REAL-TIME ASSESSMENT OF *CANDIDA* BIOFILM DISRUPTION BY *GALENIA AFRICANA*

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INTRODUCTION

- The ability of microorganisms to form biofilms poses a daunting challenge to the microbiological community, as advantages in favour of biofilm formation include protection from the environment, physical and chemical stress resistance, metabolic cooperation, and a community-based regulation of gene expression.[1]
- Biofilms particularly develop at the interface of an aqueous medium and solid surface, and are highly structured biological communities that are either surface attached or allied to one another and are enclosed by a self-produced protective extracellular matrix (ECM), exhibiting distinctive phenotypic properties, with the most notable phenotypic feature being their high resistance to antimicrobial agents.[2,3,4]
- The tendency of planktonic (free-floating) *Candida* species to display an attached biofilm mode of growth is a major virulence feature and a direct cause of therapeutic failure.[5] The most common fungal species associated with biofilm formation and infections is *Candida albicans*, which has resulted in an increased resistance to most anticandidal agents.[4,6]
- The use of fluconazole (FCZ), a routinely dispensed antifungal in the treatment of localised and systemic *Candida* infections, often leads to treatment failure due to drug resistance[7]. This increases patient morbidity and mortality and justifies the need for effective and accessible treatment alternatives.
- *Galenia africana* is an indigenous South African plant with proven antifungal properties and no toxicity to mammalian cells.[8] · In this study the activity of a *G. africana* aqueous extract against preformed *C. albicans* and *C. glabrata* biofilms was tested using the xCELLigence impedance-based real-time cell analyser (RTCA) system.

BACKGROUND

- The principle of the xCELLigence impedance-based real-time cell analyser (RTCA) system:

Employs microtiter plates with gold microelectrodes through which impedance signals (expressed as cell index (CI) values) are measured and allows for real-time monitoring of changes in cell number, size, adhesion and extracellular polymeric substances (EPS) / biofilm formation, quantitative assessment of cell adhesion and continuous and automated data analysis, thereby having an advantage over traditional biofilm staining and quantification assays. The arrangement of microelectrodes at the bottom of the wells, as used in this system, has been found to better match biofilm environmental characteristics.[9]

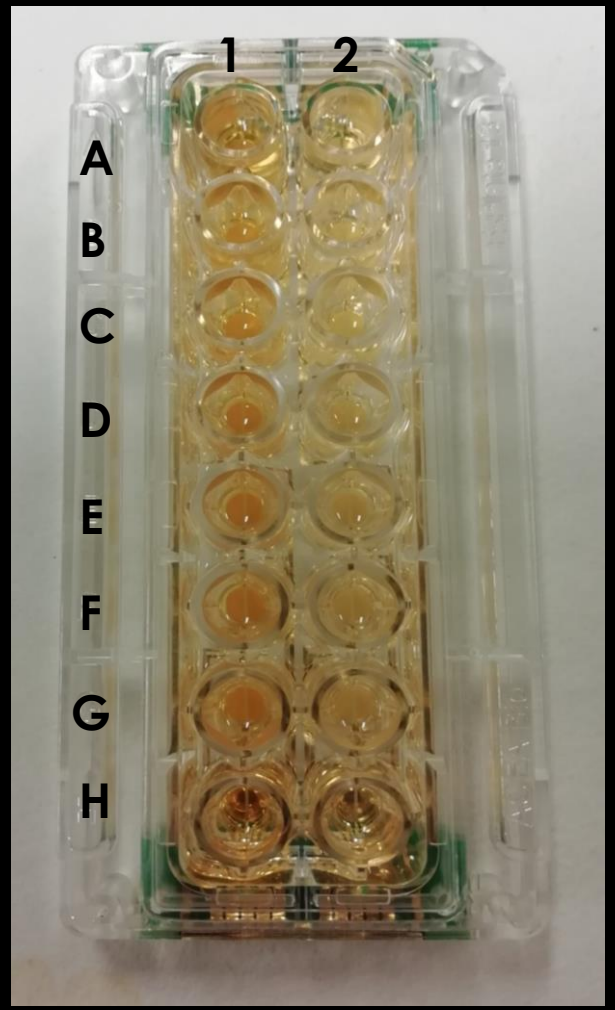
METHODS

- The type strains used in this study included *Candida albicans* (ATCC 90028) and *Candida glabrata* (ATCC 26512).

Real-time monitoring of *Candida* spp. adhesion and disruption of 10-hour preformed biofilm formation

• The real-time biofilm formation was assessed using the xCELLigence RTCA, by growing the *Candida* species for 10-hours in yeast peptone dextrose (YPD) broth on 16-well E-plates with embedded gold microelectrodes and measuring the cell adhesion / extracellular polymeric substance (EPS) formation with impedance readings set to take place at 15-minute intervals. Subsequently, after 10-hours of the initial seeding phase, the *Candida* species were treated with *Galenia africana* at varying concentrations to determine the real-time CI values for the full duration of the experiments, which were then plotted in individual graphs.

EXPERIMENTAL LAYOUT



The optimised *Candida* inoculum was added to the designated wells of the E-plates already containing 100 µl of YPD broth. Each well received 50 µl of the inoculum (as this volume provides less variation in seeding density), including the growth control well, except the sterility control well which received an additional 100 µl of YPD broth. Thus, rows A to G of columns 1 and 2 served as the experimental wells, whereas row H served as the sterility and growth control wells, respectively. Prior to adding the *G. africana* aqueous extract, all the wells contained a final volume of 150 µl/well, except the sterility control well, which contained a final volume of 200 µl/well.

Figure 1. Experimental procedure of *Candida* suspensions (50µl) and *Galenia africana* extract/FCZ (50µl) at varying concentrations on a gold microelectrode-embedded E-plate 16.

RESULTS

Disruption of 10-hour preformed *Candida albicans* adhesion and subsequent biofilm formation treated with *G. africana*

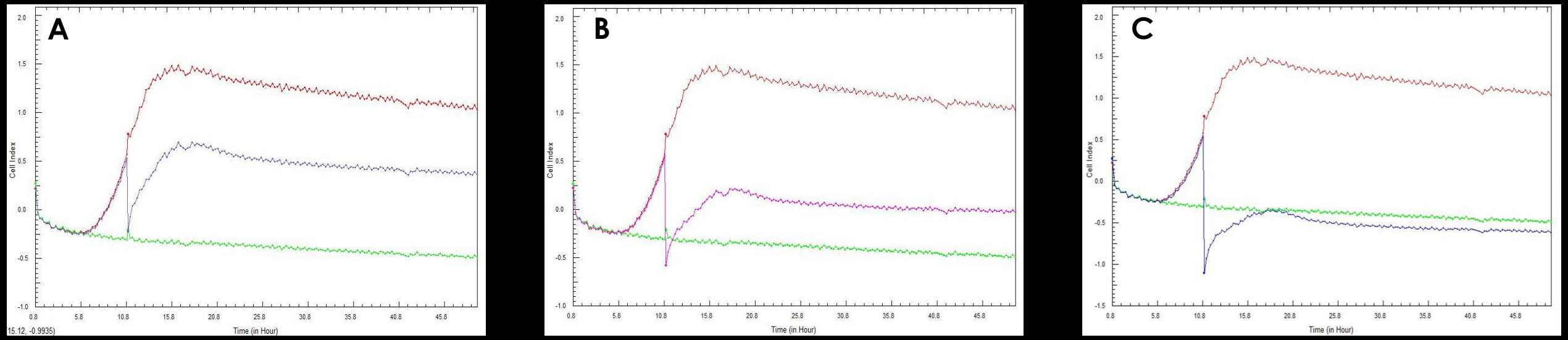


Figure 2: RTCA Cell Index (CI) growth profiles of 10-hour preformed *Candida albicans* (ATCC 90028) cell adhesion and subsequent biofilm formation treated with varying concentrations of *G. africana* aqueous extract. Each figure represents a positive control (red curve), a sterility control (green curve) and ascending concentrations of *G. africana*, where:

A. 12.5 mg/ml of *G. africana*, **B.** 25 mg/ml of *G. africana*, **C.** 50 mg/ml of *G. africana*

RESULTS

Disruption of 10-hour preformed *Candida glabrata* adhesion and subsequent biofilm formation treated with *G. africana*

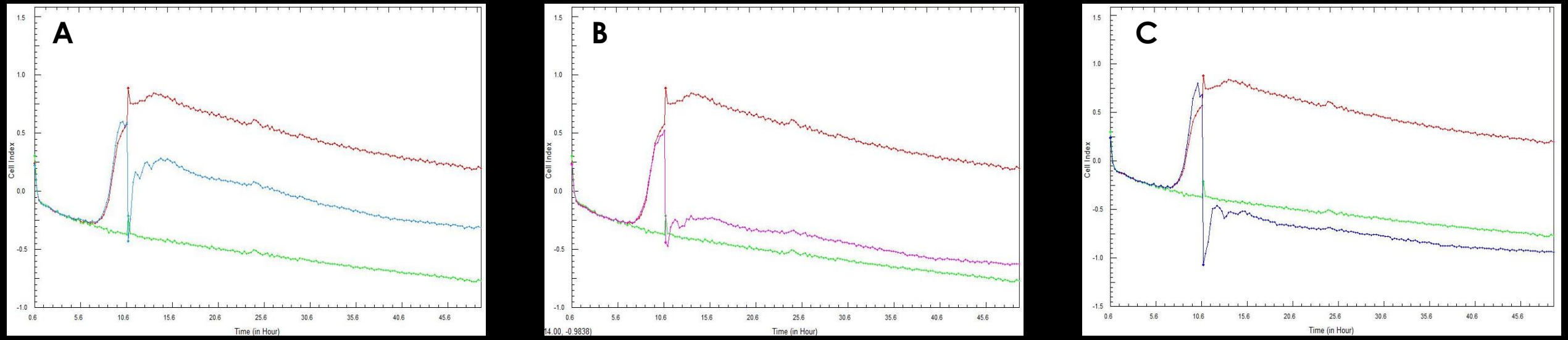


Figure 3: RTCA Cell Index (CI) growth profiles of 10-hour preformed *Candida glabrata* (ATCC 26512) cell adhesion and subsequent biofilm formation treated with varying concentrations of *G. africana* aqueous extract. Each figure represents a positive control (red curve), a sterility control (green curve) and ascending concentrations of *G. africana*, where:

A. 12.5 mg/ml of *G. africana*, **B.** 25 mg/ml of *G. africana*, **C.** 50 mg/ml of *G. africana*

CONCLUSION

- The results of this study established the effectiveness of *G. africana* as a potential anti-biofilm agent.
- RTCA technology can provide a promising method for traditional medicine and natural product research. The RTCA instrument is able to assess the ability for screening new bioactive compounds that interfere with biofilm formation or remove the preformed biofilm, as well as determining the antimicrobial effective concentration in real-time.
- The results obtained in this work further support the ability of the impedance–based RTCA instrument to monitor and validate the performance screening capability of the *Candida* species to adhere and form biofilm to the microelectrode of the E-plate in a fast, label-free, reliable and time-effective manner, which is relatively easy to use and to set-up.
- The xCELLigence RTCA instrument also validates the ability to perform screening of medicinal plant extracts in eradicating strains capable of forming biofilms, and how robustly they are able to do so.
- When comparing the preformed *Candida* biofilm disruption treated with *G. africana,* the extract was far more superior to that of FCZ-treated preformed biofilm disruption.
- To our knowledge, this is the first study on natural compounds and fungal biofilm disruption to be conducted using impedance-based technology.

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