The global rise of multidrug-resistant bacteria underscores the need for sustainable and environmentally benign antimicrobial alternatives. Biochar offers promise in this regard, yet the link between its physical structure and antimicrobial function remains poorly defined. This study evaluates the antibacterial, antibiofilm, and pharmacokinetic properties of water Hyacinth (WH)-activated biochar using in vitro assays and in silico prediction tools. Under scanning electron microscopy (SEM) coupled with EDX analysis, WH-activated biochar displayed a surface morphology that is uneven and diverse with high roughness, powerful porosity and a high oxygen content along with several mineral elements. Transmission Electron Microscopy (TEM) revealed thin, sheet like layers with disordered edges, indicative of high surface reactivity. Functionally, the WH-activated biochar exhibited strong inhibition against Listeria monocytogenes (84.22%), moderate activity against Acinetobacter sp. (59.98%), while displaying limited or no activity against most Gram-negative strains and Candida albicans. Biofilm inhibition analysis showed modest suppression against Bacillus subtilis (32.25%) and Staphylococcus aureus (20.02%), with no detectable activity against Escherichia coli or Pseudomonas aeruginosa. In silico ADME/Toxicity modeling indicated high solubility, Lipinski compliance, moderate intestinal absorption, low permeability of the blood–brain barrier, minimal CYP inhibition, rapid clearance, and low predicted cardiotoxicity. Overall, WH activated biochar demonstrates selective Gram positive antibacterial activity, modest antibiofilm potential, and a favorable …