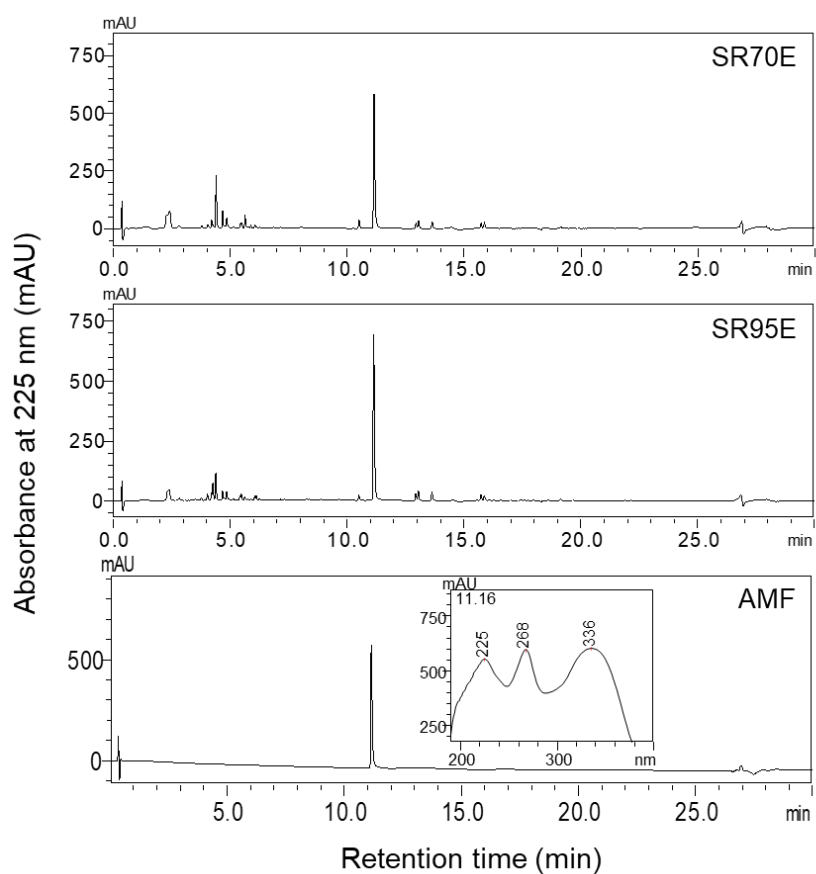


Figure S1. HPLC profiles of 70% ethanol extract of SR (SR70E), 95% ethanol extract of SR (SR95E), and amentoflavone (AMF) detected at 225 nm. Concentration of samples: SR70E (5 mg/mL); SR95E (5 mg/mL); AMF (250  $\mu$ g/mL). Injection volume 5  $\mu$ L.

The analytical HPLC profile showed that SR95E contained a large number of its natural derivatives (peaks 8-13) and different flavonoid glycosides in addition to AMF (Figure S2) [25].

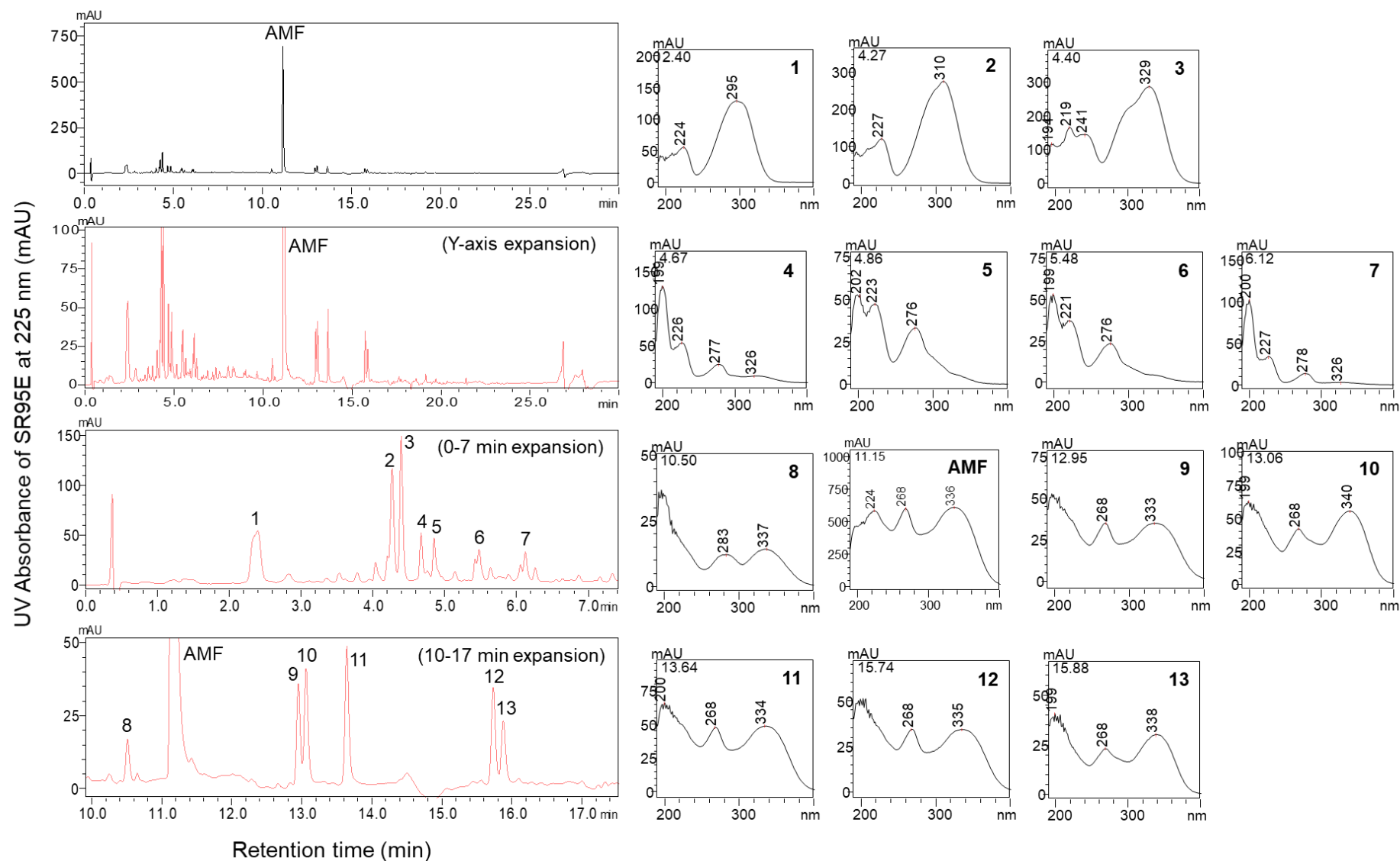


Figure S2. HPLC profiles of SR95E and UV spectra of its main phytochemical components detected at 225 nm. Concentration of samples: SR95E (5 mg/mL); Injection volume 5  $\mu$ L.

2. Primer sequences used in this article are described in Table S1.

**Table S1.** Primer sequences used for qRT-PCR.

Gene (NCBI Reference Sequence)	Forward primer	Reverse primer
<i>ACTIN</i> (NM_001101)	GGCACCACACCTTCTACAAT	GCCTGGATAGCAACGTACAT
<i>COL1A1</i> (NM_000088)	TGGCCTCGGAGGAACTTT	GCTTCCCCATCATCTCCATTC
<i>COL1A2</i> (NM_000089)	CGGTGGTGGTTATGACTTTGGT	GAAGGGTCTCAATCTGGTTGTTG
<i>MMP-1</i> (NM_001145938)	AACACATCTGACCTACAGGATTGAAA	CTTGGTGAATGTCAGAGGTGTGA
<i>MMP-2</i> (NM_001127891)	ACTGGAGCAAAAACAAGAAGACATAC	TCCATTTTCTTCTTCACCTCATTG
<i>MMP-3</i> (NM_002422)	ATGCAGAAGTTCCTTGGATTGG	GATGCCAGGAAAGGTTCTGAAG
<i>MMP-9</i> (NM_004994)	GCGCTGGGCTTAGATCATTC	GTGCCGGATGCCATTCA

## Reference

25. Yu, S.; Yan, H.; Zhang, L.; Shan, M.; Chen, P.; Ding, A.; Li, S.F.Y. A review on the phytochemistry, pharmacology, and pharmacokinetics of amentoflavone, a naturally-occurring biflavonoid. *Molecules* **2017**, *22*, 299.