

Effect of mannanoligosaccharides extracts in uropathogenic *Escherichia coli* adhesion in human bladder cells



Margarida Faustino ^a, Sara Silva ^a, Eduardo Costa ^a, Joana Odila Pereira ^{a,b}, Ana Margarida Pereira ^{a,b}, Carla F. Pereira ^a, Joana Durão ^{a,b}, Manuela E. Pintado ^a, Ana P. Carvalho ^a

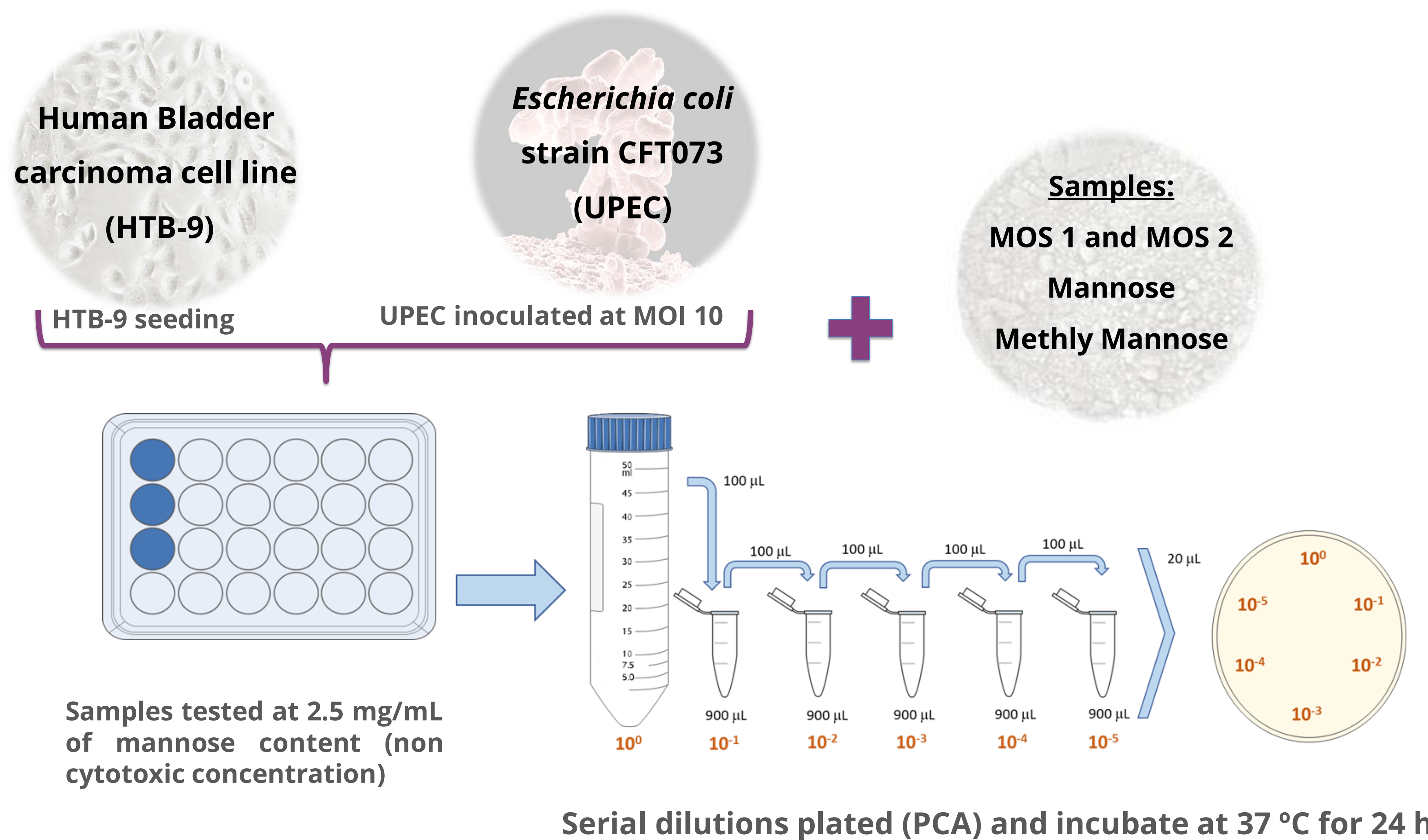
^a Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

^b Amyris Bio Products Portugal Unipessoal Lda, Portugal

Introduction / Objectives

Urinary tract infections (UTIs) are an increasingly common public health problem, with uropathogenic *Escherichia coli* (UPEC) being the most common etiological agent. The standard treatment for chronic UTIs implies long-term prophylactic use of antibiotics which promotes the development of antibiotic resistant bacteria. D-mannose and mannanoligosaccharides (MOS) are regarded as a natural alternative to the prophylactic use of antibiotics to avoid UTI development, and therefore reduce the need for antibiotics. This strategy can be used for the prevention of recurrent UTIs, thus opening a new market section for D-mannose/MOS as health supplements, the novelty regarding the use of MOS. The objective of this study was to evaluate two different MOS extracts' capacity to affect UPEC's infection of bladder cells (HTB-9), mimicking the initial infection (competition), an established infection (treatment) and prophylaxis scenarios (through quantifying the viable cells of UPEC).

Methods



Competition Assay

☐ Samples + HTB-9 + UPEC → 2 h at 37 °C with 5% CO₂

Prophylaxis Assay

☐ Samples + HTB-9 → 1, 2 and 3 h at 37 °C with 5% of CO₂
→ UPEC addition → 2 h at 37 °C with 5% CO₂

Treatment Assay

☐ HTB-9 +UPEC → 2 h at 37 °C
→ Samples addition → 2 and 24 h at 37°C with 5% CO₂

Results

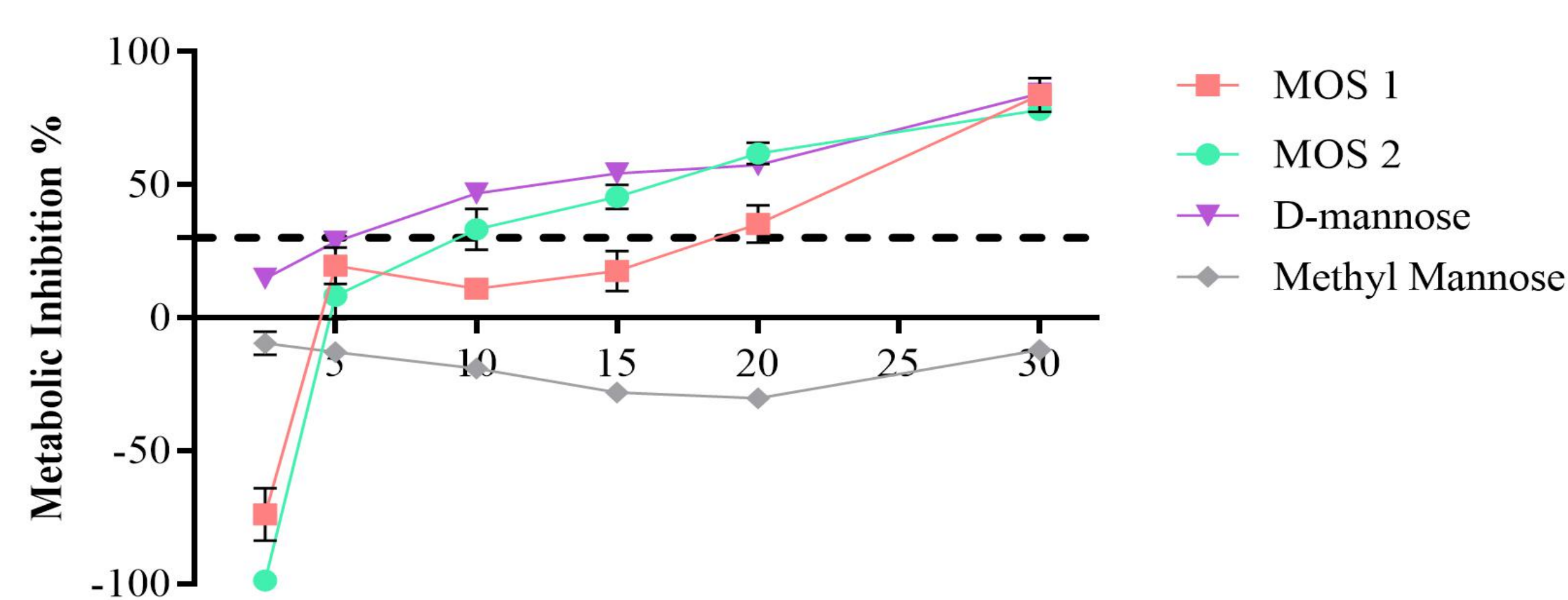


Figure 1 - Cytotoxic evaluation of HTB-9 after 24 h of contact with the samples at concentrations ranging from 2.5 to 30.0 mg/mL. Results are expressed percentage (%) metabolic inhibition in relation to the positive control. Assay in accordance with ISO 10993-5 (2009)

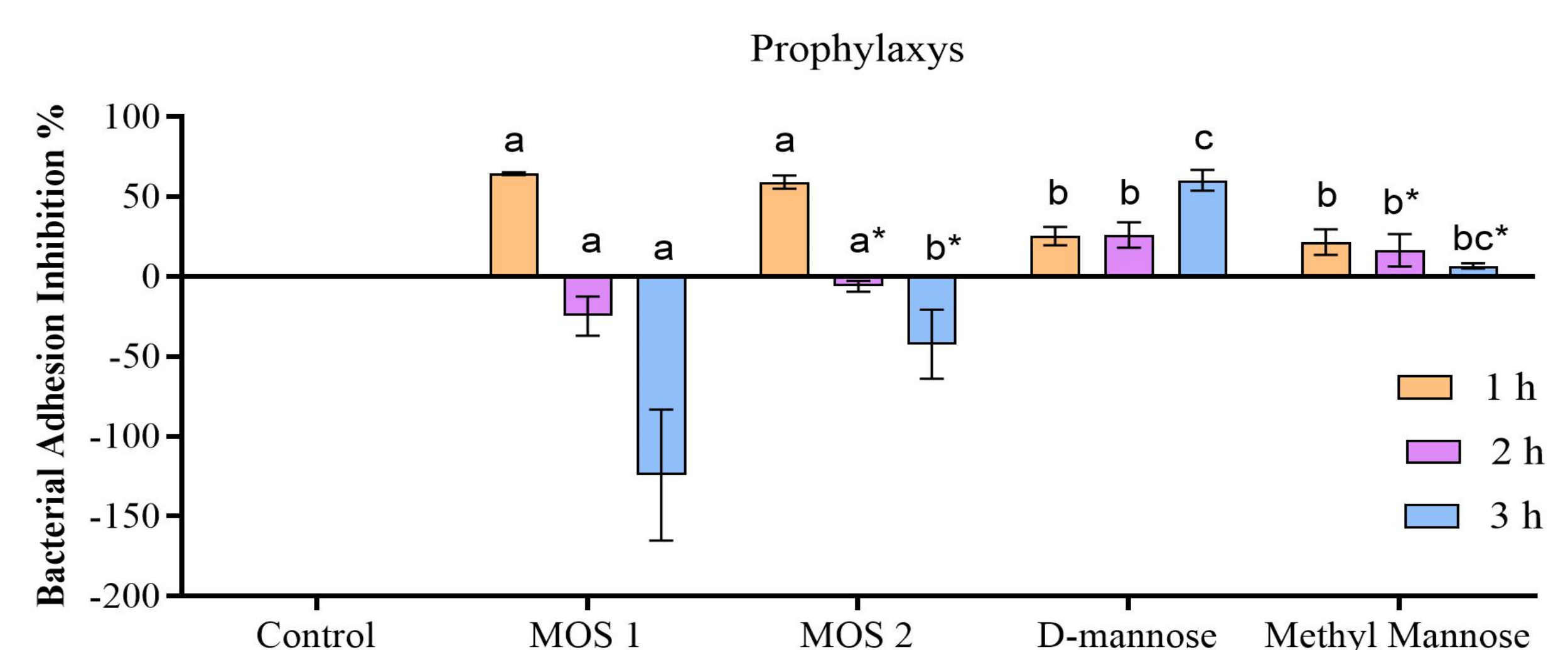


Figure 3 - Percentage (%) of adhesion inhibition of UPEC to HTB-9 after 1 h, 2 h and 3 h of HTB-9 incubation with MOS 1, MOS 2, D-mannose and methyl mannose for 1 h, 2 h and 3 h. Different letters indicate statistically significant differences ($p < 0.05$) between the different samples. Results are expressed in relation to the control.

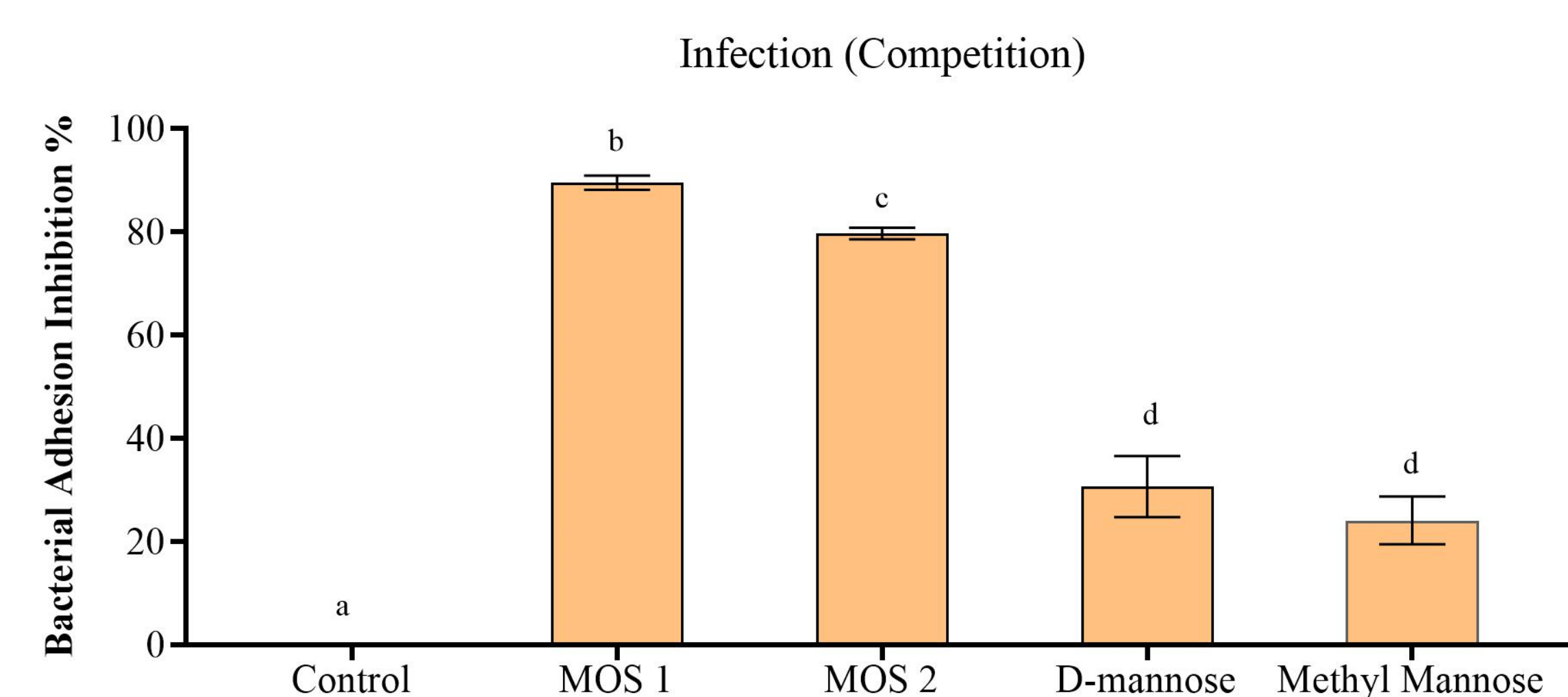


Figure 2 - Percentage (%) of adhesion inhibition of UPEC to HTB-9 after 2 h of simultaneous incubation with MOS 1, MOS 2, D-mannose and methyl mannose. Different letters indicate statistically significant differences ($p < 0.05$) between the different samples. Results are expressed in relation to the control.

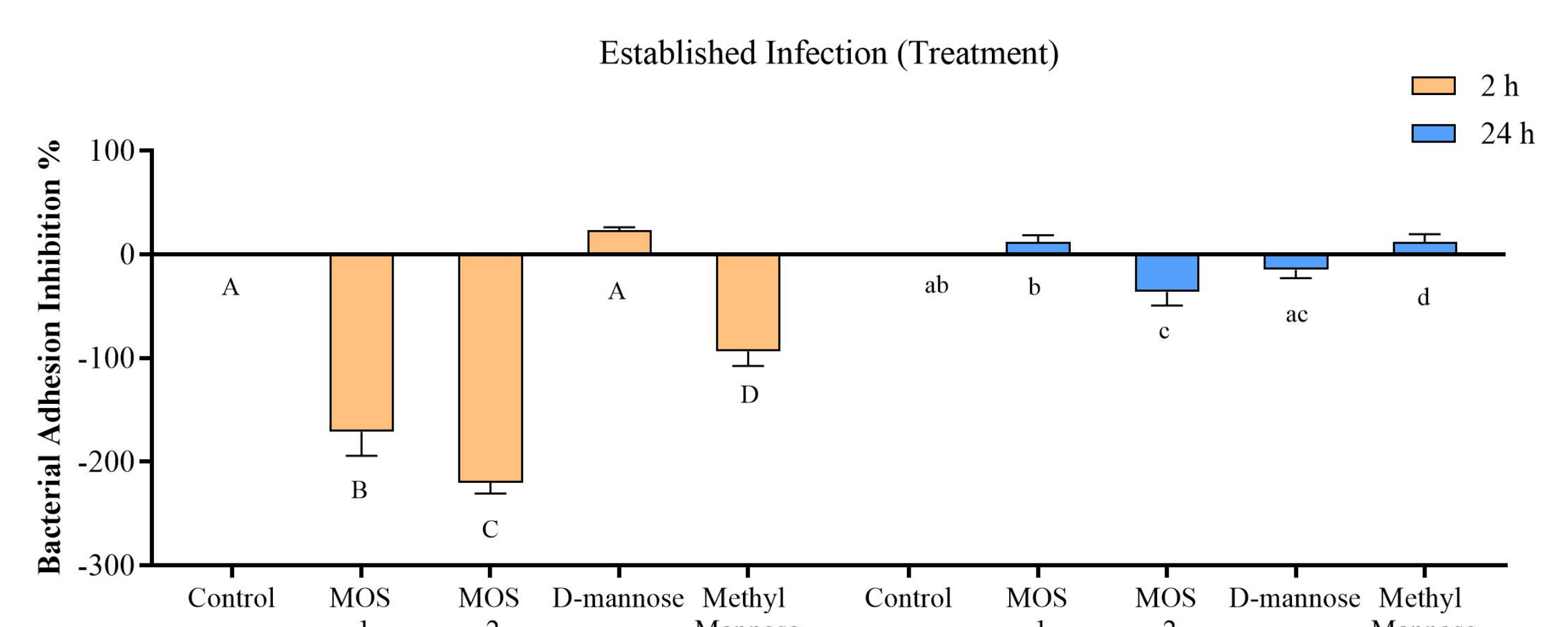


Figure 4 - Percentage (%) of inhibition of infection after 2 and 24 h in contact with MOS 1, MOS 2, D-mannose, and methyl mannose. Different letters indicate statistically significant differences ($p < 0.05$) between the different samples (A,B,C,D for 2 h and a,b,c,d for 24 h). Results are expressed in relation to the control.

Conclusions

In prophylaxis (Figure 3), 1 h of pretreatment appeared to be better than longer pre-exposure times, a likely consequence of extracts' metabolism by either the cells or bacteria. The treatment did not result in any significant reduction of the pre-established infection. MOS extracts performed better in the competition one, as 2.5 mg/mL total mannose allowed for a significant inhibition of UPEC in both extracts (MOS 1 (89.61 ± 1.37 %) and MOS 2 (79.78 ± 1.12 %)). Regardless, the extracts demonstrated an interesting potential to be used as prophylactic health supplements in the future.

Acknowledgements

This work was financially supported by Amyris Bio Products Portugal Unipessoal Lda and Escola Superior de Biotecnologia – Universidade Católica Portuguesa through Alchemy project- Capturing high value from industrial fermentation bio products (POCI-01-0247-FEDER-027578).