

Antimicrobial activity of phytochemical- antibiotic combinations against pathogenic bacteria

MARTA RIBEIRO ^{1,2,#,*}, MARIA BEATRIZ SILVA ^{1,#}, MANUEL SIMÕES ^{1,*}

¹ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; ribeiro_marta88@hotmail.com (M.R.); bia.freitas1398@gmail.com (M.B.S.); mvs@fe.up.pt (M.S.).

² CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal.

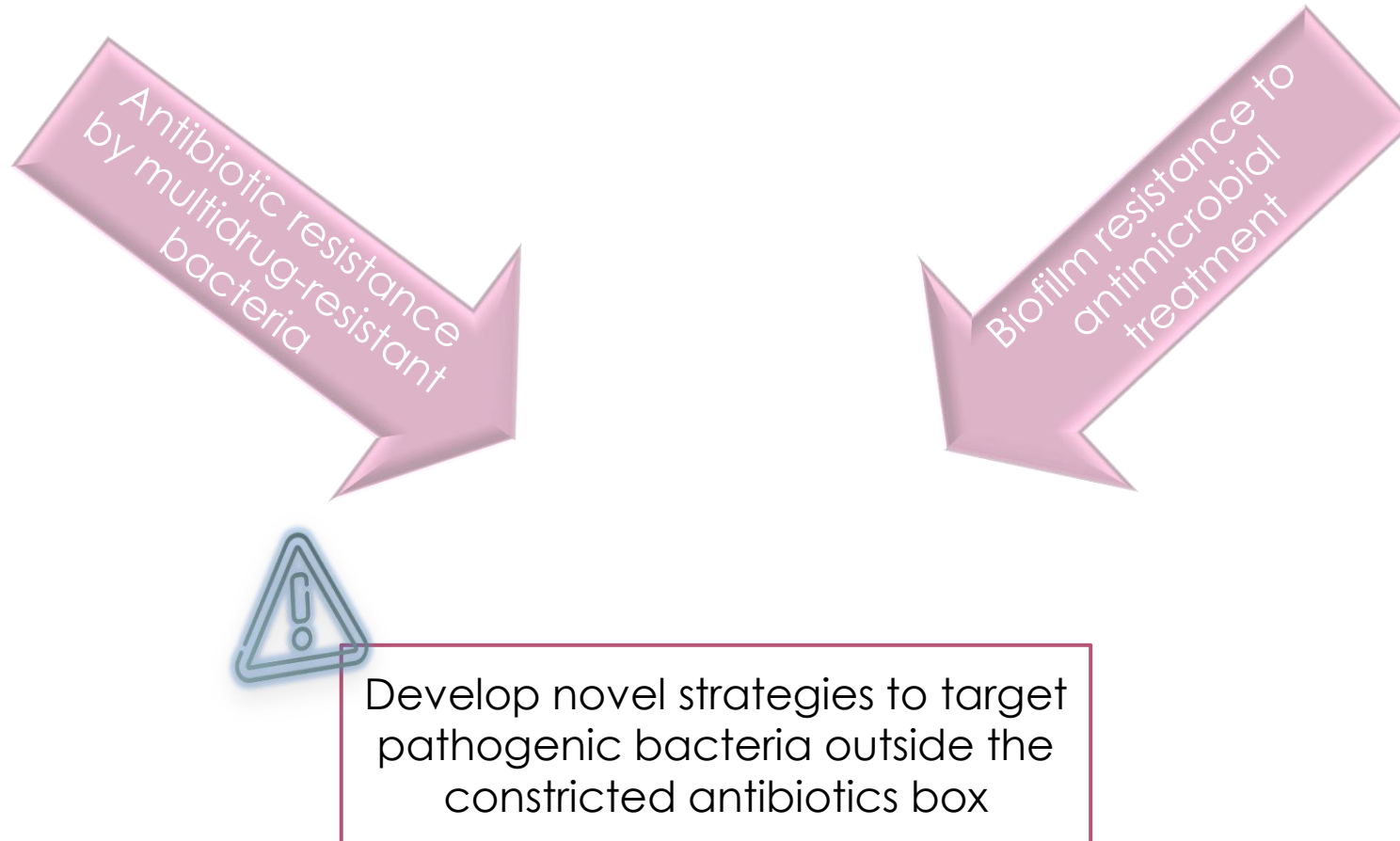
These authors contributed equally to this work.

* Correspondence: ribeiro_marta88@hotmail.com (M.R.); mvs@fe.up.pt (M.S.).

May 2021

BIOFILMS

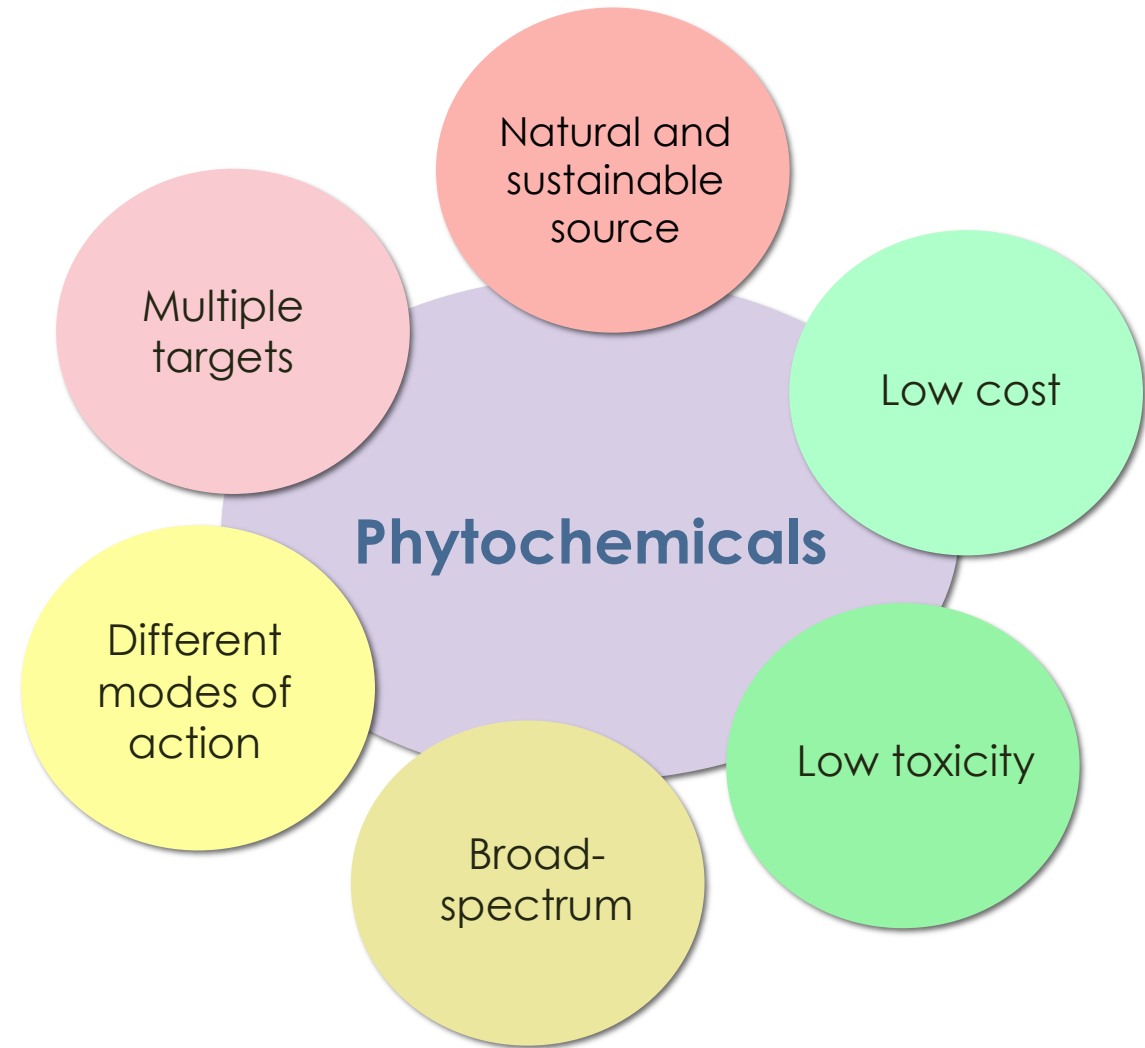
Crucial virulence factor in the pathogenesis of several medically important bacteria, including ***Staphylococcus aureus*** and ***Escherichia coli***.



NATURAL PRODUCTS: PHYTOCHEMICALS

Plant-derived natural products, also known as phytochemicals, are responsible for plant interactions with the environment.

Phytochemicals exhibit a wide range of biological properties, including antimicrobial, anti-inflammatory, antioxidant, anticancer and regenerative activities.



AIMS OF THE RESEARCH

Selection of the best phytochemicals exhibiting bactericidal activity

Selection of standard antibiotics presenting bactericidal activity

Combinatorial activity between selected phytochemicals and standard antibiotics

Phytochemicals

Citronellol (CITRO)
Cis-6-nonen-1-ol (CIS)
Citronellic acid (CA)
3-7-dimethyl-1-octanol (3,7DOC)

Antibiotics

Gentamicin (GEN)
Mupirocin (MUP)
Fusidic acid (FUS)

Combinations

CITRO + GEN
CITRO + MUP
CIS + GEN
CIS + MUP

Bacteria

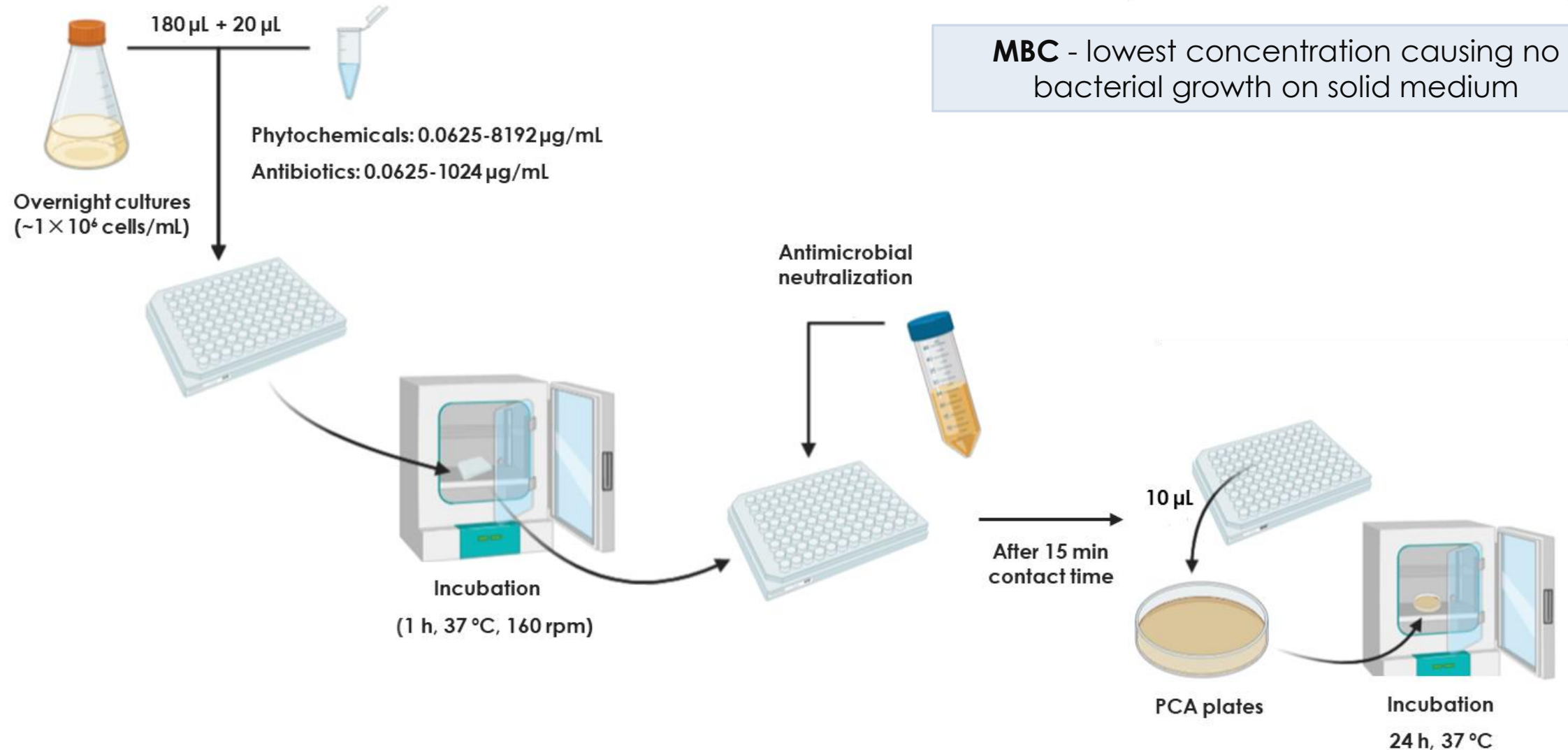
S. aureus CECT 976
methicillin-resistant *S. aureus* (MRSA) XU212

E. coli CECT 434 – Gram-negative bacteria

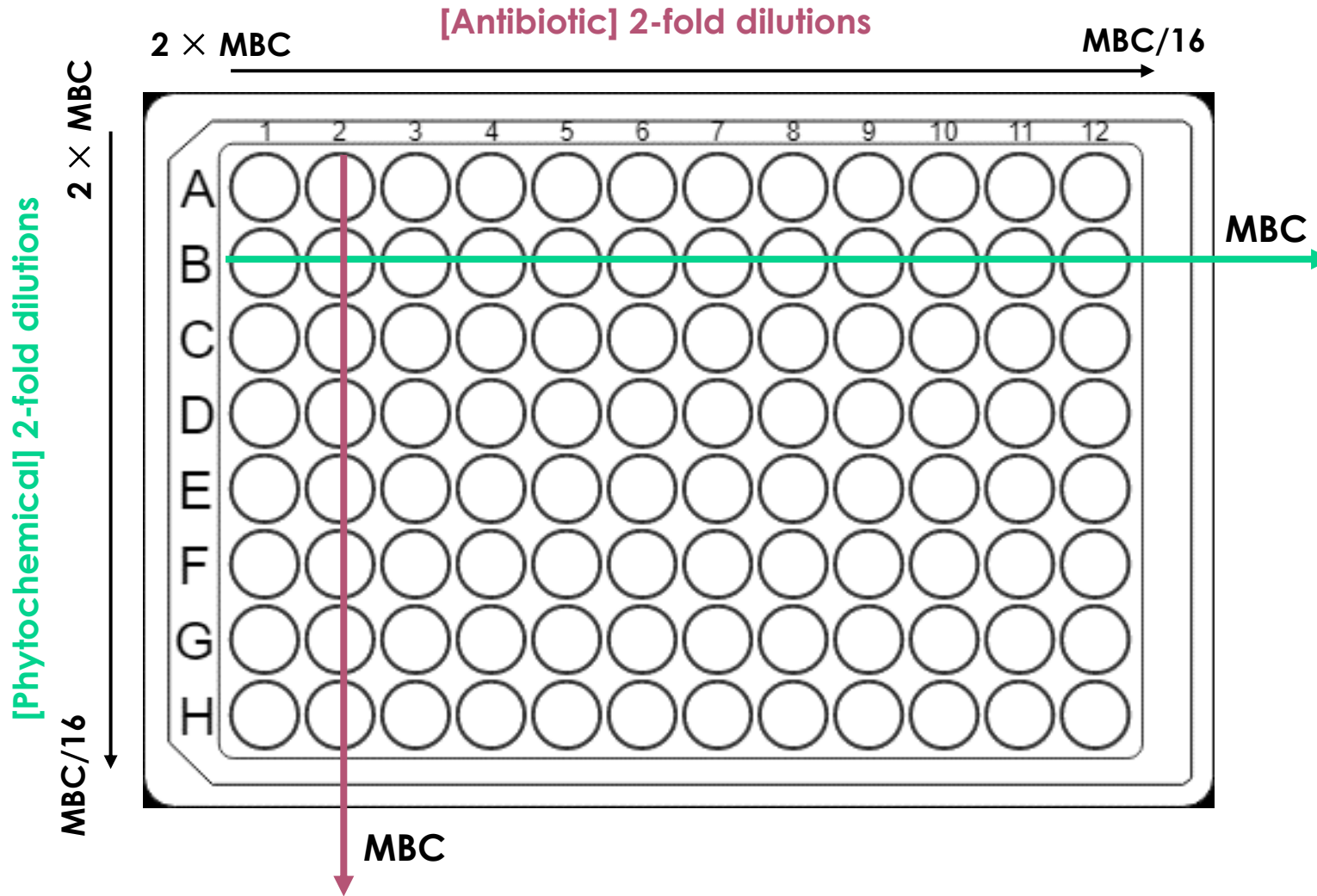
} Gram-positive bacteria

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)

MBC - lowest concentration causing no bacterial growth on solid medium



DETERMINATION OF FRACTIONAL BACTERICIDAL CONCENTRATION (FBC)



The **FBC index** was established to understand the effect between combinations of phytochemicals and antibiotics.

$$FBC \text{ index} = FBC_A + FBC_B$$

$$FBC_A = \frac{MBC_A \text{ in the presence of } B}{MBC_A \text{ alone}}$$

$$FBC_B = \frac{MBC_B \text{ in the presence of } A}{MBC_B \text{ alone}}$$

Compound A – Antibiotic; Compound B – Phytochemical

Table 1. Correlation between FBC index and the effect of the combination of compounds.

Synergy	Additive	Indifference	Antagonism
≤ 0.5	≤ 1	> 1 and < 2	≥ 2

BACTERICIDAL ACTIVITY OF PHYTOCHEMICALS AND ANTIBIOTICS

Selection of the best phytochemicals exhibiting bactericidal activity

CITRO
CIS
CA
3,7DOC

Selection of standard antibiotics presenting bactericidal activity

GEN
MUP
FUS

Table 2. MBC ($\mu\text{g}/\text{mL}$) of selected phytochemicals and standard antibiotics against pathogenic bacteria.

		<i>S. aureus</i> CECT 976	MRSA XU212	<i>E. coli</i> CECT 434
Antibiotics	GEN	8	NA	64
	MUP	32	64	NA
	FUS	128	NA	NA
Phytochemicals	CITRO	512	NA	2048
	CIS	1024	2048	1024
	CA	2048	NA	4096
	3,7DOC	NA	NA	NA

COMBINATORIAL ACTIVITY BETWEEN PHYTOCHEMICALS AND ANTIBIOTICS

Combinatorial activity
between selected
phytochemicals and
standard antibiotics

CITRO + GEN
CITRO + MUP
CIS + GEN
CIS + MUP

Table 3. FBC index of the different combinations between selected phytochemicals and standard antibiotics against pathogenic bacteria.

		<i>S. aureus</i> CECT 976	MRSA XU212	<i>E. coli</i> CECT 434
CITRO	GEN	1.125 (I)	NA	0.188 (S)
	MUP	0.562 (A)	*	–
CIS	GEN	0.75 (A)	–	0.25 (S)
	MUP	0.75 (A)	0.562 (A)	–

NA: no activity; S: synergy; A: additive; I: indifference; – no significant bactericidal effect when combining the compounds (compared with the compound alone at MBC); * the concentration of MUP was reduced from 64 µg/mL (MUP alone) to 32 µg/mL in the presence of CITRO (NA alone against MRSA).

This work showed the antimicrobial efficacy of selected phytochemicals to be used as an alternative to and/or in combination with standard antibiotics against pathogenic bacteria.



Besides the possibility of reducing toxicity of the compounds when used in combination, the side effects occurred by phytochemicals are considerably less as they are derived from plants.

This research was financially supported by: Base Funding - UIDB/00511/2020 funded by national funds through the FCT/MCTES (PIDDAC); Project Biocide_for_Biofilm - PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219, ABFISH - PTDC/ASP-PES/28397/2017 - POCI-01-0145-FEDER-028397 and ALGAVALOR - POCI-01-0247-FEDER-035234, funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES.

