

Discovering novel plant-derived drug leads for the treatment of HIV through an integrated approach

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INTRODUCTION

HIV/AIDS is now the leading cause of death in Sub-Saharan Africa and has moved up to fourth place among all causes of death worldwide. According to estimates from the UNAIDS 2009 report (UNAIDS 2009) on the global AIDS epidemic, around 33.4 million people were living with HIV and 2 million died of AIDS related causes in 2008. Current prevention strategies are inadequate to stop the rapid spread of the epidemic. The search for safe, affordable treatments for HIV thus remains a major challenge and a key towards controlling the disease.

A collaborative research programme was initiated between the Council for Scientific and Industrial Research (CSIR) in South Africa and Esperanza Medicines Foundation (EMF), InPheno in Switzerland to evaluate South African medicinal plants for the identification of potentially new anti-HIV drug leads.

METHOD

Procedure for plant selection

Potential plant based candidates were selected after undertaking an extensive literature study to identify plants reportedly used for the treatment of HIV or related illnesses including “boosting the immune system”. The various databases of the CSIR were searched including publications, books in the public domain and information received from indigenous knowledge holders.

Bioassay screening

EMF and InPheno provide a state-of-the-art cellular-based HIV infection system “decipher, deCIPh” (Figure 1) to measure HIV replication (including various subtypes with particular relevance for Africa such as subtypes D, D/C, A/E, B and HIV variants using either of the dominant cellular co-receptor types for HIV: CCR5 and CXCR4, or dual-tropic). These, combined with assays for cellular cytotoxicity are used to define a potential candidate for further development.

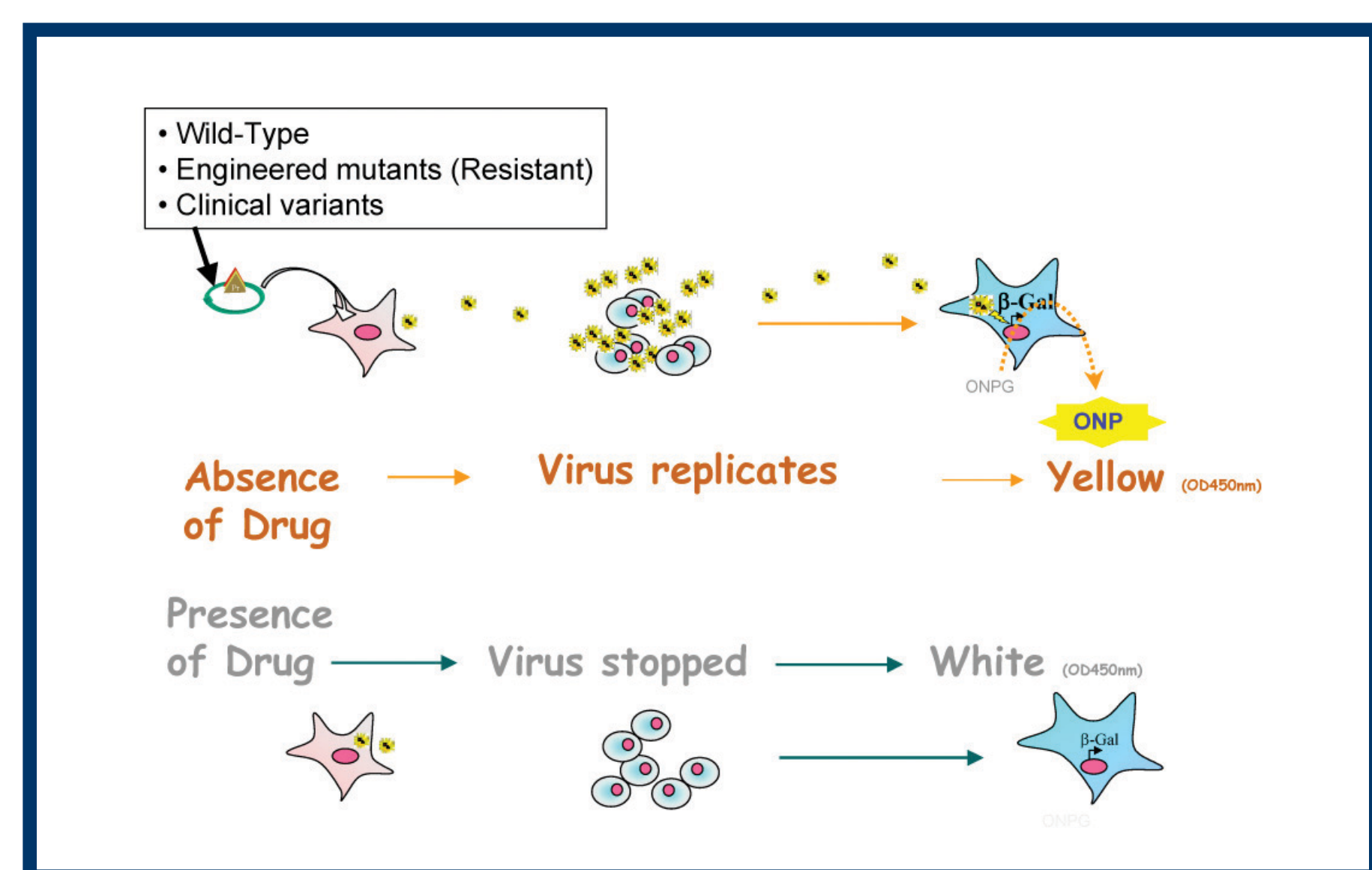


Figure 1: Standard protocol of Cell-infection for Phenotyping resistance using the decipher system

The screening results were classified as follows:

- $IC_{50} \leq 11 \mu\text{g/ml}$ in both co-receptors; the extract was considered potent
- $70 \mu\text{g/ml} \leq IC_{50} < 111 \mu\text{g/ml}$ in both co-receptors; the extract was considered moderate
- $1000 \mu\text{g/ml} < IC_{50} < 70 \mu\text{g/ml}$ in both co-receptors; the extract was considered weak
- $IC_{50} \geq 1000 \mu\text{g/ml}$ in at least both co-receptors; the extract was considered inactive.

An accelerated compound identification approach

In an effort to speed up the process of identifying HIV drug leads or active compounds, an accelerated approach was adopted (Figure 2). This approach is based on 96 well-fractionation using semi-preparative HPLC and LC-MS de-replication technology targeted at the identification of a significant proportion of compounds within botanical extracts.

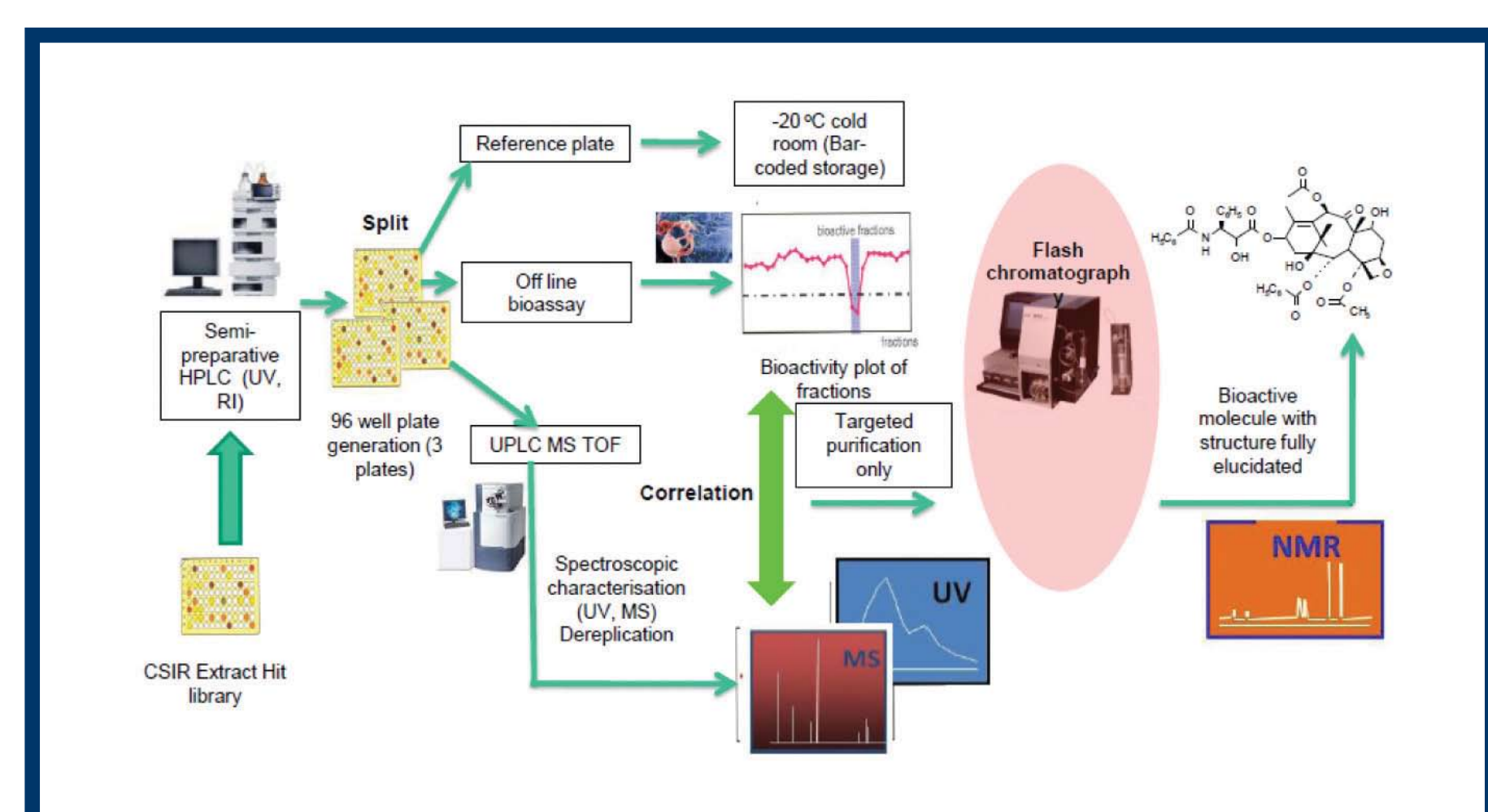


Figure 2: Accelerated approach to active ingredient identification

RESULTS AND DISCUSSION

Twenty-nine plants were selected; collected and extracted resulting into 48 plant extracts which were screened using the HIV assay. The screening results are shown in Figures 3, 4 and 5. Inhibition of 50% viral replication is reported as IC_{50} while concentration of extract provoking 50% of cell death after a 4-day time-window is reported by CD_{50} (cytotoxicity). The cytotherapeutic window (CtW) being the ratio between CD_{50}/IC_{50} was determined. The results showed that most of the plant extracts demonstrated specific anti-viral activity in a concentration range that would also significantly affect cell viability that is, a low CtW. However, three extracts, *Alepidea amatymbica* (Figure 5 no. 35), *Terminalia sericea* (Figure 5 no. 39) and *Schefflera umbellifera* (Figure 5 no. 42) were identified as potential hits for further development as these had CtW values that were sufficiently large to warrant their further development.

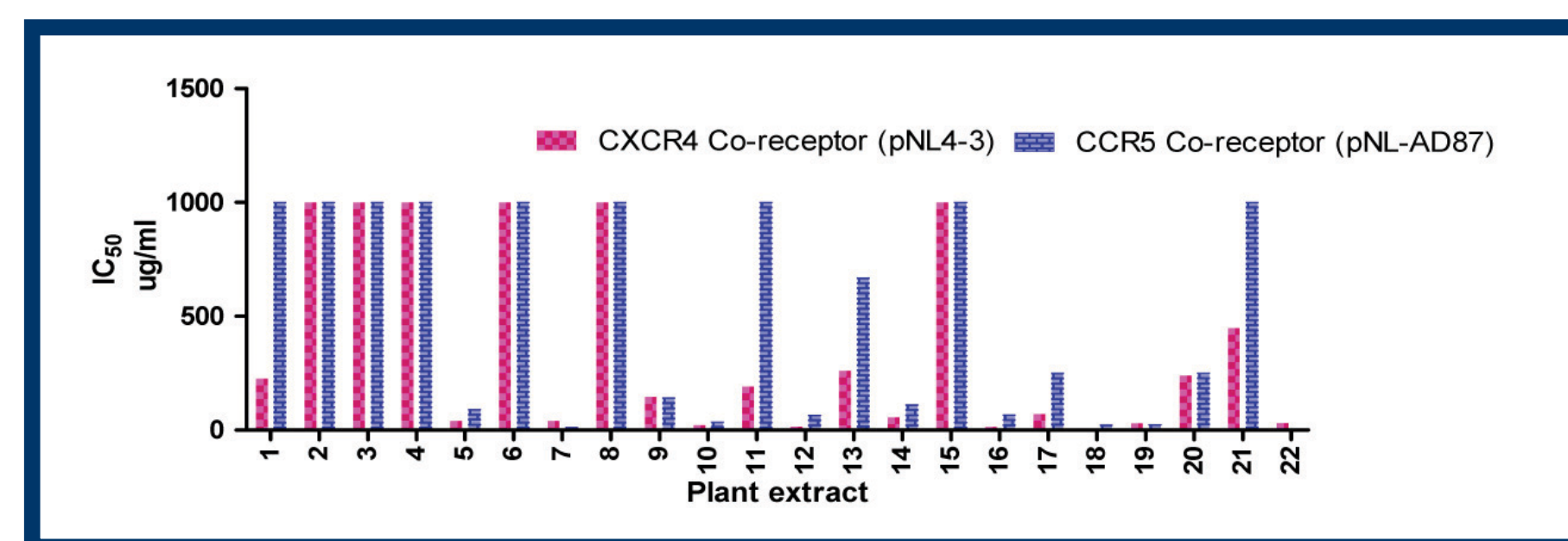


Figure 3: IC_{50} values of extract no. 1 to 22 screened in the CXCR4 and R5 virus using decipher system

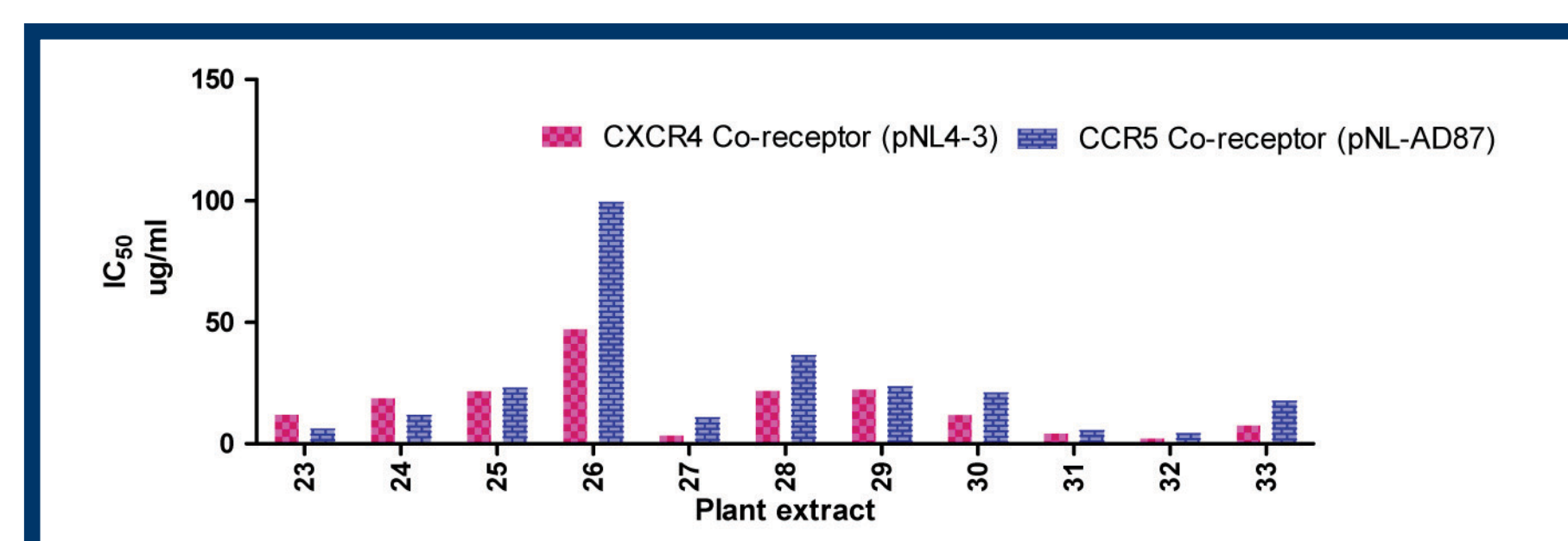


Figure 4: IC_{50} values of extract no. 23 to 33 screened in the CXCR4 and R5 virus using decipher system

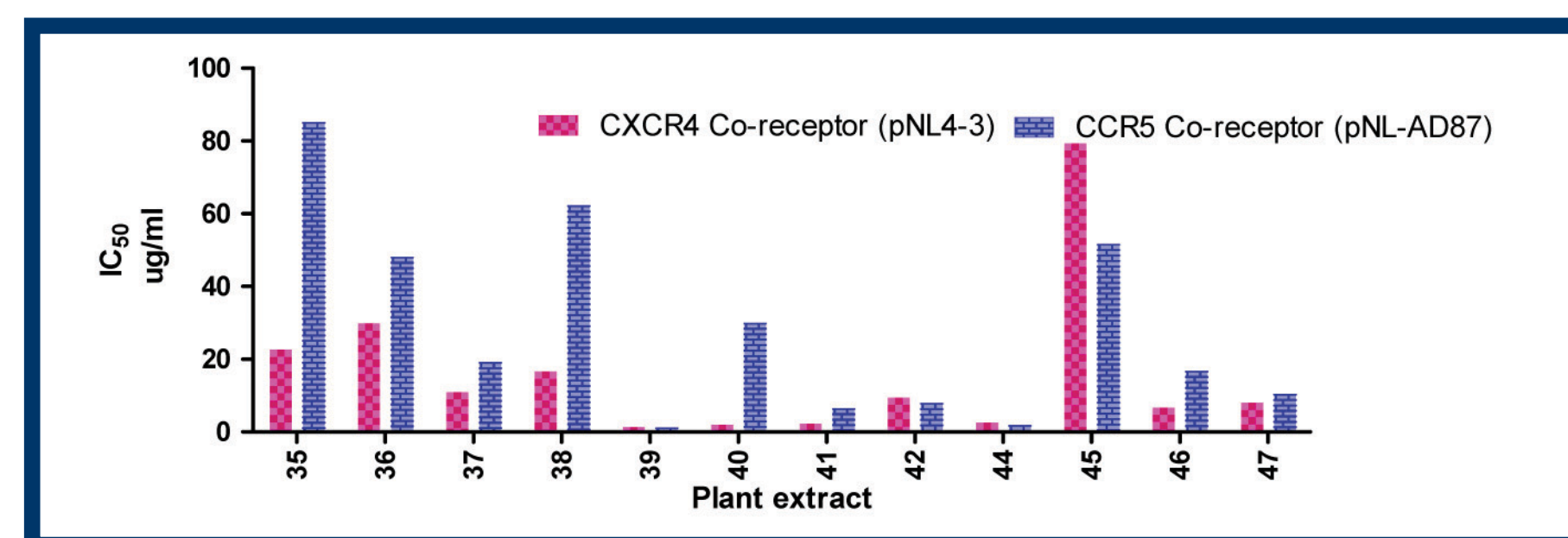


Figure 5: IC_{50} values of extract no. 34 to 48 screened in the CXCR4 and R5 virus using decipher system

The most promising extract was found to be the aqueous extract of *A. amatymbica* which showed an IC_{50} value of $22.61 \mu\text{g/ml}$ against the pNL-3 for CXCR4 usage and $85 \mu\text{g/ml}$ against the pNL-AD87 for CCR5 usage and also showed no levels of toxicity. The extract was fractionated using an accelerated approach (Figure 2) and led to the isolation of two compounds namely, rosmarinic and caffeic acid which were tentatively identified using UV/MS data (Figure 6) and literature information.

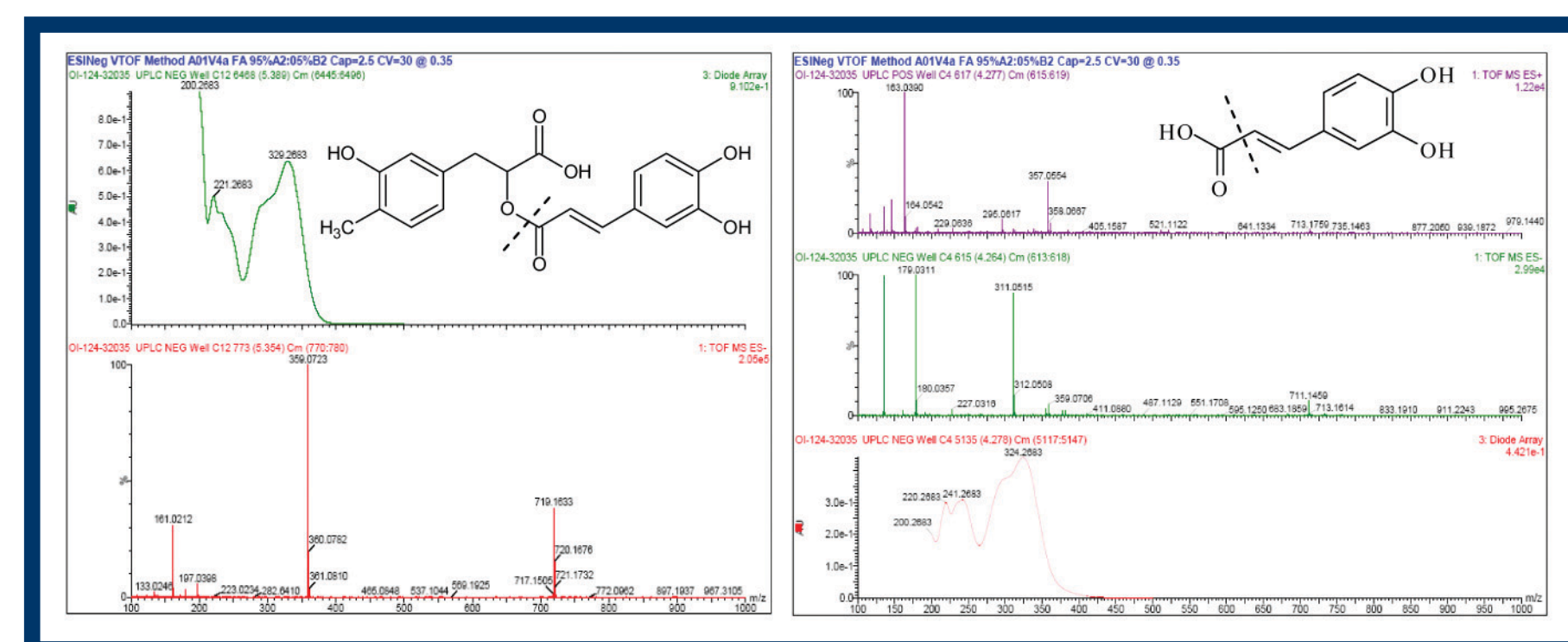
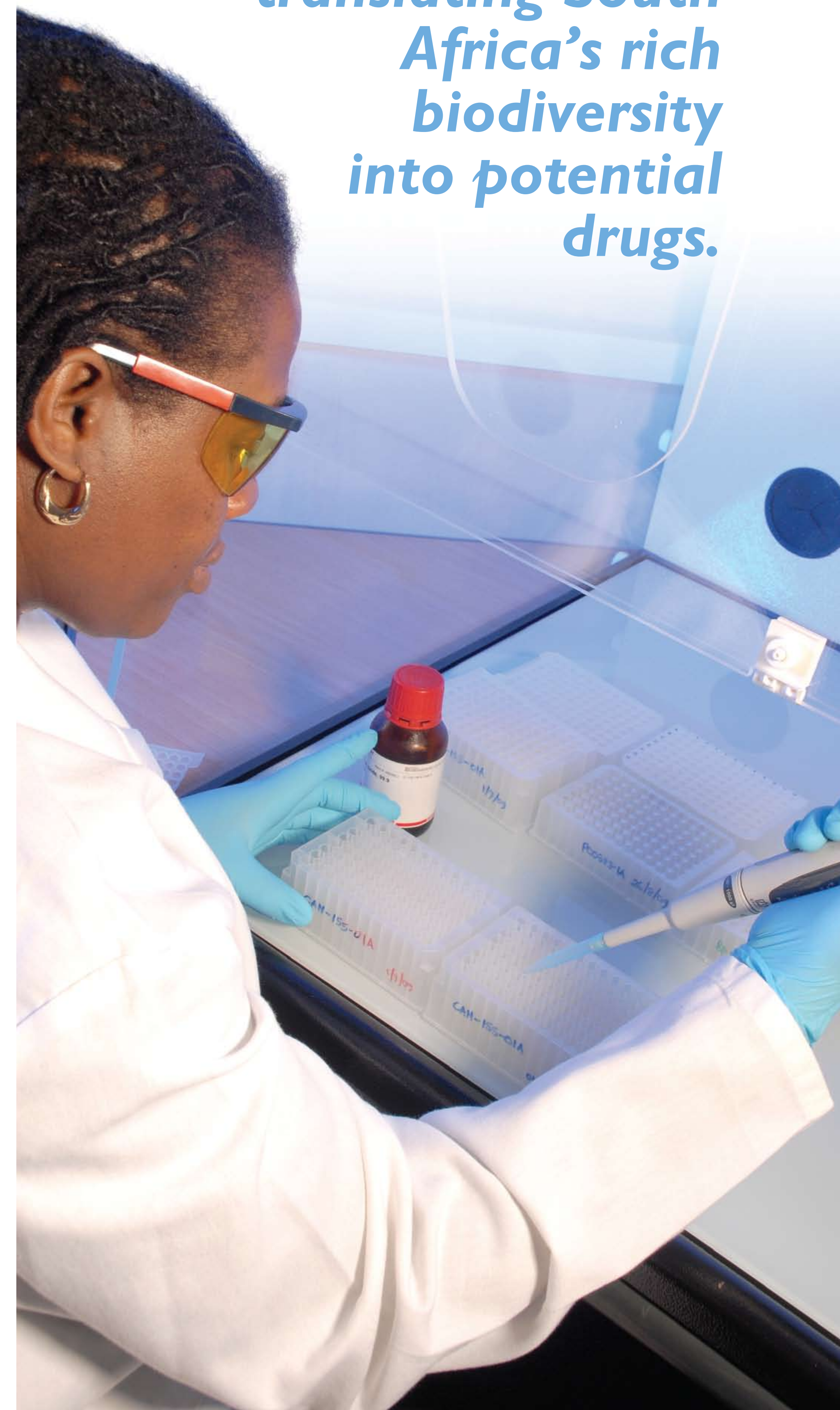


Figure 6: UV and MS data of rosmarinic and caffeic acid

CONCLUSION

Three hits have been identified as potential HIV candidates and are currently being further investigated. A multidisciplinary approach to drug discovery is one of the key success factors to identifying and developing new pharmaceuticals. Such a collaborative approach was successfully established between the CSIR and EMF. The approach to accelerate the identification of potentially new anti-HIV leads from complex plant extracts has proved successful and de-replication has led to the identification of rosmarinic and caffeic acid from the aqueous extract of *A. amatymbica* which are known compounds exhibiting weak anti-HIV activity.

Bio-scientists at the CSIR are set to screen one of the largest natural product extract libraries against HIV, based on the country's unique plant biodiversity thereby translating South Africa's rich biodiversity into potential drugs.



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