Supporting Information:

Isolation and characterization of allomelanin from pathogenic black knot fungus – a sustainable source of melanin

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Supporting Figures

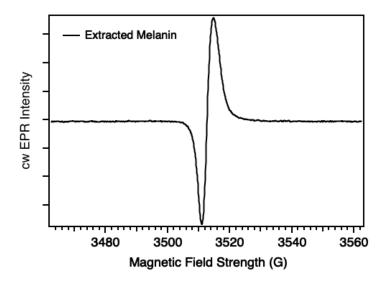


Figure S1: Electron paramagnetic resonance (EPR) spectrum of melanin extracted from black knots collected with the Bruker EMX continuous wave (cw) EPR spectrometer using a microwave frequency of 9.63 GHz. The solid powder sample was loaded into a quartz capillary tube to collect the EPR spectrum.

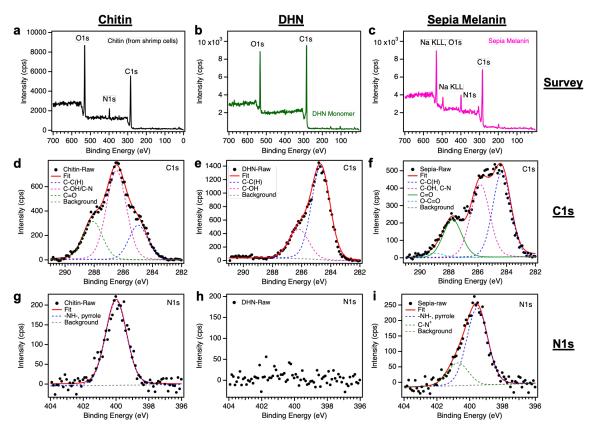


Figure S2: XPS survey scans (a, b, c), high-resolution carbon C1s (d, e, f), and high-resolution nitrogen N1s (g, h, i) spectra for chitin (from shrimp cells), 1, 8-dihyroxynapthalene (DHN) monomer, and sepia melanin (from Sigma-Aldrich). The bold line (in red) represents the fit to the experimental spectrum (black solid circles), and dotted lines represent the bands from the curve fitting.

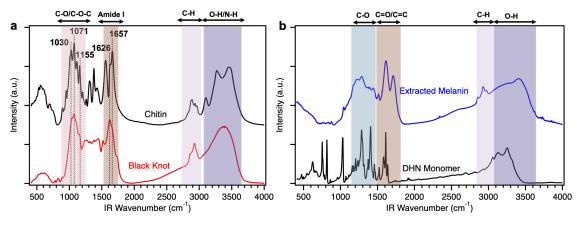


Figure S3: (a) Comparison of FTIR spectra of black knot powder and shrimp chitin. (b) Comparison of FTIR spectra of extracted melanin and DHN monomer. The spectra have been vertically offset for clarity.

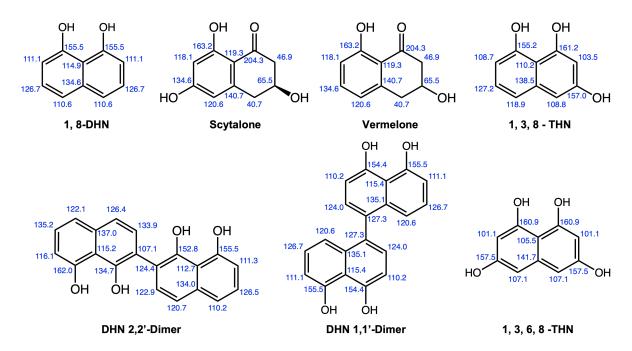


Figure S4: ¹³C chemical shift predictions for intermediates in DHN bio-synthetic pathway generated using ChemNMR in ChemDraw v.20.0 (Perkin-Elmer). These predictions match with the experimental chemical shift values observed for extracted melanin spectra shown in Figures 6.

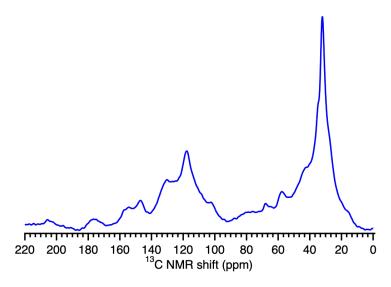


Figure S5: 13 C ssNMR spectrum of melanin extracted from black knot fungus acquired using 750 MHz Magnex Scientific magnet and Bruker spectrometer.

Supporting Tables

Table S1: Atomic percentage ($\% \pm SD; n>=2$) of different elements detected with XPS from black knot powder, extracted melanin, chitin, 1,8-DHN monomer, and sepia melanin.

Sample	C	О	N	Na	Cl
Black Knot	71.45 ± 5.03	24.70 ± 4.50	3.55 ± 0.95	-	-
Extracted melanin	74.55 ± 0.95	24.53 ± 1.16	0.90 ± 0.50	-	-
Chitin	61.35 ± 0.21	31.95 ± 0.78	6.15 ± 1.20	-	-
1, 8-DHN Monomer	79.55 ± 1.77	19.10 ± 0.99	0.20 ± 0.28	_	_
Sepia melanin	64.90 ± 2.55	22.05 ± 0.21	10.65 ± 1.20	1.15 ± 0.49	1.25 ± 0.64

Table S2: Percentage ($\% \pm SD, n=2$) of different types of carbon bonds in black knot powder, extracted melanin, chitin, 1,8-DHN monomer, and sepia melanin extracted using deconvolution of C1s XPS spectra via Multipak software and X-ray Photoelectron Spectroscopy Tool (XPST).

Sample	C-C(H)	C-OH, C-N	C=O	O-C=O
Black Knot	60.55 ± 3.02	31.52 ± 1.80	7.93 ± 1.47	-
Extracted melanin	58.23 ± 5.45	33.93 ± 1.62	5.64 ± 1.34	2.2 ± 2.51
Chitin	24.39 ± 4.38	50.27 ± 6.26	25.34 ± 1.92	-
1, 8-DHN Monomer	73.96 ± 2.02	26.04 ± 2.02	-	-
Sepia melanin	46.77 ± 4.74	34.30 ± 2.22	17.60 ± 2.04	1.33 ± 0.85

Table S3: Assignment of signals in the FTIR spectrum of extracted melanin.

IR Wavenumber (cm ⁻¹)	Peak Assignment		
3100-3600	O-H stretching		
2800-3100	aliphatic/aromatic C-H stretching		
1550-1700	C=O stretching and C=C stretching		
1200-1400	C-O residues		