WORLD CONGRESS ON INFECTIOUS DISEASES

IN SILICO DESIGN OF ANALOGOUS PEPTIDES DERIVED OF *BOMBINA* SPECIES WITH IMPROVED ANTIMYCOBACTERIAL ACTIVITY *IN VITRO*

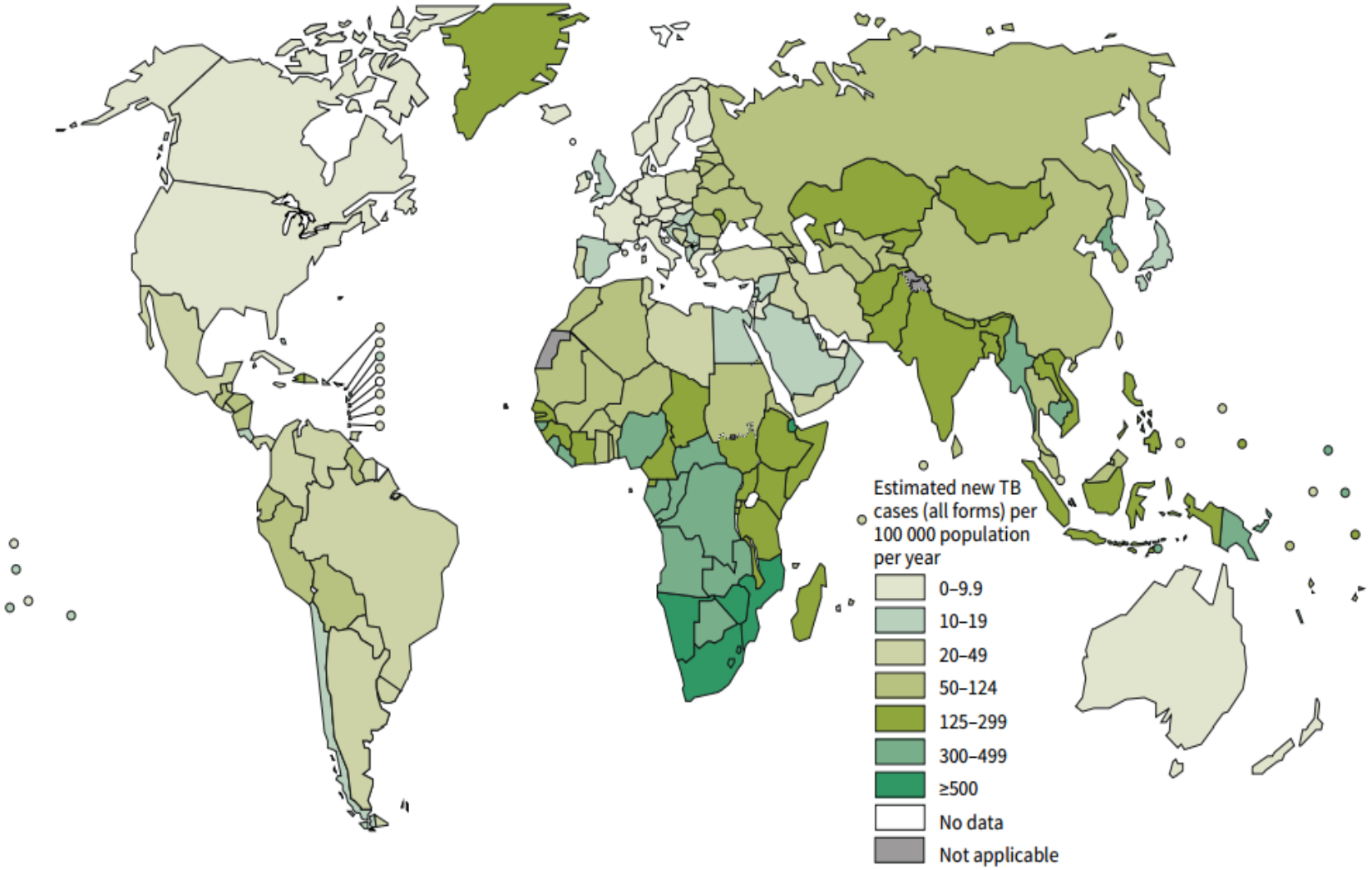
***Sandra M. Chingaté , Carlos Yesid Soto, Luz Mary Salazar**

***PhD student**

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF MYCOBACTERIA

Faculty of Sciences, Universidad Nacional de Colombia

Estimated number of TB cases, (OMS 2013)



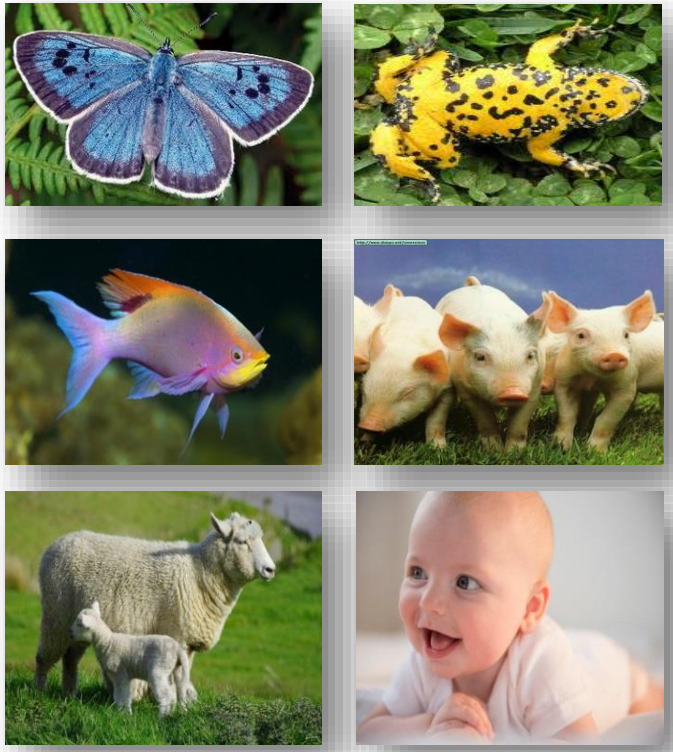
WHO Report 2013/Global Tuberculosis control. The Global Plan to Stop TBC, 2006–2015. Geneva, Stop TBC Partnership and World Health Organization, 2012 (WHO/HTM/ STB/2012.6).

Problems With Controling TB.

- Co-infection with HIV
- Low efficiency of BCG vaccine
- Fault accessibility rapid diagnostic methods
- Emergence of resistant strains of *M. tuberculosis* to anti-TB drugs

ANTIMICROBIAL PEPTIDES

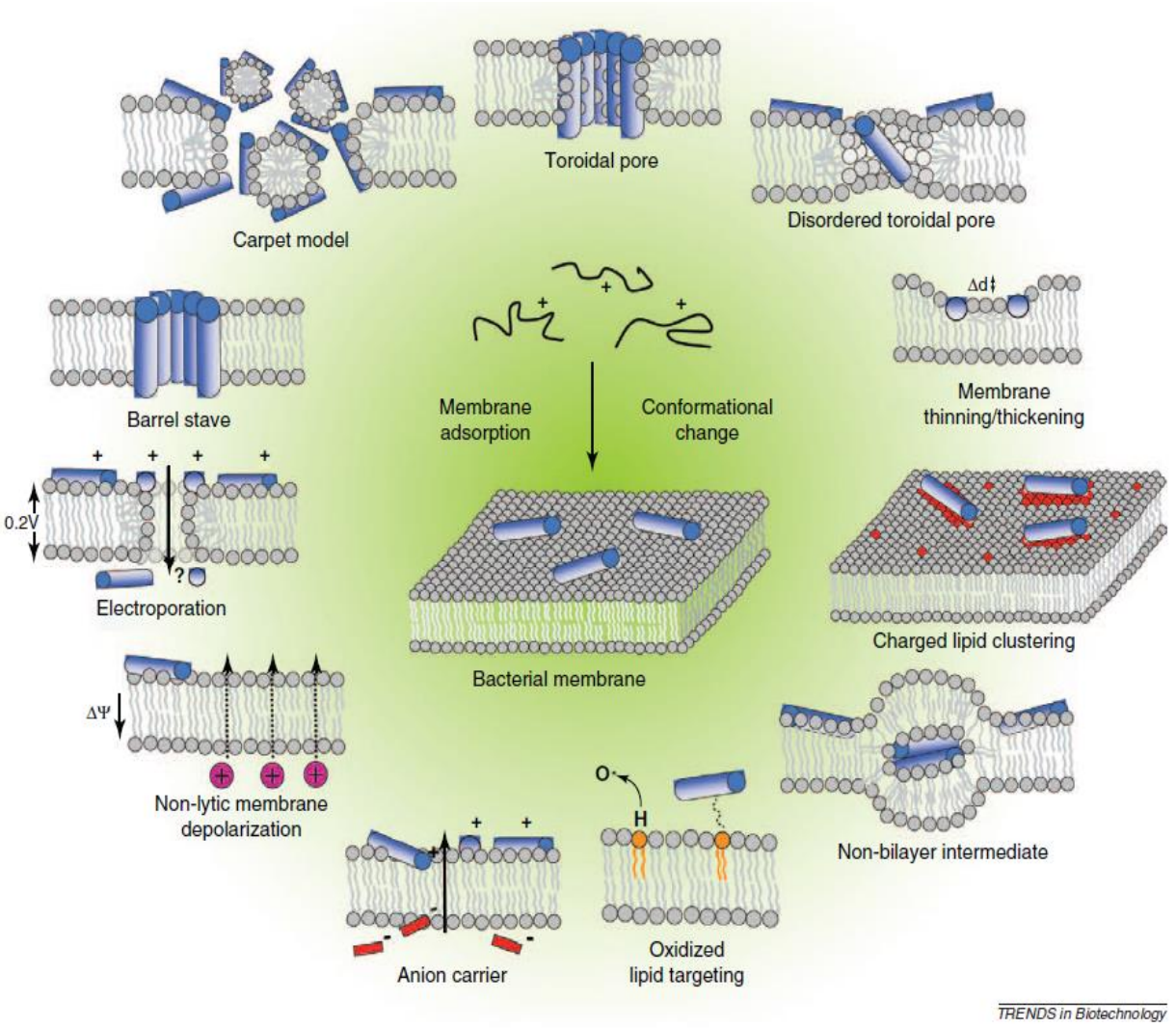
- Innate immune response.
- Tissues exposed to microbial attack are synthesized.
- Inhibit or slow the growth and help as allies in mechanisms of natural and adaptive immunity.



Antibacterial, antifungal, antitumor and anticancer

ACTION MECHANISM

- Length
- Sequency
- Charge
- Secundary Structure
- Amphipathicity
- Hydrophobicity



Nguyen, L. T., Haney, E. F., & Vogel, H. J. (2011). The expanding scope of antimicrobial peptide structures and their modes of action. *Trends in biotechnology*, *29*(9), 464–72.

EXPERIMENTAL STRATEGY

Bioinformatic design

- Antibacterial activity
- Helical Structure
- Amphipathicity
- Positive Charge

synthesis and Chemical characterization

- Peptide synthesis fmoc/tBu
- Cromatography Sepak; HPLC-RP
- MALDI-TOF
- Circular dichroism

Biological tests

- MIC (resazurina)
- Hemolytic activity
- Inhibition Basal ATPase Activity
- Citotoxity activity

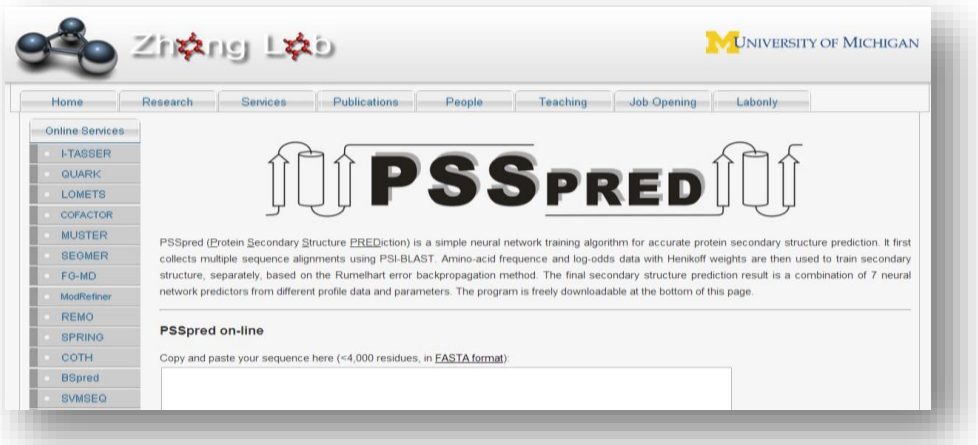
IN SILICO DESIGN OF ANTIMICROBIAL PEPTIDE ANALOGOUS

BIOINFORMATIC DESIGN: Native peptides

Secundary structure prediction

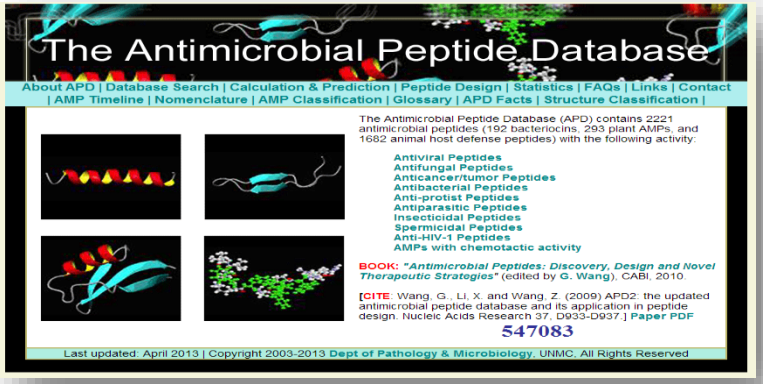
Peptide	Sequency/Secundary structure
Bombinin	**GIGALSAKGALKGLAKGLAQHFAN**
Maximin 1	**GIGTKILGGVKTALKGALKELASTYAN**





BIOINFORMATIC DESIGN

APD2 : 2429 antimicrobial peptides



POSITIVE CHARGE

C-t : Membrane Interaction

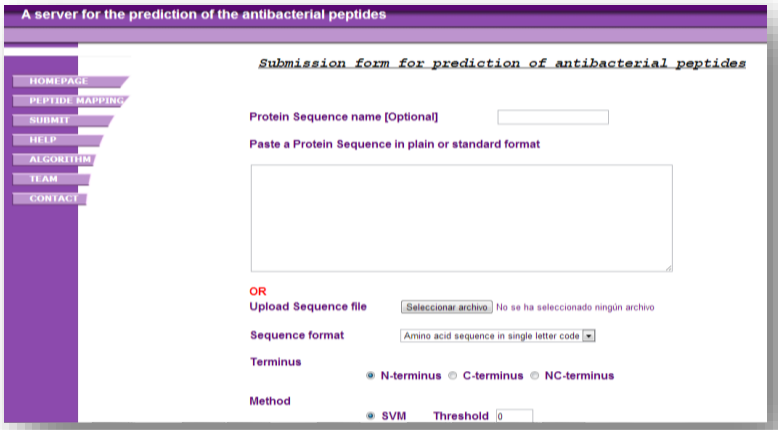
N-t: DNA or RNA Interaction

N-terminal :G,I,K,L

C-terminal :K,G,C,R

N and C terminal high frequency : K y R

AntiBP Server



AntiBP server mapping the native peptide sequence by 15 a.a length

Antimicrobial Score

http://aps.unmc.edu/AP/main.php

http://www.imtech.res.in/raghava/antibp/index.html

in silico Bombinin analysis

Sequence	Antibacterial Score	Charge
GIGALSAKGALKGLAKGLAQHFAN CCCCCCHHHHHHHHHHHHHHHHC	N.A	3
GIGALSAKGALKGLA	1,000	2
IGALSAKGALKGLAK	-0.318	3
GALSAKGALKGLAKG	1,101	3
ALSAKGALKGLAKGL	0,705	3
LSAKGALKGLAKGLA	-0.692	3
SAKGALKGLAKGLAQ	0,638	3
AKGALKGLAKGLAQH	0,327	3
GALKGLAKGLAQHFA	0,437	2
ALKGLAKGLAQHFAN	0,103	2

Blank peptide (Bombinin-A)

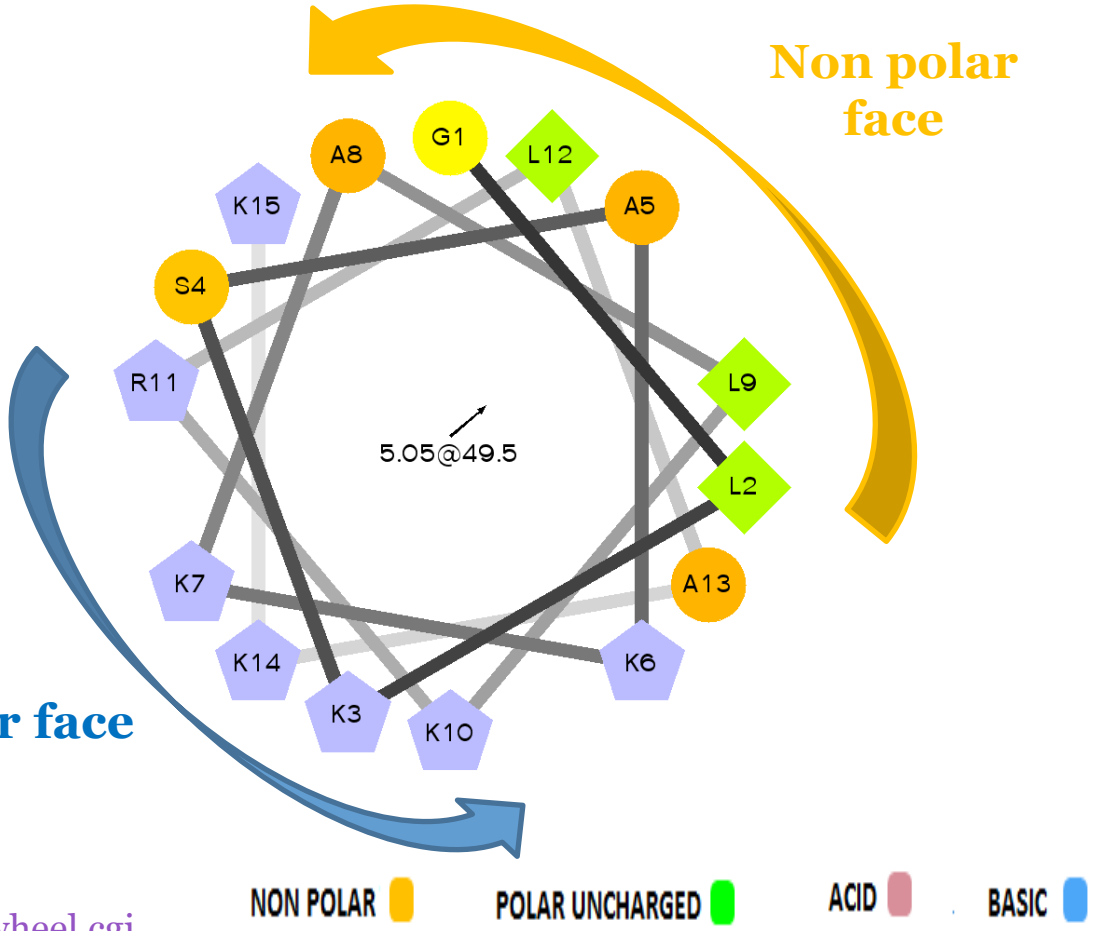
Analogous peptide (Bombinin-B)

in silico Bombinin analysis

a) GALSAKGALKGLAKG (1,101) Bombinin-A (+3) **Blank peptide**
CCCCHHHHHHHHCCC

b) GLKSAKKALKRLAKK (2,715) Bombinin-B (+7) **Analogous peptide**
CCHHHHHHHHHHHHC

Helical Wheel representation: amphipathic structure



http://rzlab.ucr.edu/scripts/wheel/wheel.cgi

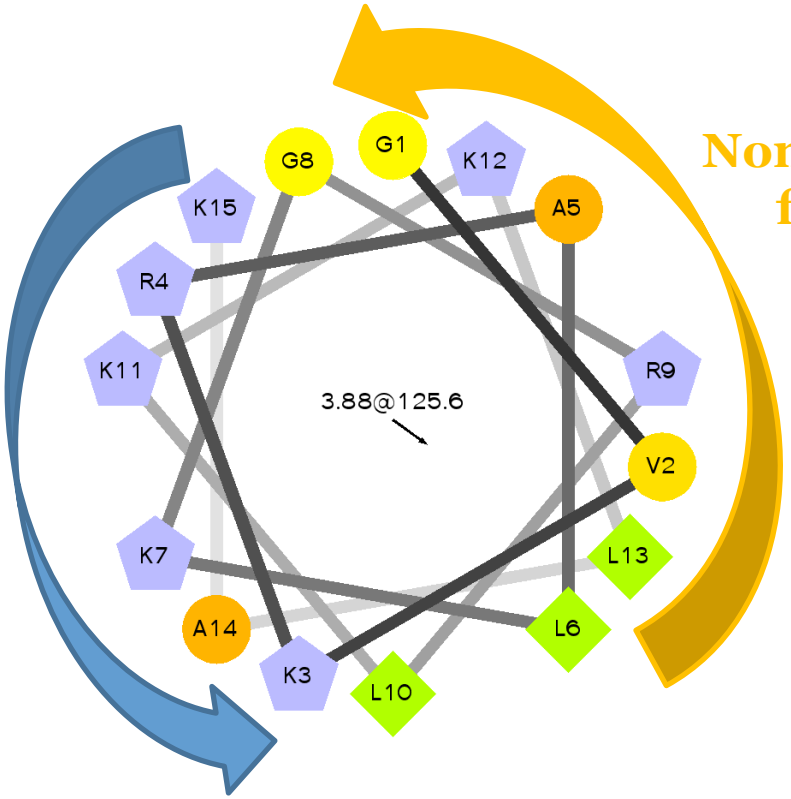
in silico Maximin 1 analysis

GIGTKILGGVKTALKGALKELASTYAN
CCCCHHHHHHHHHHHHHHHHHHHHCC

a) **GVKTALKGALKELAS** (1,054) *Maximin 1*-A (+2) **Blank peptide**
CCCHHHHHHHHHHC

b) **GVKRALKGRLKKLAK** (3,028) *Maximin 1*-B (+7) **Analogous peptide**
CHHHHHHHHHHHHC

Helical Wheel representation: amphipathic structure

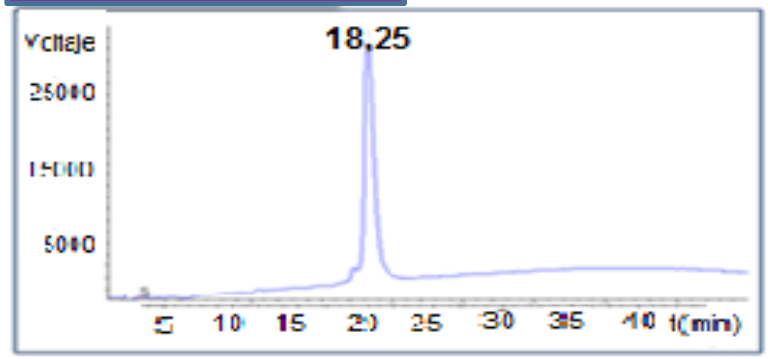


http://rzlab.ucr.edu/scripts/wheel/wheel.cgi

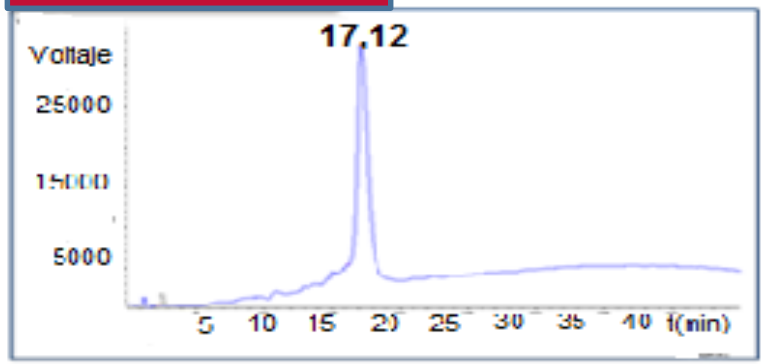
CHEMICAL CHARACTERIZATION

HPLC-RP

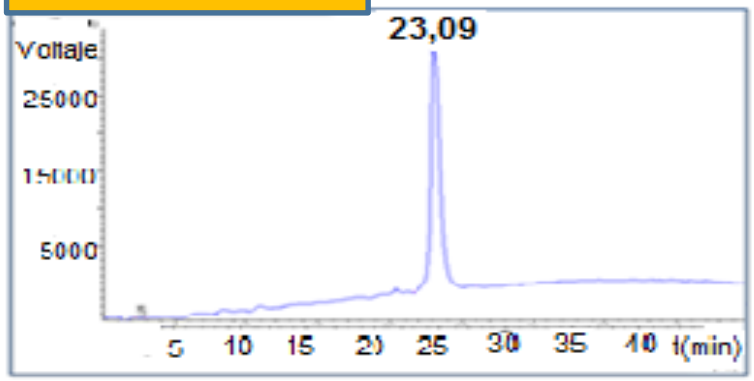
Bombinin-A



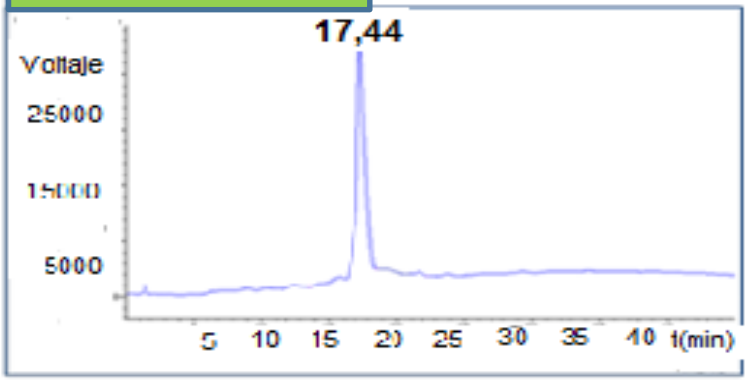
Bombinin-B



Maximin 1-A



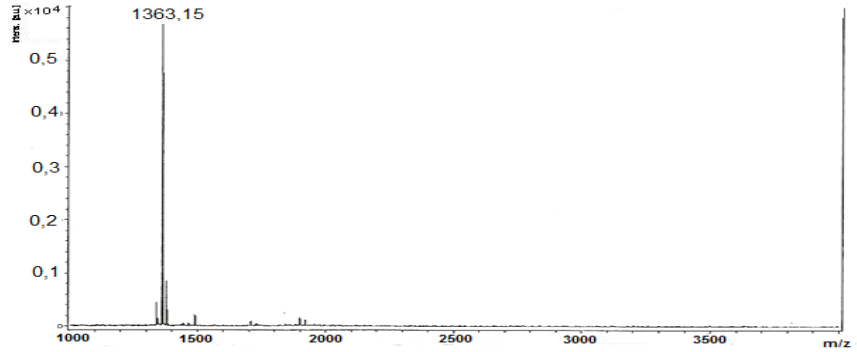
Maximin 1-B



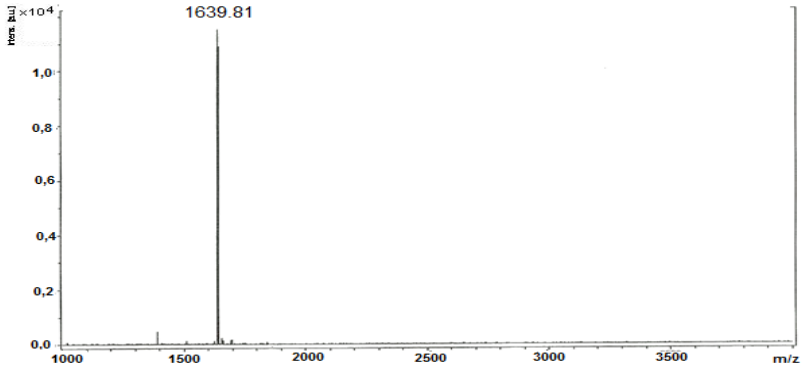
C-18 column and Sepak C-18 column

MALDI-TOF

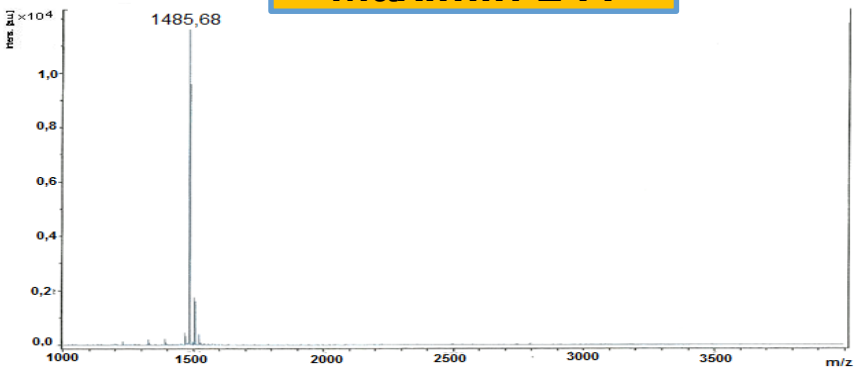
Bombinin-A



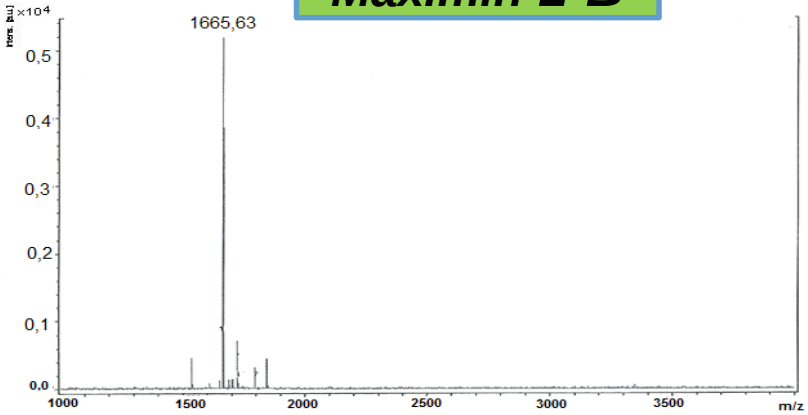
Bombinin-B



Maximin 1-A



Maximin 1-B

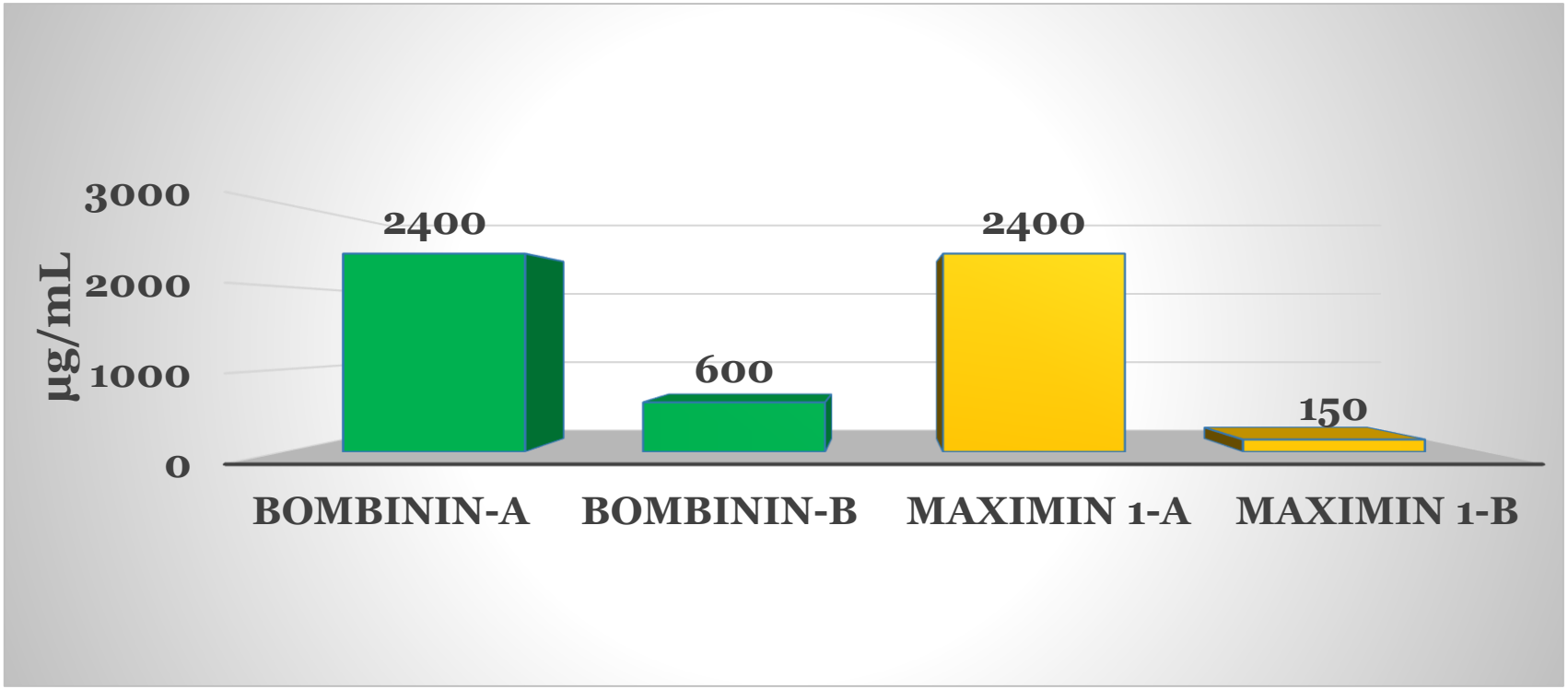


PEPTIDE	MW (Da)
Bombinin-A	1341,8
Bombinin-B	1640,3
Maximin 1-A	1485,68
Maximin 1-B	1665,63



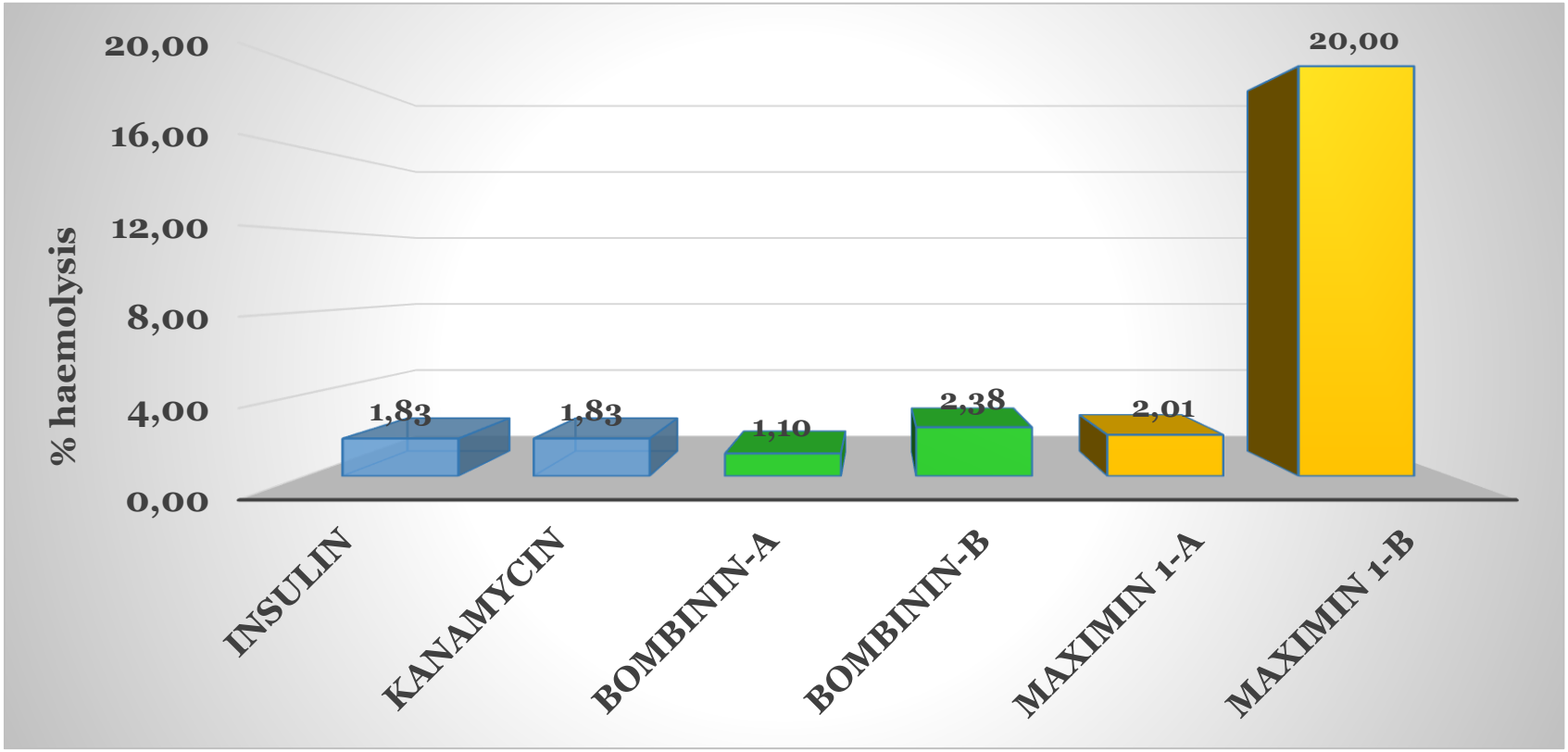
BIOLOGICAL TESTS

M. smegmatis mc²155 viability in presence of Bombinin and Maximin 1 modified



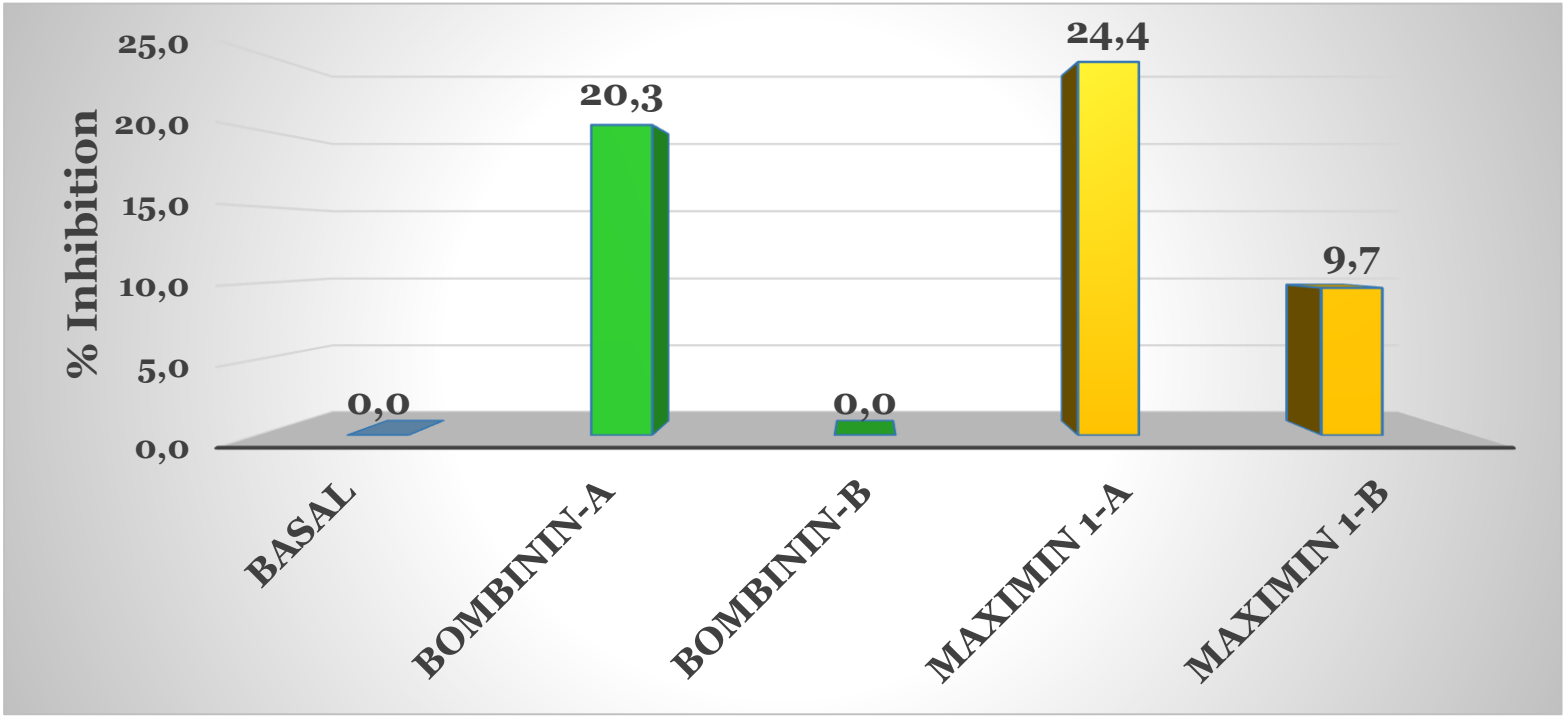
Estimated by MTT assay. The analogue peptide Bombinin-B has 600 µg/mL and Maximin 1-A 150 µg/mL, it shows the increasing positive charge and helical structure improvement antimycobacterial activity.

Hemolytic activity in presence of Bombinin and Maximin 1 modified



In the hemolytic assay, Insulin was used as unrelated peptide and kanamycin as reference control therapy against TB .The haemolysis percentages not surpassed 10% in the most of peptides.

The modified peptides don't shows inhibition of Basal ATPase activity in the *M. smegmatis* mc²155 membrane



The antimycobacterial activity of the peptides depends of inhibition of some ATPase enzymes, which are essential for the cell viability and holding proceses crucial to cell function.

Citotoxity activity over murine macrophages in presence of Maximin 1 modified

Peptide	CL_{50} (µg/mL)
Bombinin-A	8366
Bombinin-A	3211
Maximin 1-A	4286
Maximin 1-B	532,9

All peptides shows citotocitiicy using higher concentration comparate with the results in minimal concentration inhibitory test

Murine macrophages Celular line J774

Alamar Blue method

CONCLUSION

Overall, it was determined that the design of peptides doing changes in its primary structure to improve features like helical structure, antibacterial and charge value, allows the development of new strategies for designing antimicrobial peptides as antituberculosis compounds.

Thank you very much