Antimicrobial activity of the alkaloid cephaeline obtained through an in vitro biotechnological process against multidrug-resistant pathogens

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Antimicrobial resistance (AMR) represents one of the most significant global health threats today. Recent estimates indicate that AMR may cause 1.91 million direct deaths by 2050. Among the primary challenges are the critical priority pathogens defined by the World Health Organization (WHO), including carbapenem-resistant *Acinetobacter baumannii* and third-generation cephalosporin-resistant *Enterobacterales*. The 2024 Political Declaration of the United Nations General Assembly established a global goal of a 10% reduction in AMR-related mortality by 2030, encouraging both the pharmaceutical industry and academia to develop new agents. In this context, the present study aimed to evaluate the antimicrobial activity of cephaeline, an isoquinoline alkaloid obtained through an *in vitro* plant biotechnological process, against clinically relevant pathogens, including multidrug-resistant strains and mature biofilms. Cephaeline was obtained from adventitious root cultures of *Carapichea ipecacuanha*, produced under controlled conditions, followed by extraction, purification, and structural characterization. The isolated compound was identified via high-resolution mass spectrometry and nuclear magnetic resonance as the alkaloid cephaeline. The substance was converted into its free base form following treatment with sodium hydroxide for the assays. Antimicrobial activity was determined by minimum inhibitory concentration (MIC) assays using 96-well plate microdilution, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Strains from the ESKAPE group were evaluated, including *Enterobacter cloacae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus faecium*, as well as clinical isolates of *A. baumannii* with Extensively Drug-Resistant (XDR) and Pandrug-Resistant (PDR) profiles. For the evaluation of antimicrobial potential in biofilms, a standard strain of *A. baumannii* was utilized, cultured for seven days on acrylic specimens to ensure matrix maturity. Following the culture period, cephaeline was added and colony-forming units were counted, comparing the results with those obtained using the antimicrobials meropenem and piperacillin + tazobactam. The results demonstrated antimicrobial activity of cephaeline against all tested strains, except P. *aeruginosa*, with MIC values below 3.42 mg/mL. In biofilm assays, a 53% reduction in viable cell counts was observed, with performance equivalent to the antimicrobials meropenem and piperacillin/tazobactam, showing no statistically significant difference between treatments. These findings highlight the potential of cephaeline as an active antimicrobial agent against clinically relevant pathogens, including multidrug-resistant strains and mature biofilms. The results of this study led to the filing of two patent applications with the National Institute of Industrial Property (INPI), underscoring the innovative nature of cephaeline as an antimicrobial agent (BR1020260051691; BR102060051748). It is concluded that cephaeline exhibits significant antimicrobial activity, including against multidrug-resistant strains and mature biofilms, positioning it as an innovative and promising candidate for the development of new

therapeutic strategies against global antimicrobial resistance.

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