Electronic Supplementary Material (ESI) for MedChemComm.
This journal is © The Royal Society of Chemistry 2018

Flush with a flash: Natural three-component antimicrobial combinations based on *S*-nitrosothiols, controlled superoxide formation and "domino" reactions leading to peroxynitrite

Rama Alhasan[a†], Ammar Kharma[a†], Muhammad Jawad Nasim[a], Ahmad Yaman Abdin[a], Justine Bonetti[b], Philippe Giummelli[b], Chukwunonso E.C.C. Ejike[a,c], Pierre Leroy[b], Caroline Gaucher[b] and Claus Jacob*[a]

1. The Nitroblue Tetrazolium (NBT) assay

1.1 The riboflavin/day light system

In this system $O_2^{\bullet-}$ was generated by photo-sensitization of riboflavin employing the visible day light, and the generation of superoxide was confirmed using the NBT assay according to the procedure described in the literature with a few modifications.[1, 2].

A mixture of 1000 μM riboflavin, 200 μM NBT and 20 mM phosphate buffer (pH 7.4) was illuminated by a direct light source, whereby $O_2^{\bullet-}$ reduces the yellow nitroblue tetrazolium (NBT) into blue formazan, which can be monitored spectrophotometrically at 560 nm on a Cary50 Bio UV/VIS spectrophotometer (Varian Australia Pty Ltd., Mulgrave, Australia), with 60 min time intervals. The highest absorbance peak (λ_{max}) was recorded and the optimal concentrations were fixed and chosen according to the speed and intensity of the reaction.

1.2 The xanthine/xanthine oxidase system

$O_2^{\bullet-}$ was generated by enzymatic methods employing a xanthine and xanthine oxidase system according to the procedure described in the literature with a few modifications.[1, 2] A mixture of 400 μM xanthine, 200 μM NBT, 50 mU/ml xanthine oxidase and 20 mM phosphate buffer (pH

7.4) was prepared, $O_2^{\bullet-}$ reduces the yellow nitroblue tetrazolium (NBT) into blue formazan, which can be monitored spectrophotometrically at 560 nm on a Cary50 Bio UV/VIS spectrophotometer (Varian Australia Pty Ltd., Mulgrave, Australia), with three minute time intervals. The highest absorbance peak (λ_{max}) was recorded and the optimal concentrations were chosen according to the speed and intensity of the reaction.

2. Antimicrobial activity against *Candida tropicalis*

The antimicrobial activity was carried out according to the general procedure described in the literature with few modifications.[3-5] The peroxynitrite formed in the *S*-nitrosothiol/riboflavin + day light system inhibited the growth of *C. tropicalis* by almost 50 % at higher concentrations, while there was no activity in the absence of the light source. The activity is less apparent in the *S*-nitrosothiol/xanthine + xanthine oxidase system due to the obvious sensitivity of microorganism against xanthine alone. A concentration dependence has also been observed for the riboflavin + day light system. The results are provided in Figures 1-6.

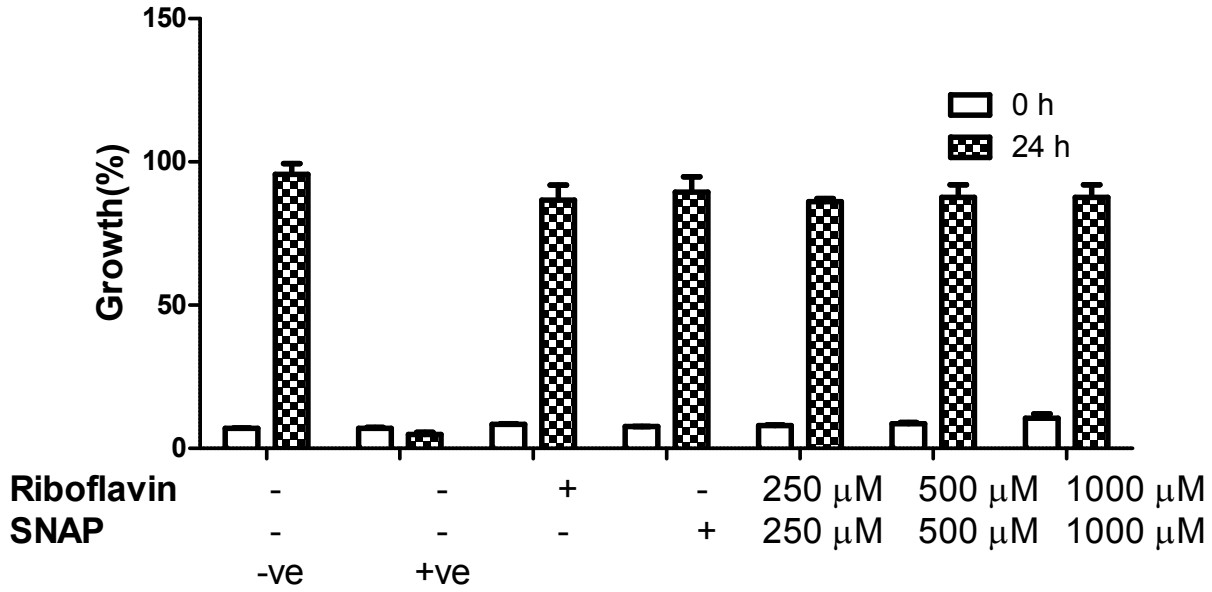

Figure 1: Activities of the three-component system containing SNAP and riboflavin (no day light) at 250 μM, 500 μM and 1000 μM concentrations against *C. tropicalis*.

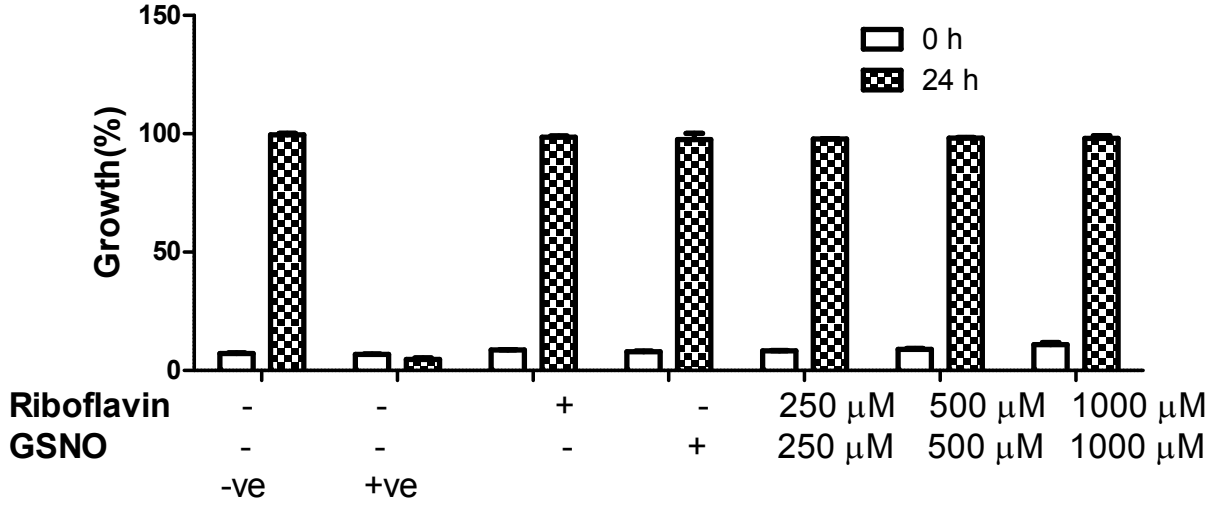

Figure 2: Activities of the three-component system containing GSNO and riboflavin (no day light) at 250 μM, 500 μM and 1000 μM concentrations against *C. tropicalis*.

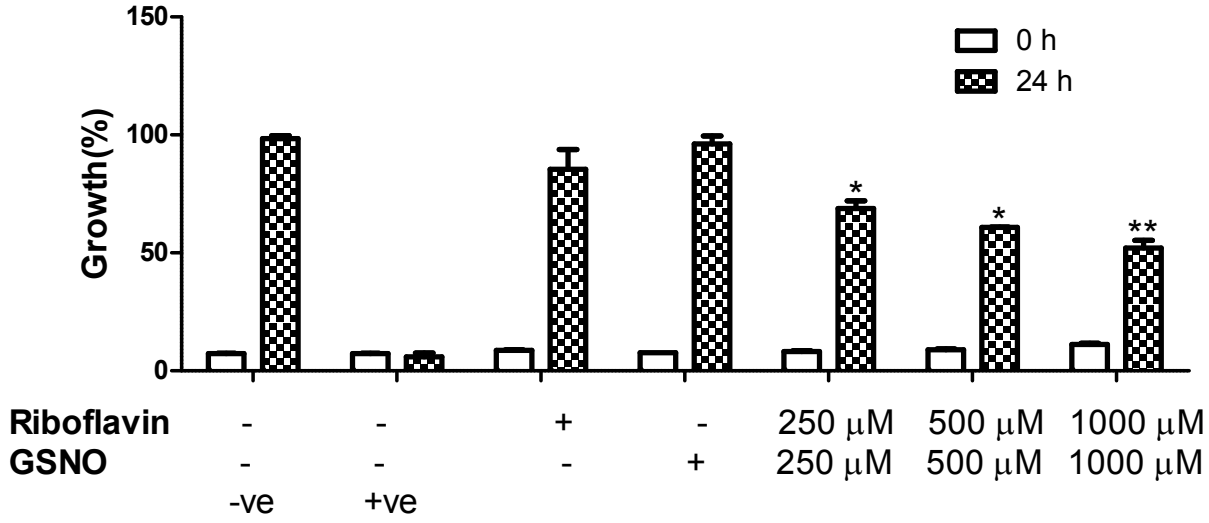

Figure 3: Activities of the three-component system containing GSNO and riboflavin + day light at 250 μM, 500 μM and 1000 μM concentrations against *C. tropicalis*.

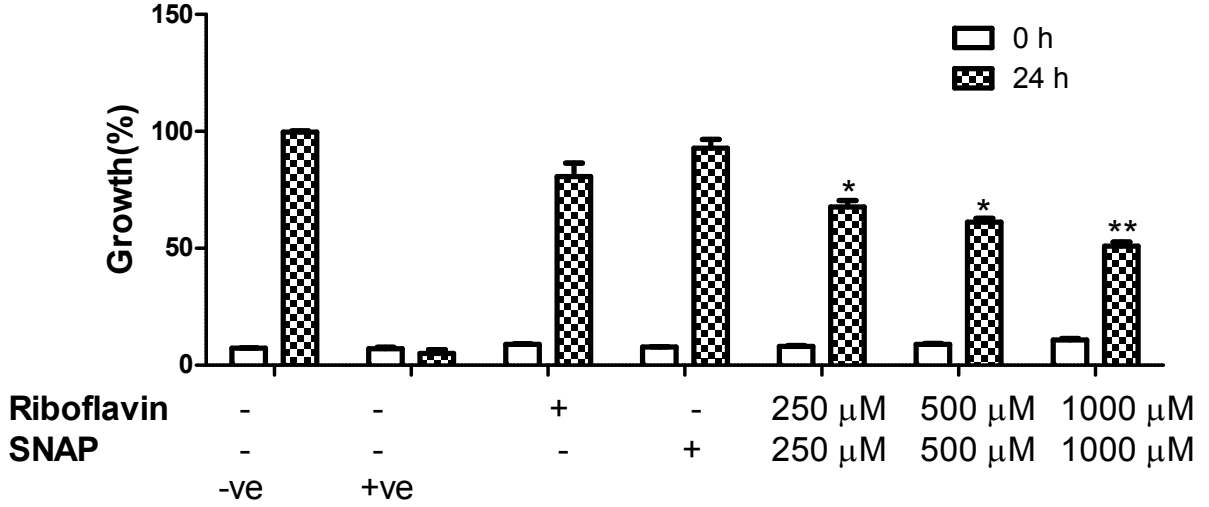

Figure 4: Activities of the three-component system containing SNAP and riboflavin + day light at 250 μM, 500 μM and 1000 μM concentrations against *C. tropicalis*.

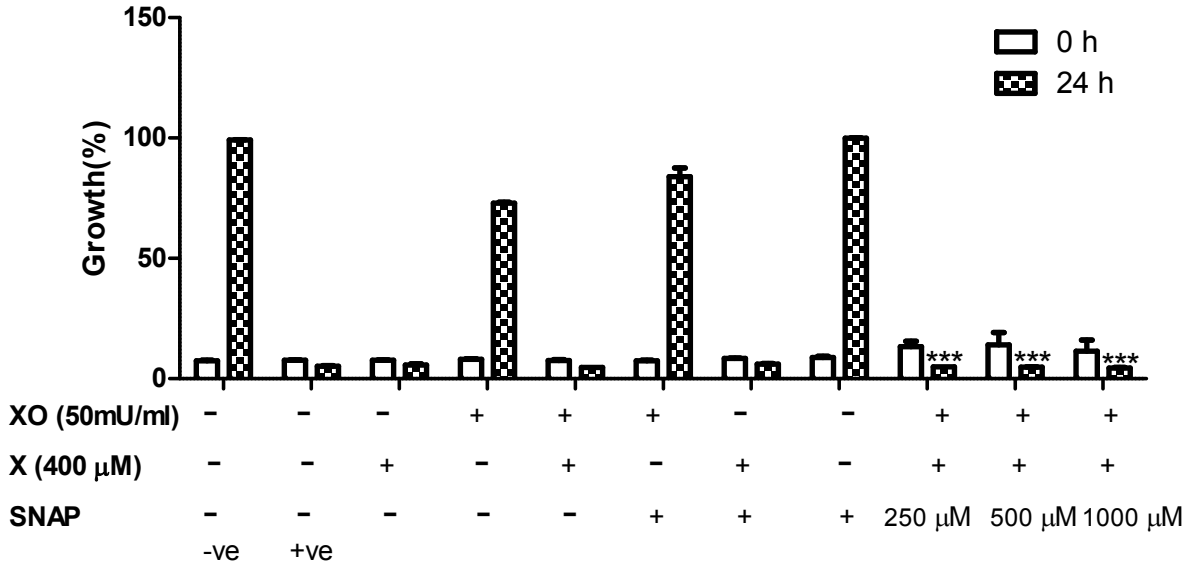

Figure 5: Activities of the three-component system containing SNAP and xanthine + xanthine oxidase against *C. tropicalis*.

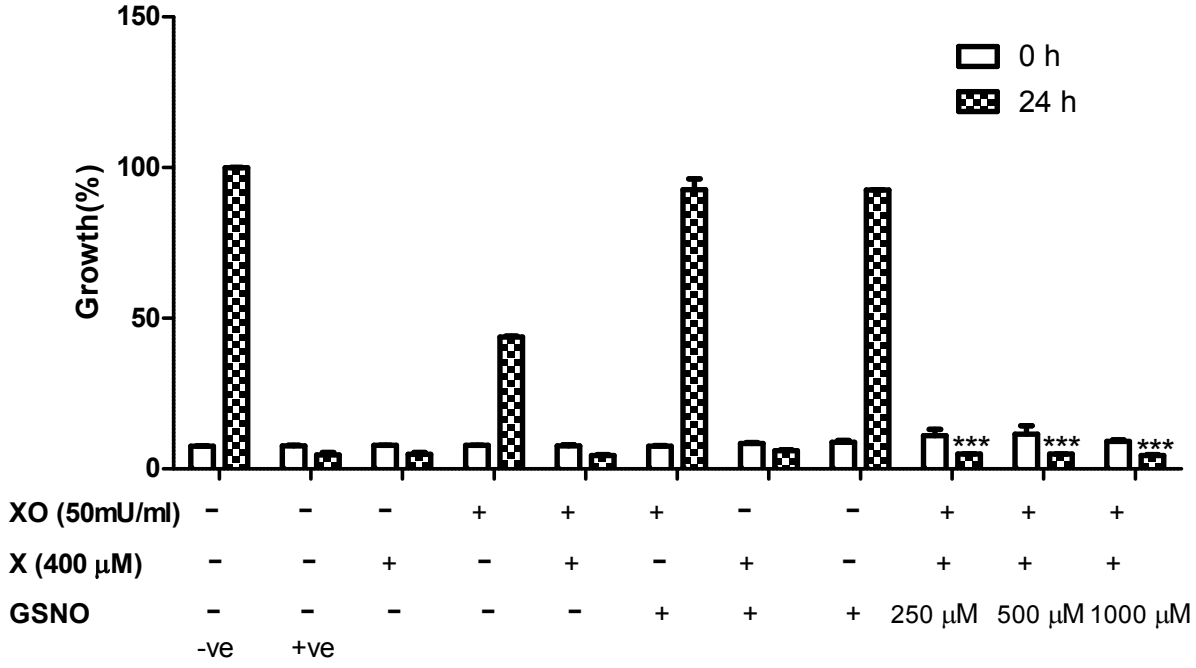

Figure 6: Activities of the three-component system containing GSNO and xanthine + xanthine oxidase against *C. tropicalis*.

Antimicrobial activity against *B. cereus*:

The antimicrobial activity was carried out according to the general procedure described in the literature with few modifications.[3-5] The Xanthine and Xanthine oxidase system could not be applied to all microorganisms, as some of them were sensitive to 400μM concentrations of xanthine. In such cases, a concentration of 200 μM xanthine was employed instead.

The following graphs provide the activity against the Gram-positive bacterium *Bacillus cereus*. Once the concentrations of xanthine were decreased, lower quantities of $O_2^{\bullet-}$ were generated, which in turn caused a decrease in the $ONOO^-$ concentrations to ineffective levels.

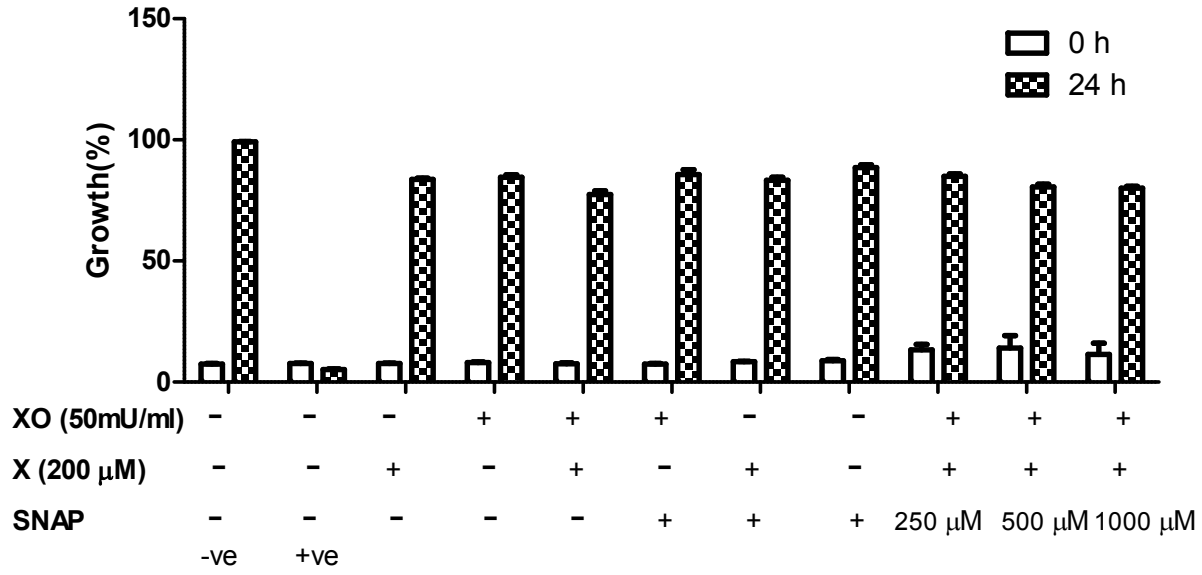

Figure 7: Activities of the three-component system containing SNAP and xanthine + xanthine oxidase against *B. cereus*.

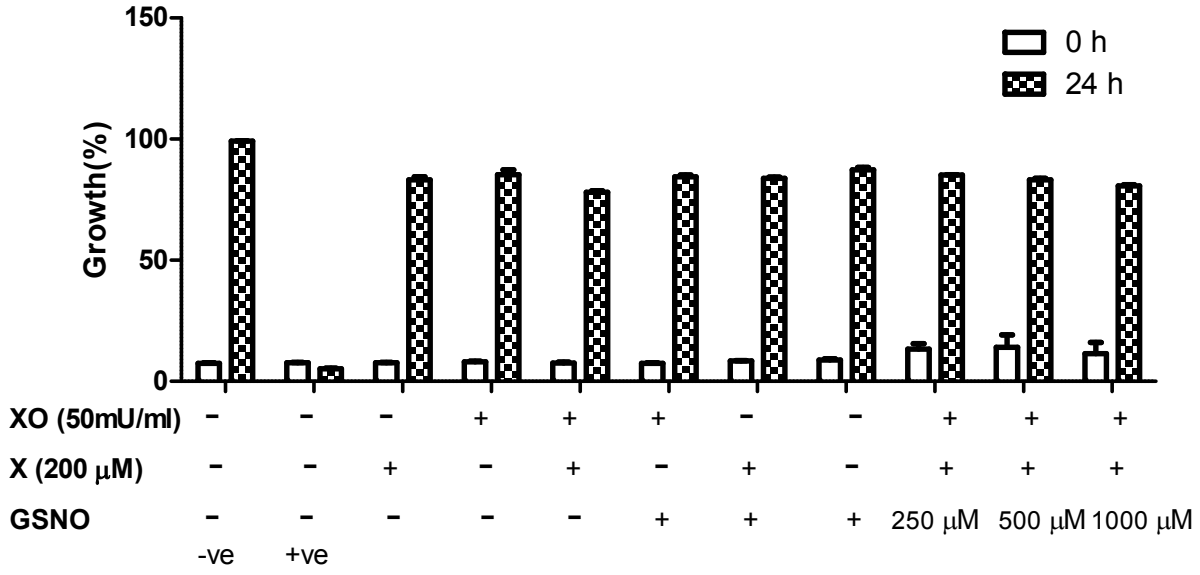

Figure 8: Activities of the three-component system containing GSNO and xanthine + xanthine oxidase against *B. cereus*.

Literature

1. A. Barik, B. Mishra, L. Shen, H. Mohan, R. M. Kadam, S. Dutta, H. Y. Zhang and K. I. Priyadarsini, *Free Rad. Biol. Med.*, 2005, 39, 811-822.
2. H. R. Ibrahim, M. I. Hoq and T. Aoki, *Int. J. Biol. Macromol.*, 2007, 41, 631-640.
3. S. Griffin, R. Alkhayer, S. Mirzoyan, A. Turabyan, P. Zucca, M. Sarfraz, M. Nasim, A. Trchounian, A. Rescigno, C. Keck and C. Jacob, *Inventions*, 2017, 2, 24.
4. S. Griffin, M. Sarfraz, S. F. Hartmann, S. R. Pinnapireddy, M. J. Nasim, U. Bakowsky, C. M. Keck and C. Jacob, *Antioxidants*, 2018, 7.
5. M. Sarfraz, S. Griffin, T. Gabour Sad, R. Alhasan, M. J. Nasim, M. Irfan Masood, K. H. Schafer, C. Ejike, C. M. Keck, C. Jacob and A. P. Ebokaiwe, *Antioxidants*, 2018, 7.