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PRELIMINARY STUDIES REGARDING THE CYTOTOXICITY OF RED POLYKETIDES USED AS A DYE IN THE FOOD INDUSTRY

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Abstract

Red yeast rice is currently used in Asia as a dye for meat, fish, and other food products, as well as a food supplement due to its statin content. Reports worldwide have documented adverse effects, particularly in individuals with pre-existing health conditions. In this context, we conducted in vitro tests to assess the cytotoxicity of three red dyes derived from different types of red yeast rice. In our study, a normal human standardized cell line was exposed to these red dyes for 24 and 48 hours. The results obtained during these studies revealed that the red polyketides derived from Monascus ruber and Monascus purpureus display cytotoxicity for the studied cell line after 24 hours of exposure, cytotoxicity that persists after 48 hours of exposure only for Monascus purpureus. Red polyketides obtained from high-productivity Monascus sp do not exhibit cytotoxicity in vitro for the studied cell line

Key words*:* cytotoxicity, *Monascus sp*., red polyketides.

INTRODUCTION

Microorganisms of the *Monascus* genus are used in Asia as dietary supplements; however, they are most commonly utilized as pigments in the food industry. These pigments, known as red yeast rice, are natural polyketides biosynthesized by fungi of the *Monascus* genus (Dufosse, 2006; Radu et al., 2010a; Silbir & Goksungur, 2019; Sen et al., 2019). The most well-known are the red pigments monascorubramine and rubropunctamine, the orange pigments monascorubrin and rubropunctatin, and the yellow pigments monascin and ankaflavin. These bioproducts have been and continue to be widely used in the food industry in Asian countries for coloring fermented foods, meat, beverages, and confectionery products. Data reported in 2023 by Egea et al. indicated that the pigments derived from *Monascus purpureus* are used as additives in ice cream, cheese, poultry products, and pork products (Egea et al., 2023). Pigments derived from *Monascus ruber* are used as additives in yogurt, cheese, bread, red beer, lollipops, and jellies (Egea et al., 2023) or as an alternative to synthetic colorants in food products such as sausages, ham, meat, fruit beverages, wines, and teas (Fabre et al., 1993; Vendruscolo et al., 2016; Gong et al., 2023; Albișoru et al., 2024). In silico studies conducted by Albișoru et al. have shown that red polyketides (monascorubrin and rubropunctamine) inhibit most CYP-type liver enzymes responsible for detoxification processes in the liver (monascorubramine inhibits the enzymes encoded by CYP1A2, CYP2C19, CYP2C9, CYPD6, CYP3A4; rubropunctamine inhibits CYP1A2, CYP2C19, CYP2C9). Moreover, these two polyketides exhibit immunotoxicity and toxicity for the brain, because these molecules can cross the blood-brain barrier (Albișoru et al., 2024). Since red pigments are the most commonly used in the

food industry, this study aimed to evaluate the cytotoxicity induced by exposing a normal HaCaT cell line to bioproducts containing red pigments produced by three *Monascus* species.

MATERIALS AND METHODS

In this study, polyketides biosynthesized by three *Monascus* species were used, as follows: *Monascus purpureus* DSM 1379, *Monascus ruber* MUCL 28962, and a *Monascus* species with high productivity (MM), the last one has been received as a gift from Prof. Octavian Duliu of the Institute of Atomic Physics, Măgurele, Romania (Albișoru et al., 2024). The red polyketides were obtained in agreement with the methodology presented by Albișoru et al. (Albișoru et al., 2024), using the pigments biosynthesized by each of the aforementioned Monascus species. Cytotoxicity tests were conducted on a standardized HaCaT cell line, obtained from Cell Line Service GmbH (cat. no. 330493, Eppenheim, Germany), using the methodology reported by Bostan et al. (Bostan et al., 2021). The data obtained from the in vitro tests were used to evaluate the IC50 value, employing linear methods provided by Excel Office 2021 and nonlinear models performed using Systat 4.0 software (Inpixon, Palo Alto, USA).

The concentrations of the crude red polyketides from the solution of ethanol 75% used in this study were as follows: *Monascus purpureus* (MP) red polyketides: 425.10 mg/L; *Monascus ruber* (MR) red polyketides:110.21 mgL mg/L; *Monascus* high productive (MM) red polyketides: 1075 mg/L (Albisoru et al., 2024). All bioproducts were tested using the same volumetric concentration range (0-100 µL/mL). The values of IC50 obtained were reported in µg/mL, keeping into account the crude bioproduct concentration of each solution tested.

RESULTS AND DISCUSSIONS

The results obtained after 24 hours of exposure to red polyketides derived from the three Monascus species by applying a linear math model, were the following: in the case of red polyketides biosynthesized by *Monascus ruber* (Figure 1a, b, c), IC50=26.88 µg/ml (R^2=0.81) (Fig. 1b); for red polyketides biosynthesized by *Monascus purpureus*, IC50=98.66 µg/ml (R^2 = 0.75) (Figure 1a); and for red polyketides biosynthesized by the high-productivity *Monascus* species, IC50=584.07 µg/ml (R^2 =0.75) (Figure 1c). The analysis of these data indicates that the highest cytotoxic potential is exhibited by the red pigment isolated from *Monascus ruber*, followed by the red pigments isolated from *Monascus purpureus* and, lastly, those from the high-productivity *Monascus* species. It is important to note that, in all three cases, the correlation coefficient generated by the software used ranged between 0.75 and 0.81. The data obtained through the mathematical modeling of in vitro cytotoxicity data using nonlinear models (Figure 2a, b, c) showed that: for red polyketides obtained from *Monascus ruber*, IC50=20.60 µg/mL; in the case of red polyketides biosynthesized by *Monascus purpureus*, the program generated an IC50=152.15 µg/mL; for red polyketides biosynthesized by the high-productivity Monascus species, the program generated an IC50 value of 21.29×10^3 µg/mL. It is important to note that the math functions generated by the SYSTAT program had correlation coefficient values ranging between 0.91 and 0.99.

Analyzing the data obtained at 24 hours, it can be observed that, regardless of the mathematical model used, the cytotoxicity of the red polyketides (Table 1) obtained from the three Monascus species increases in the following order: *Monascus ruber* > *Monascus purpureus* > *Monascus* high productive.

The math modelling of the results obtained from cytotoxicity tests performed at 48 hours showed the following: in the case of linear models (Figure 3a, b, c), for the polyketides biosynthesized by *Monascus ruber*, an IC50 of 20.75 µg/mL was obtained (Figure 3b). In the case of the red polyketides biosynthesized by *Monascus purpureus*, an IC50 of 65.47 µg/mL (R^2 = 0.70) is obtained (Figure 3a). For the red polyketides biosynthesized by the high-productivity *Monascus* species, an IC50 of 352.64 µg/mL (R^2 = 0.75) was obtained (Figure 3c).

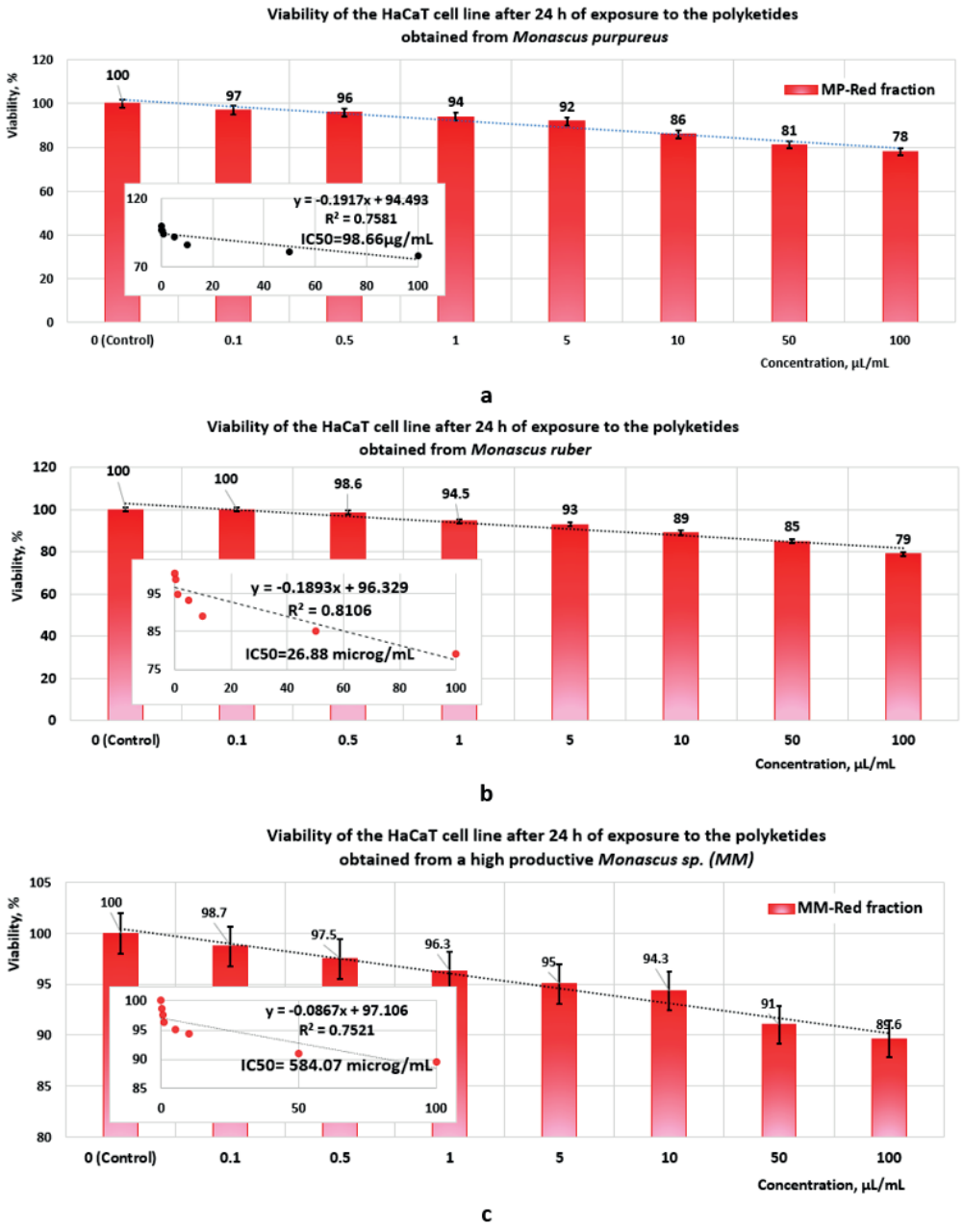
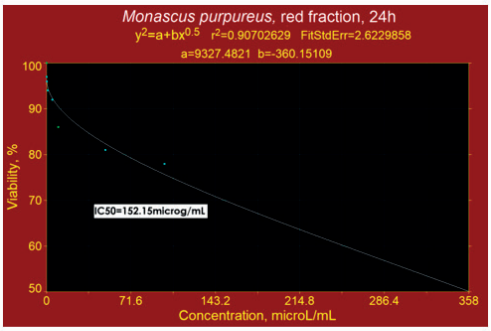


Figure 1. Proliferation studies performed *in vitro* on the human standardized cell lines (HaCaT) exposed for 24 h to red polyketides: a) estimated value of IC50 for red polyketides biosynthesized by MP using linear models; b) estimated value of IC50 for red polyketides biosynthesized by MR using linear models; c) estimated value of IC50 for red polyketides biosynthesized by MM using linear models



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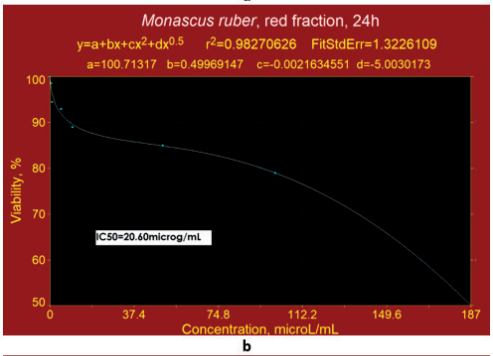

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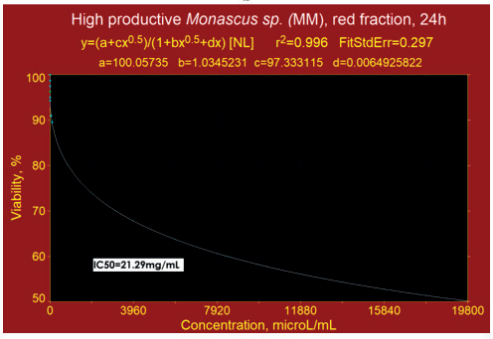

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Figure 2. Proliferation studies of the human standardized cell lines (HaCaT) exposed for 24 h to red polyketides: a) estimated value of IC50 for red polyketides biosynthesized by MP using nonlinear models; b) estimated value of IC50 for red polyketides biosynthesized by MR using nonlinear models; c) estimated value of IC50 for red polyketides biosynthesized by MM using non-linear models

The analysis of these data indicates that after 24 hours of exposure of the HaCaT cell line to the studied red polyketides, the highest cytotoxicity is exhibited by red pigment isolated from *Monascus ruber*, followed by the red pigments isolated from *Monascus purpureus*, and finally those derived from the high-productivity *Monascus* species. In all three cases, the correlation coefficient generated by the software used ranged between 0.66 and 0.70 (Figure 3a, b, c, Table 1).

The data obtained from the mathematical modeling of the in vitro cytotoxicity data using nonlinear models (Figure 4a, b, c) led to the following results: for the red polyketides obtained from *Monascus ruber*, an infinity IC50 value was obtained. In the case of the red polyketides biosynthesized by *Monascus purpureus*, the program generated an IC50 of 34.16 µg/mL. For the red polyketides biosynthesized by the high-productivity *Monascus* species, the program generated an IC50 value of 1763×10^3 µg/mL. The math functions generated by the SYSTAT program had correlation coefficients ranging between 0.94 and 0.99 (Figure 4a, b, c, Table 1). Analyzing the data obtained after 48 hours, it can be observed that the best correlation coefficients are obtained for nonlinear models (Figure 4a, b, c, Table 1). Both types of models used generate IC50 values for the polyketides biosynthesized by *Monascus purpureus* that are relatively close, and the values obtained in both cases categorize these as substances with moderate cytotoxicity (Figure 3a, Figure 4a, Table 1). Analyzing the IC50 values generated by the mathematical models used for the red polyketides biosynthesized by high-productivity *Monascus*, it is found that these bioproducts can be classified as non-toxic substances. The results obtained for the red polyketides from *Monascus ruber* after 48 hours of exposure are entirely different, depending on the mathematical model used to evaluate the IC50. Thus, if the IC50 is evaluated with a linear function, the obtained IC50 value classifies the bioproduct containing red polyketides biosynthesized by *Monascus ruber* as toxic (with the observation that the obtained value is generated with a function for which the correlation coefficient is 0.70). However, if the IC50 is evaluated using more complex math models (nonlinear functions), it is observed that the obtained IC50 value is infinitely large (a value obtained with a correlation coefficient of 0.98), which classifies this bioproduct in the category of non-toxic substances.

Table 1. *In vitro* evaluation of the cytotoxicity of red polyketides for HaCaT cells line

Source of red polyketide	IC50 value , µg/mL		Cytotoxicity	Observations	References
	Linear model	Nonlinear model			
Exposure time: 24 h					
Monascus ruber	**26.88** R^2=0.81	**20.60** R^2=0.98	Very cytotoxic	IC50 value appropriate for both models	Ciric et al., 2023
Monascus purpureus	**98.66** R^2=0.75	**152.15** R^2=0.90	Moderate cytotoxicity	IC50 values relatively appropriate for both models	Ciric et al., 2023
Monascus sp. high productive	**584.07** R^2=0.75	**21290** R^2=0.99	No toxicity	Both models indicate that red polyketides obtained from *Monascus* sp. high productive do not exhibit cytotoxicity *in vitro* on HaCaT cell line	Ciric et al., 2023
Exposure time: 48 h					
Monascus ruber	**20.75** R^2=0.70	**Infinitely** R^2=0.98	no cytotoxicity	More probably, after 48h of exposure the red polyketides are decomposed in compounds without cytotoxicity	Ciric et al., 2023
Monascus purpureus	**65.47** R^2=0.81	**34.16** R^2=0.81	High cytotoxicity	IC50 values relatively appropriate for both models	Ciric et al., 2023
Monascus sp. high productive	**352.64** R^2=0.67	**1763x10^3** R^2=0.94	No cytotoxicity	Both models indicate that red polyketides obtained from *Monascus* sp. high productive do not exhibit cytotoxicity *in vitro* on HaCaT cell line	Ciric et al., 2023

This behavior can be explained by the fact that, after 48 hours of exposure, under the specific conditions used for the HaCaT cell line, the red polyketides biosynthesized by *Monascus ruber* are transformed into compounds with no cytotoxicity. The results obtained are also supported by tests performed by Mahmoud et al. (2020) on *Allium cepa,* which reported mitotic indices ranging from 1 to 1.77 for tests carried out with polyketides derived from *Monascus* (dissolved in alcohol), compared to the negative control (aqueous alcohol solutions), where mitotic indices ranged from 1 to 1.74. The chromosomal aberrations detected in the case of exposure to solutions containing *Monascus* pigments ranged from 0.91 to 2.4, compared to the negative control, where chromosomal aberrations ranged from 1.87 to 6.53 (Mahmoud et al., 2020). The tests conducted by Chaudhary et al. (2024) demonstrated that polyketides separated from Monascus species do not contain cardiac glycosides or alkaloids, but do contain polyphenolic compounds, which impart antioxidant properties to the *Monascus*-derived bioproducts. The advantages of using biosynthesized pigments from Monascus species in the food industry are as follows: a) these represent a natural alternative to synthetic colorants such as Red 40 (Caro et al., 2015); b) red polyketides have health benefits due to their: antioxidant effects (Radu et al., 2012a; Radu et al., 2016a; Chaudhary et al., 2024); antimicrobial properties (Albisoru et al., 2024); anti-proliferative effects (Radu et al., 2012b; Wu et al., 2021); and biostimulatory properties (Radu et al., 2010a; Radu et al., 2012c; Radu et al., 2016b).

The use microorganisms with high productivity as a source of pigments for the food industry also aligns with the principles of circular bioeconomy, as the exhausted biomass resulting from the extraction of the targeted polyketides can be developed into biosorbents for water pollution remediation (Radu et al., 2006; Dima et al., 2007) or used as fillers in the production of new types of biofertilizers (Radu & Meghea, 2007; Radu et al., 2020). Further developments of red polyketides biosynthesized by *Monascus* species may include pharmaceutical applications. Due to their antimicrobial properties these bioproducts could be used in the development of antimicrobial composite biomaterials, with matrices made from animal (Ioan et al., 2020) or plant-based biopolymers (Radu et al., 2010b; Zaharie et al., 2022).

To meet the growing demand for red pigments in the food industry, as an alternative to chemically synthesized red pigments, further studies, including in vivo evaluations, are necessary to assess the toxicity of red polyketides biosynthesized by *Monascus* species.

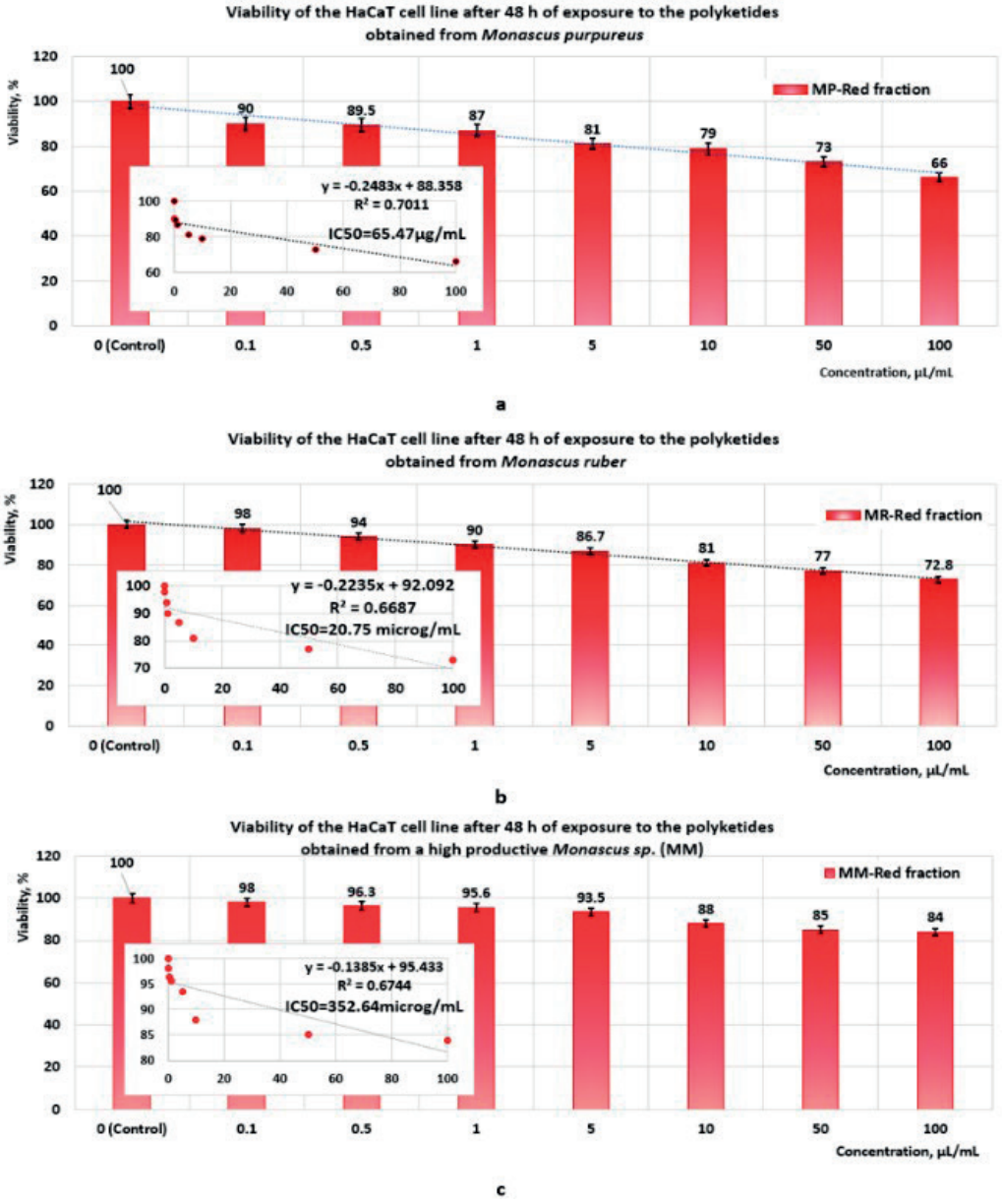
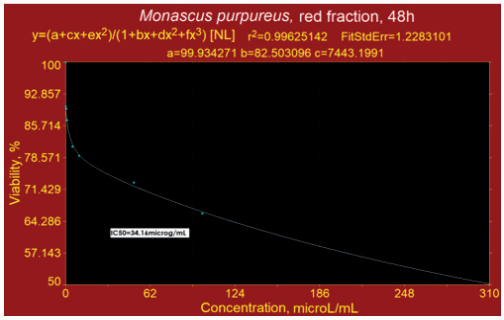


Figure 3. Proliferation studies performed in vitro on the human standardized cell lines (HaCaT) exposed for 48 h to red poliketides: a) estimated value of IC50 for red polyketides biosynthesized by MP using linear models; b) estimated value of IC50 for red polyketides biosynthesized by MR using linear models; c) estimated value of IC50 for red polyketides biosynthesized by *MM* strain using linear models



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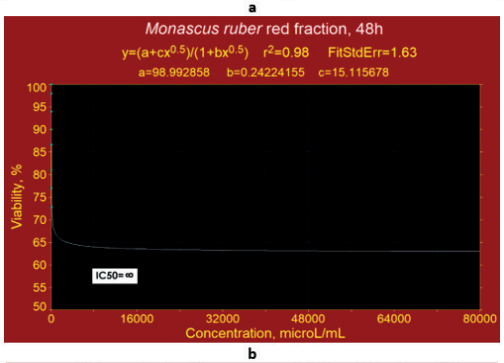

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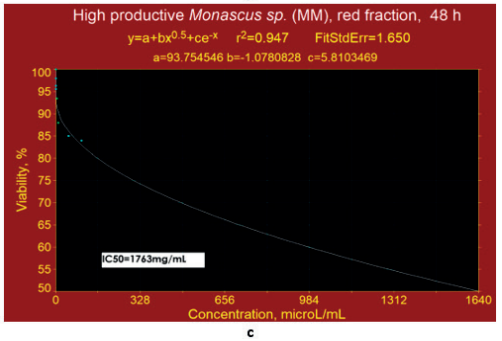

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Figure 4 . Proliferation studies of the human standardized cell lines (HaCaT) exposed for 48 h to red polyketides: a) estimated value of IC50 for red polyketides biosynthesized by MP using nonlinear models; b) estimated value of IC50 for red polyketides biosynthesized by MR using nonlinear models; c) estimated value of IC50 for red polyketides biosynthesized by MM using non-linear models

CONCLUSIONS

The data obtained from the evaluation of the IC50 parameter, showed that the red polyketides obtained from high-productivity *Monascus* do not exhibit cytotoxicity in vitro for the studied cell line. Polyketides derived from *Monascus ruber* and *Monascus purpureus* display cytotoxicity for the studied cell line after 24 hours of exposure, with cytotoxicity that persists after 48 hours of exposure only for *Monascus purpureus*.

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