

Aarón Andrés Santana Hernández, Alejandro Yam Puc, Elizabeth Ortiz-Vázquez
Tecnológico Nacional de México Campus Mérida

Background

The therapeutic use of bee products has gained relevance in response to the increasing development of antimicrobial-resistant strains. Propolis collected by *Melipona beecheii*, a stingless bee native to Central America and the southeast of Mexico, has been used in Mayan traditional medicine due to its biological properties, including its antimicrobial activity. Its chemical composition is diverse, spotlighting pentacyclic triterpenes as metabolites with potential antibacterial use. This research aims to determine which metabolites are responsible for the antibacterial activity in *M. beecheii* propolis.

Problem description

Antimicrobial resistance is a global problem that requires new therapeutic agents. Although propolis possesses bioactive properties, its chemical variability—shaped by environmental factors—hinders its standardization and clinical application. This study focuses on the isolation and antibacterial evaluation of pentacyclic triterpenes from *M. beecheii* propolis against clinically relevant pathogens.

Methodology

Propolis samples were collected in Maní, Yucatán. The hexanic fraction obtained from an ethanolic propolis extract—rich in pentacyclic triterpenes—was separated and purified by column chromatography using hexane and ethyl ether mixtures. Antibacterial activity was evaluated by Kirby-Bauer disk diffusion and microdilution methods against *Escherichia coli* and *Staphylococcus aureus*.

Results and conclusion

Successive purifications of the hexanic fraction revealed the presence of four principal bands (I-IV), observable by thin-layer chromatography. These bands were rich in pentacyclic triterpenes with a characteristic functional group at carbon 3: I acetylated (β -amyrin, germanicol, moretenol, and cycloartanol acetates), II ketone (marsformosanone, β -amyrenone, germanicone, and lupenone) and hydroxylated (III glutinol, α -amyrin and IV α -amyrin and β -amyrin).

The ethanolic extract and fractions I and IV exhibited antibacterial activity. Microdilution assays showed that 1.5 mg/ml of ethanolic extract inhibited up to 90% of *S. aureus* growth. These results show that *M. beecheii* propolis is abundant in pentacyclic triterpenes with antibacterial activity, especially against Gram-positive strains.

Practical implications for beekeepers

The findings of this study highlight the value of *M. beecheii* propolis, promoting its sustainable collection and commercialization. Standardizing the bioactive compounds in this propolis could enhance its potential applications in the pharmaceutical and food industries. Furthermore, promoting meliponiculture positively impacts biodiversity and the local economy, reinforcing it as a sustainable development practice.

Antibacterial Properties of Pentacyclic Triterpenes from *Melipona beecheii* Propolis



By MSc. Aarón Santana Hernández
Applied and Molecular Microbiology
Laboratory
Instituto Tecnológico de Mérida
México

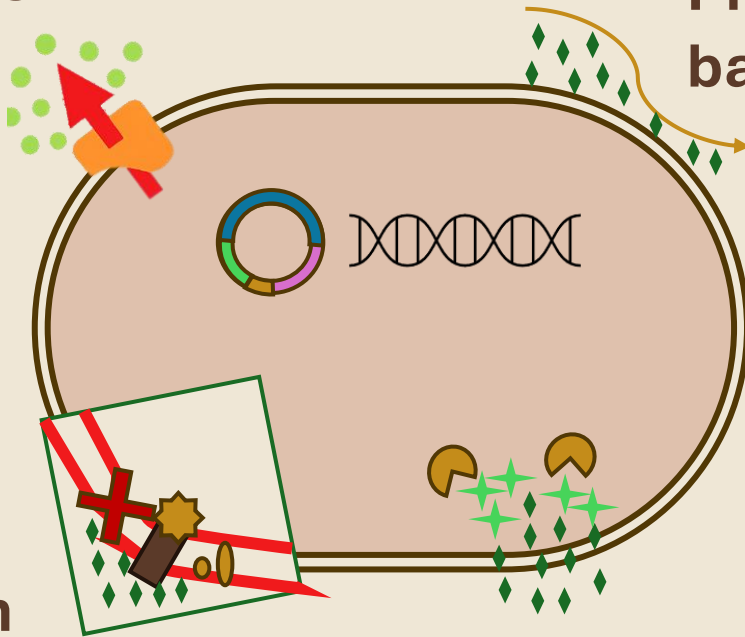


Introduction

Antimicrobial resistance (AMR)

Efflux pumps

Physical
barriers



Modification
of drug target

Inactivation
of drugs

Causes

- The misuse and overuse of antimicrobials
- Insufficient sanitary control
- Limited education on the matter

Consequences

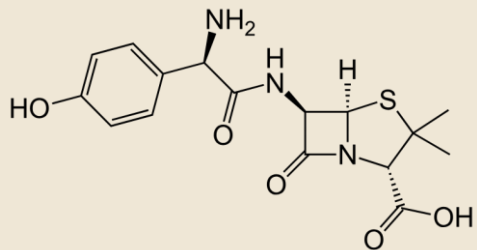


>1.7 Million of deaths are attributed to AMR in 2019

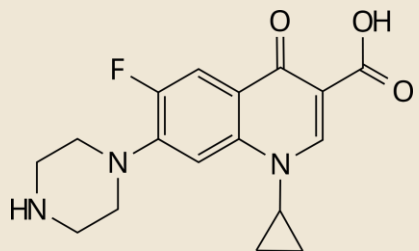


Longer and more costly hospitalizations

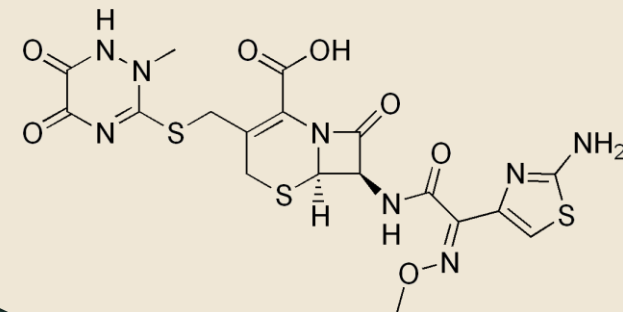
Escherichia coli



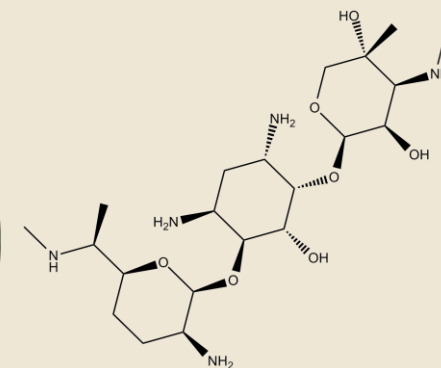
(AMX) Amoxicillin



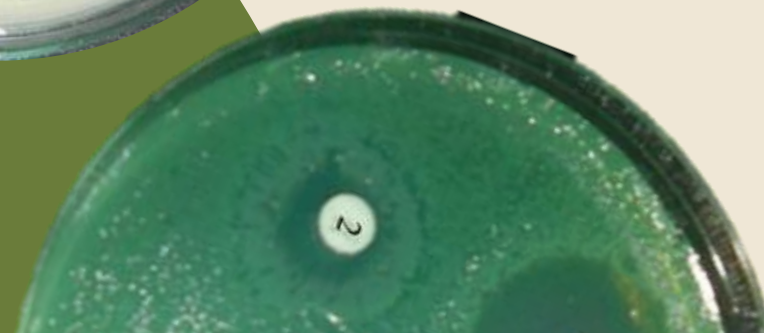
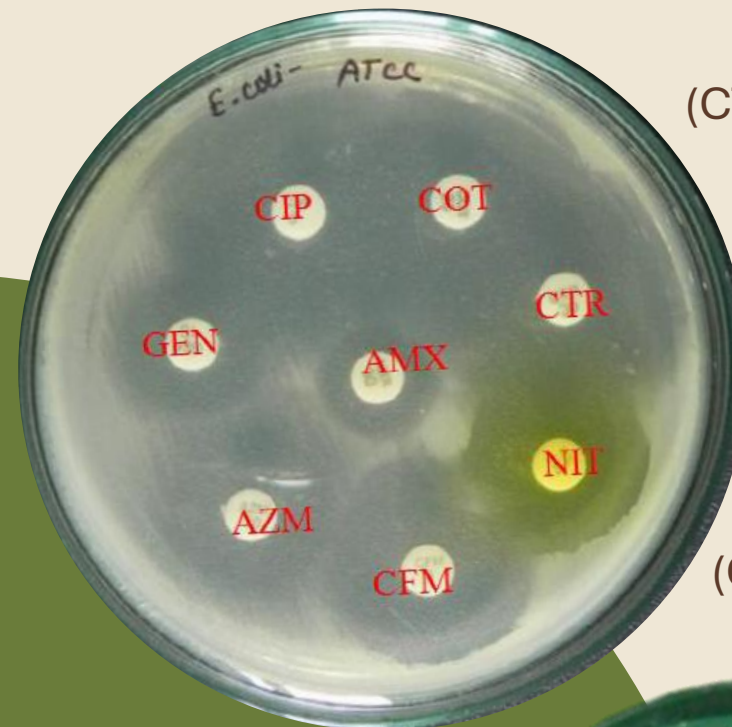
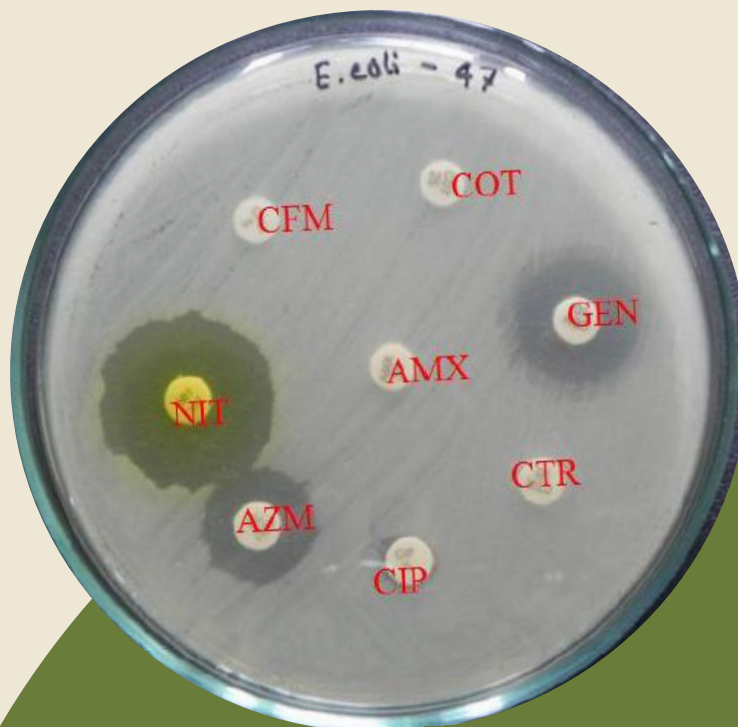
(CIP) Ciprofloxacin



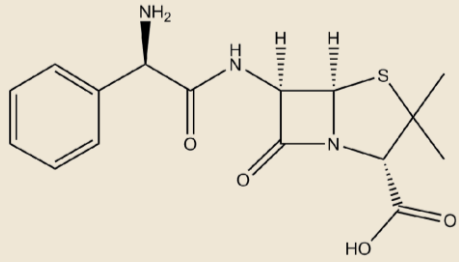
(CTR) *Ceftriaxone*



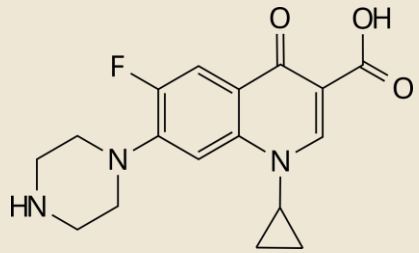
(GEN) Gentamycin



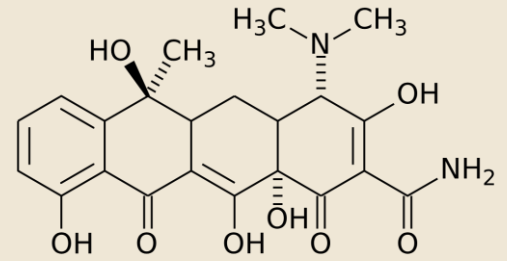
Pseudomonas aeruginosa



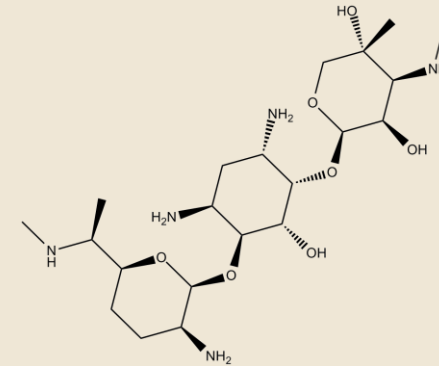
(1) Ampicillin



(5) Ciprofloxacin



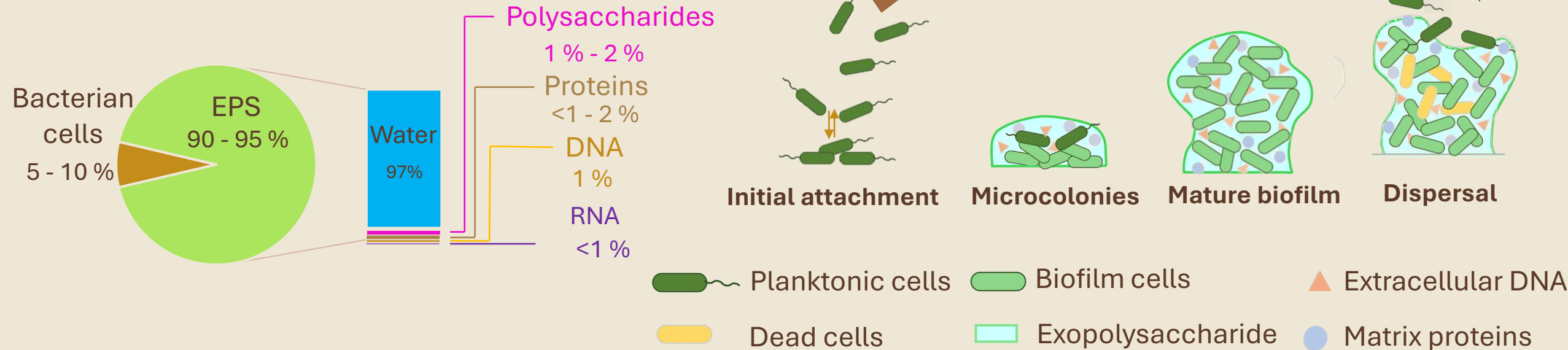
(2) Tetracycline



(4) Gentamycin



Introduction Biofilm



Biofilm functions

- Protection
- Nutrition
- Communication
- Adaptation

*Pseudomonas
aeruginosa*

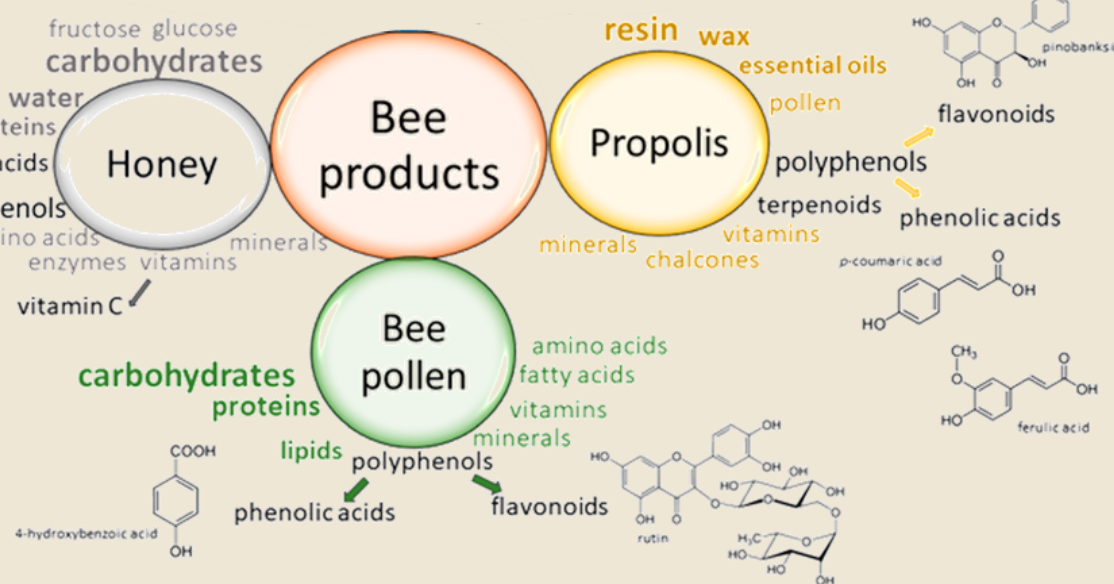
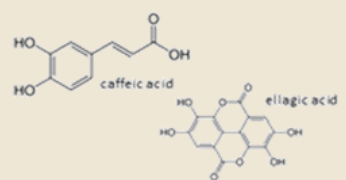
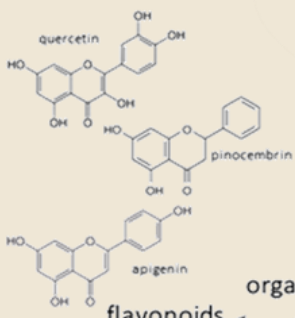
Exopolysaccharides
Psl
Pel
Alginate

Quorum sensing
Las
Rhl
PQS
IQS

Melipona beecheii hive products as antibacterial agents



Xunan Kaab
Melipona beecheii



Melipona beecheii's propolis



Rhizophora mangle



Bursera simaruba



Xunan Kaab
Melipona beecheii



resin wax

essential oils

pollen

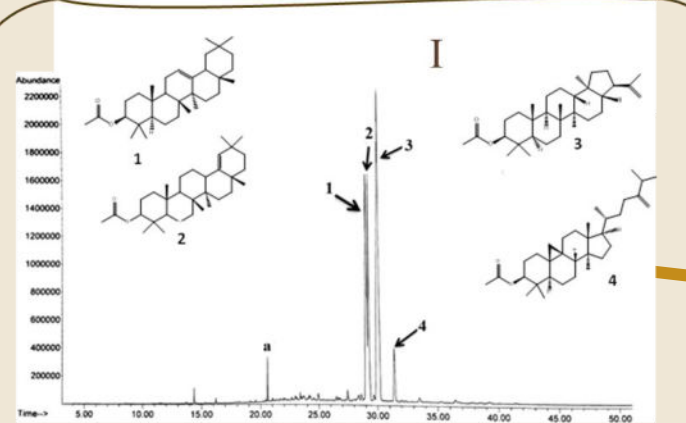
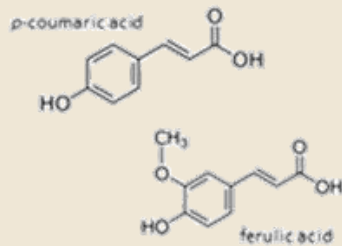
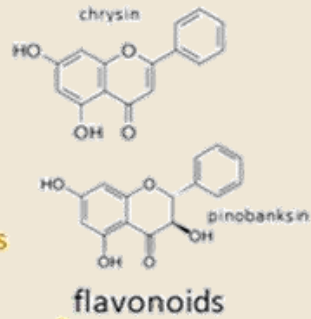
Propolis

polyphenols

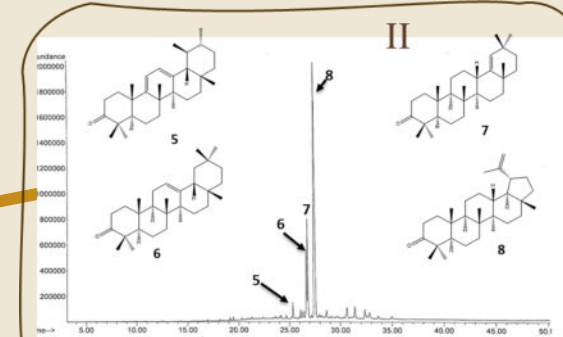
terpenoids

vitamins

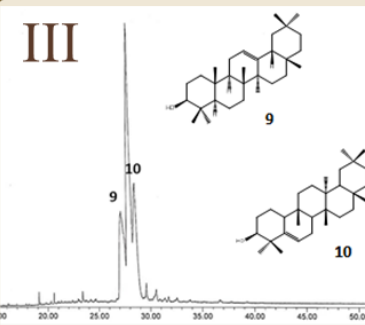
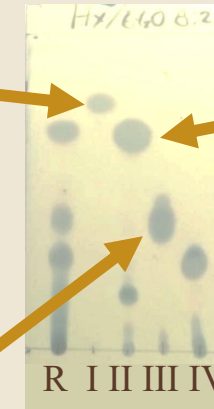
chalcones



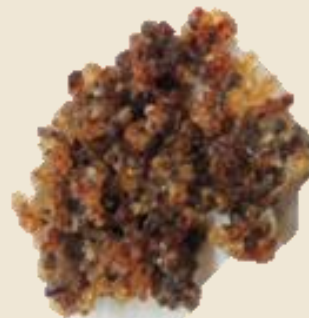
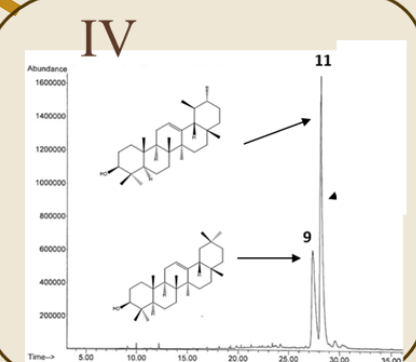
- 1) β -amyrin acetate
2) Germanicol acetate
3) Moretenol acetate
4) Cycloartenol acetate



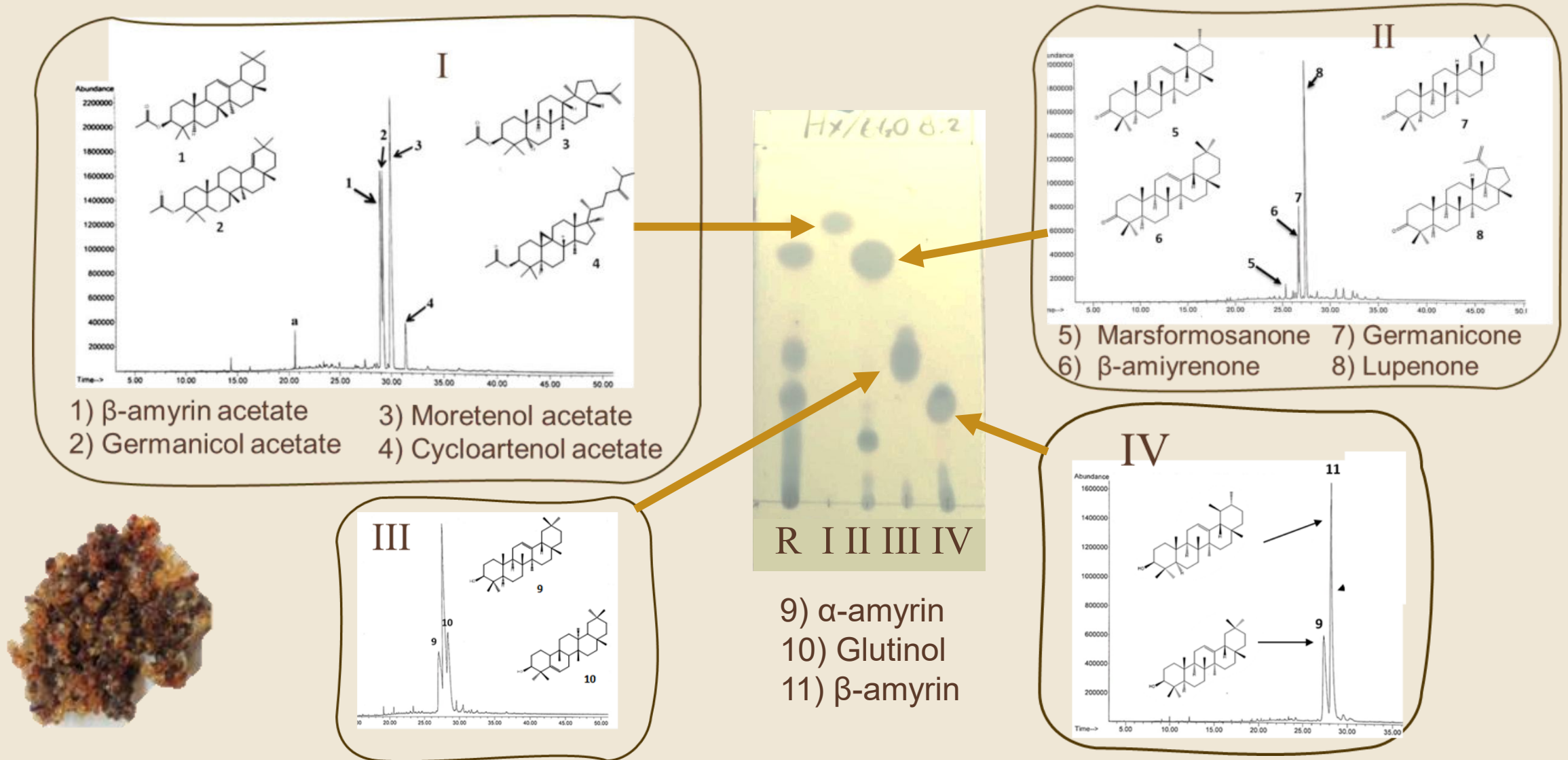
- 5) Marsformosanone
6) β -amyrinone
7) Germanicone
8) Lupenone



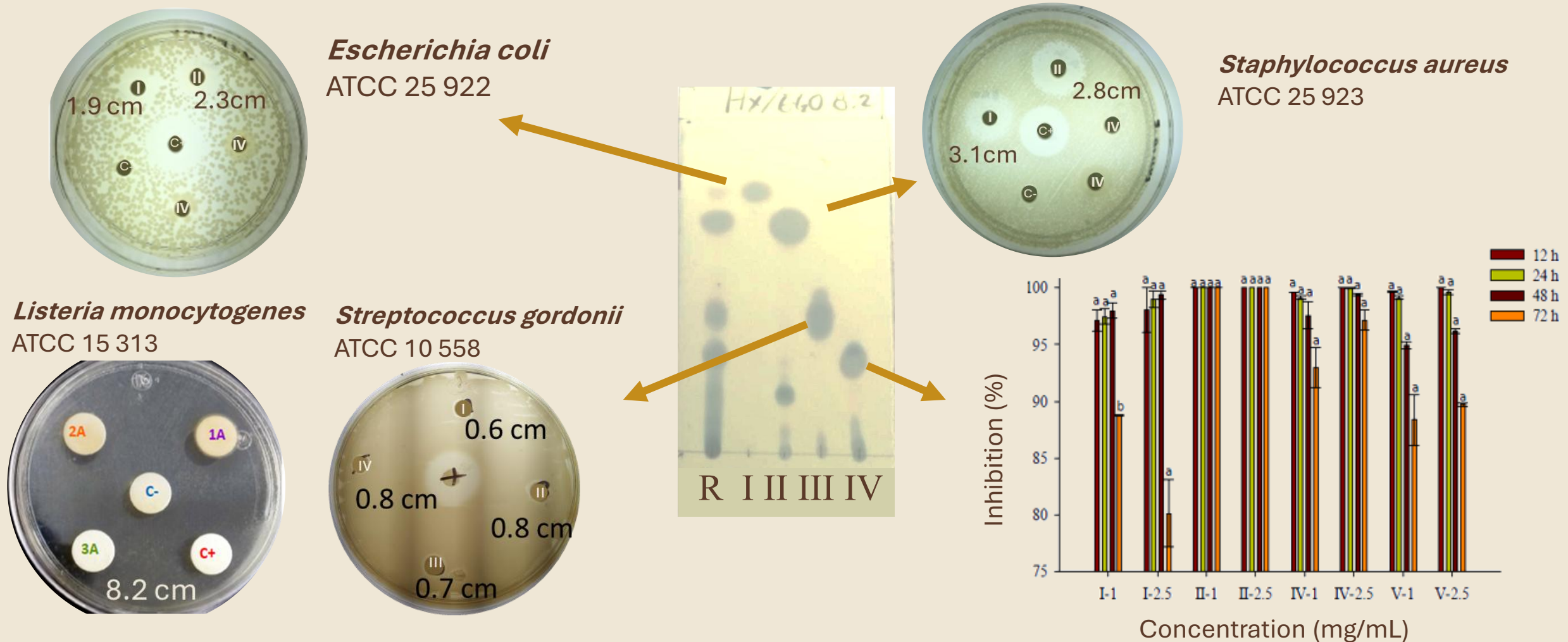
- 9) α -amyrin
10) Glutinol
11) β -amyrin



Pentacyclic triterpenes in *Melipona beecheii* propolis



Antibacterial Properties of Pentacyclic Triterpenes from *Melipona beecheii* Propolis



The project

Propolis origin: Maní Yucatán



Meliponary “U Náajil Yum Kin”, property of Luis Armando Quintal Medina
Video from: Gustavo Muñoz Pallas



Maceration in
ethanol w:v 1:10
24 h (x3)

Ethanollic
Extract of propolis
(EEP)



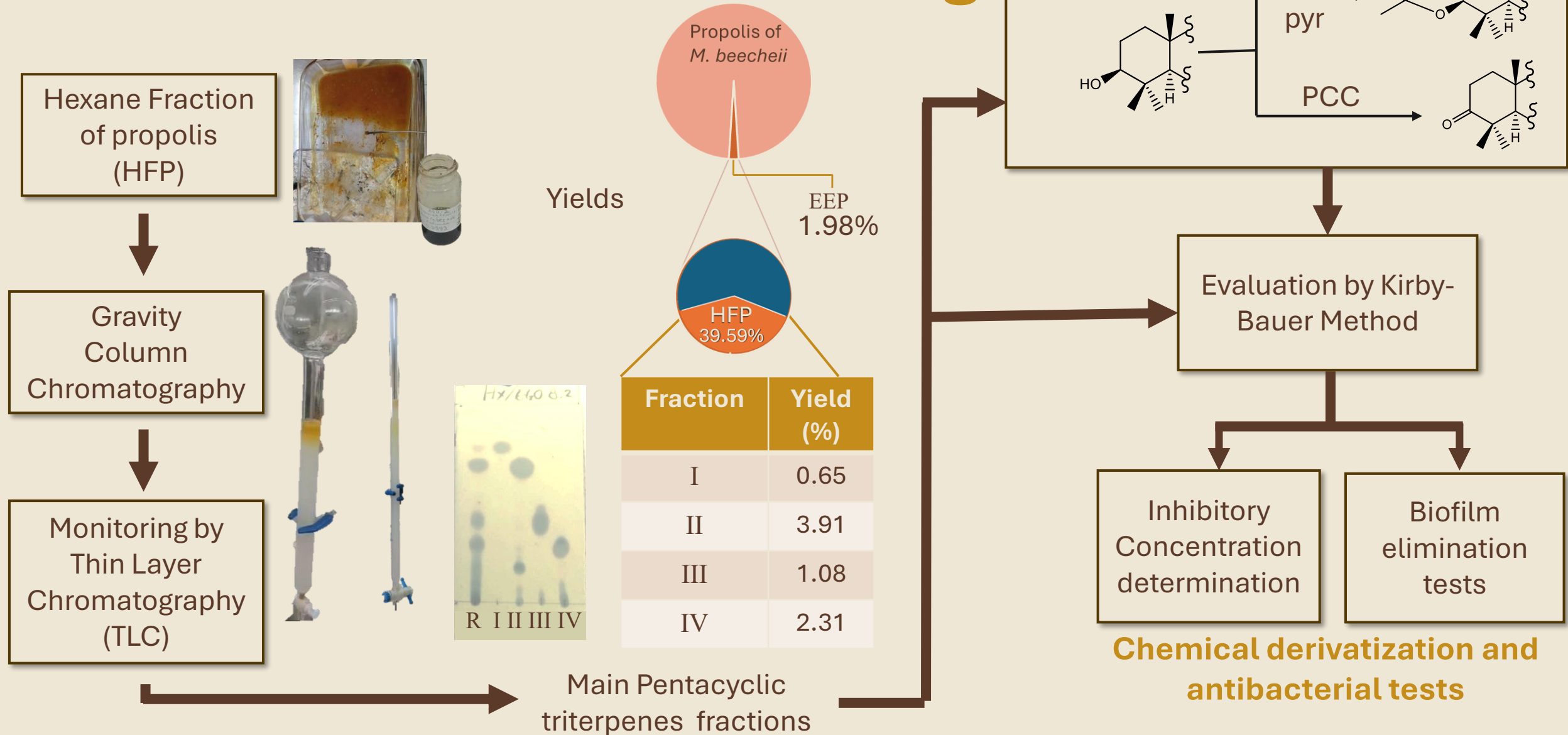
Hexane Fraction
of propolis
(HFP)



Purification and monitoring

The project

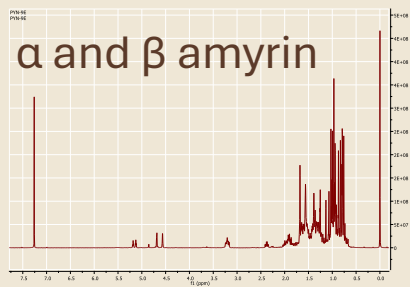
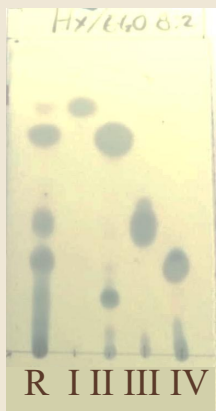
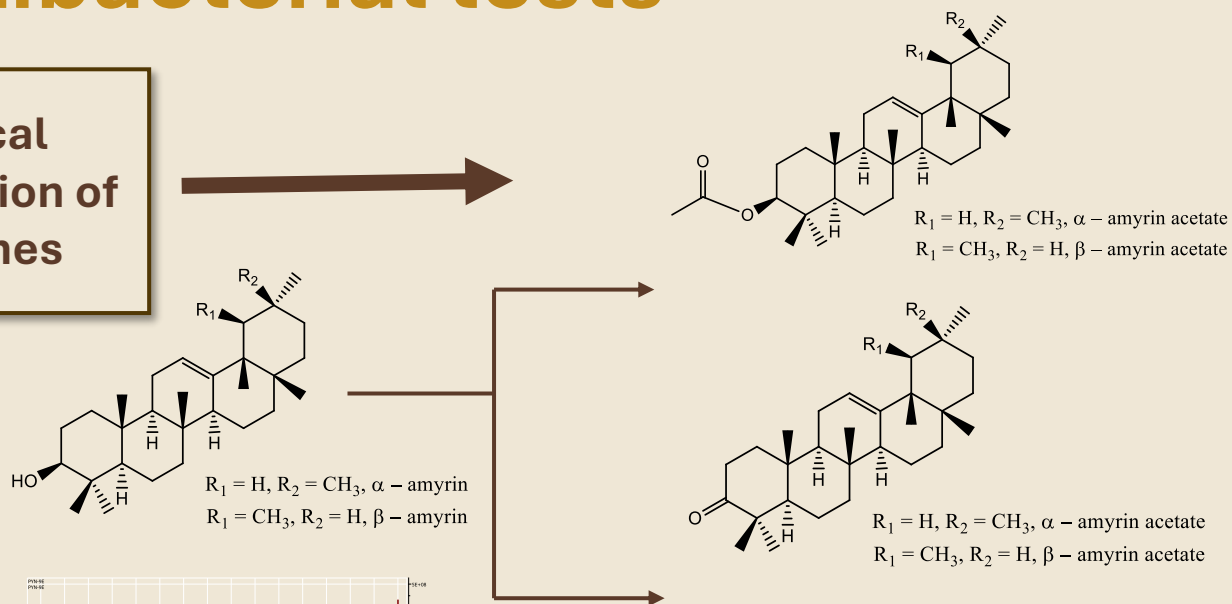
Purification and monitoring



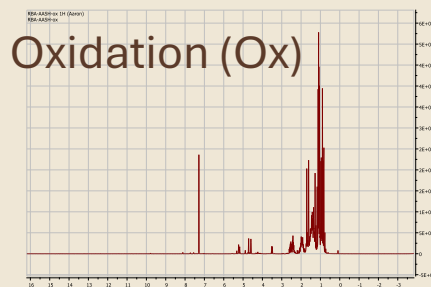
The project

Chemical derivatization and antibacterial tests

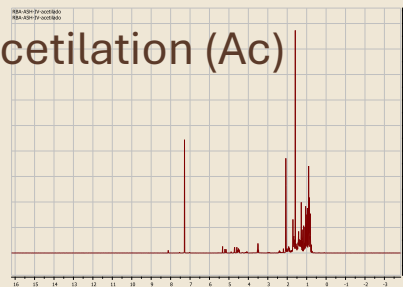
Chemical
derivatization of
triterpenes



Oxidation (Ox)



Acetylation (Ac)

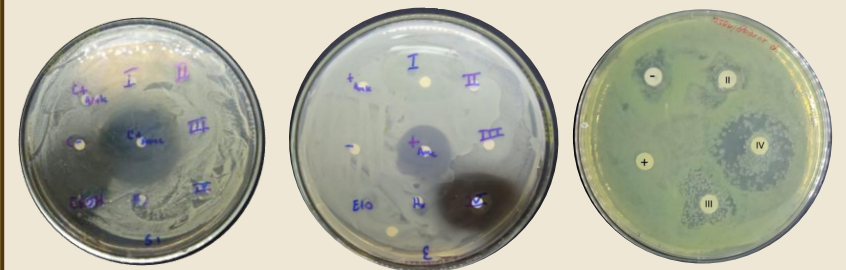


Confirmation by ^1H -NMR and TLC

Unpublished data

Evaluation by Kirby-Bauer Method

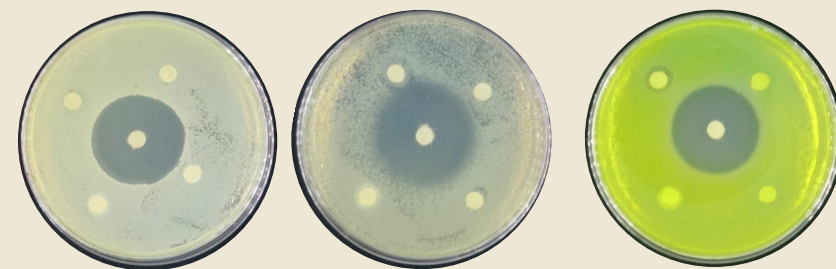
Before derivatization



S. aureus

E. coli

P. aeruginosa



After derivatization

Inhibitory
Concentration
determination

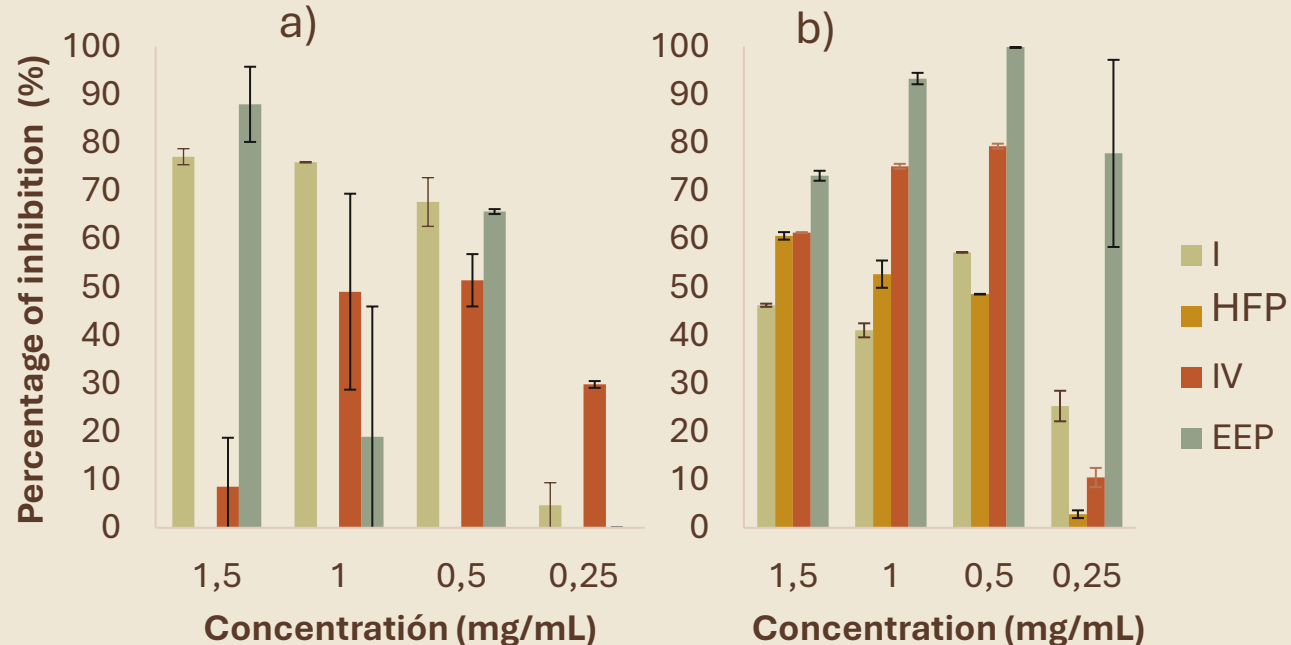
Biofilm
elimination
proves

The project

Antibacterial tests

Inhibitory Concentration determination

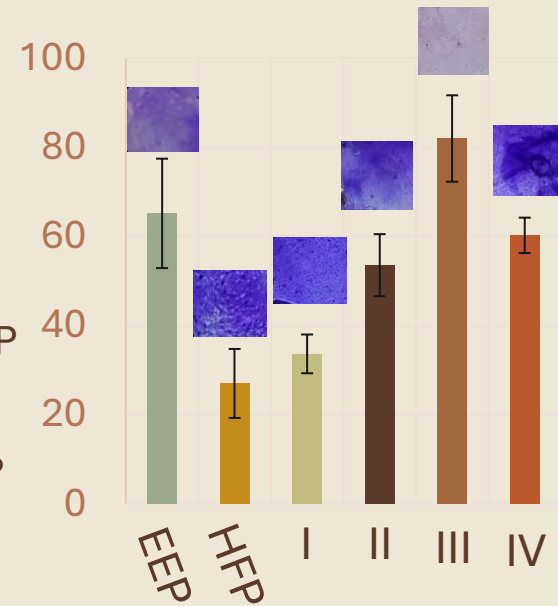
Percentage of inhibition of *S. aureus* (a) and *E. coli* (b) using different propolis extracts and fractions



Unpublished data

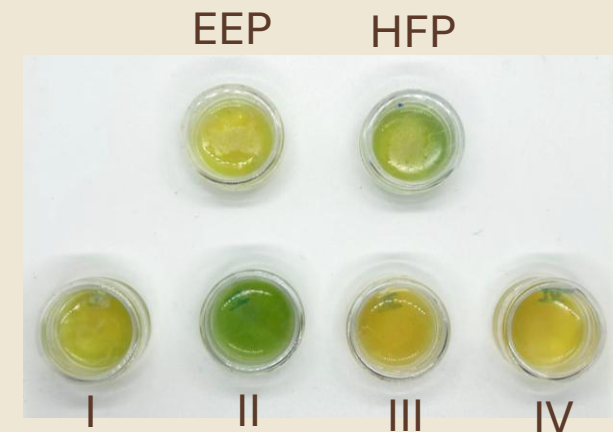
Biofilm elimination tests

Biofilm elimination 3 mg/mL



Fractions proved against *P. aeruginosa* biofilm

Incubation plates with different concentrations of pyocyanin

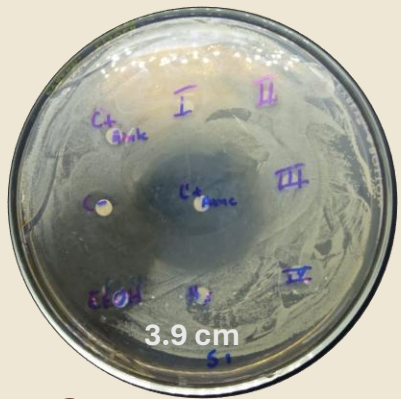


The project

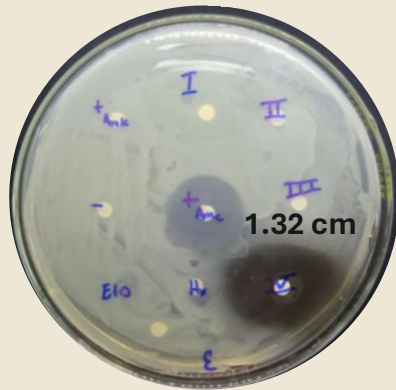
Chemical derivatization and antibacterial tests

Evaluation by Kirby-Bauer Method

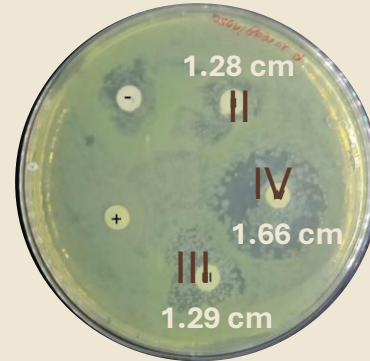
Before derivatization, Concentration: 3 mg/mL, + Control Amk 15 µg/mL



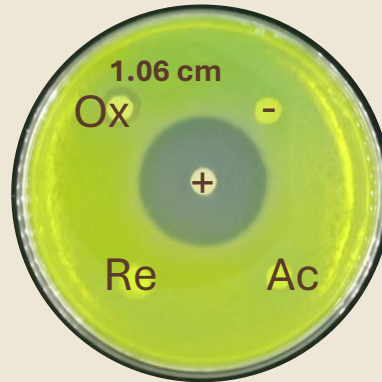
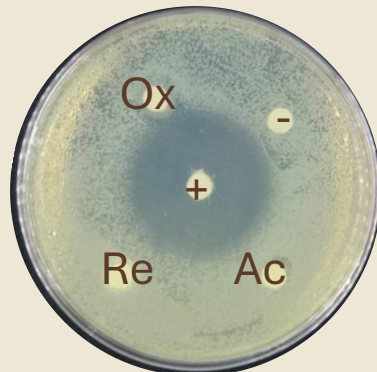
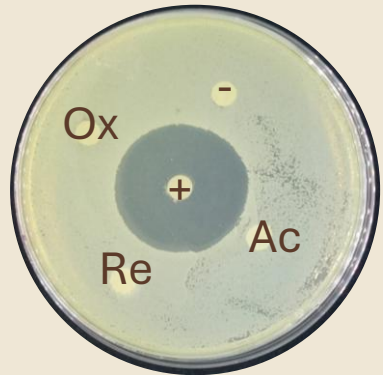
S. aureus



E. coli

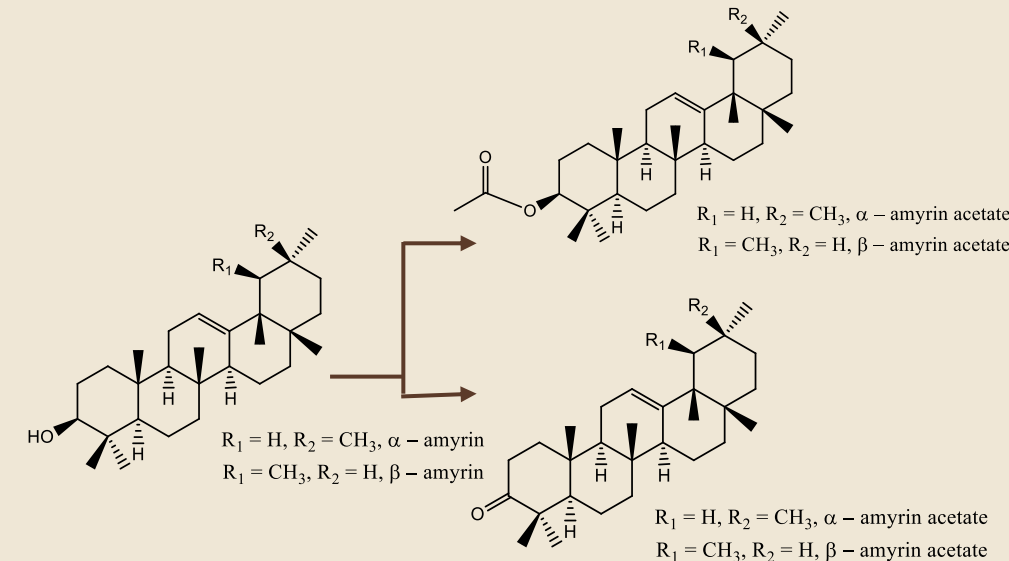


P. aeruginosa



After derivatization, Concentration: 18 mg/mL, + Control Amk 15 µg/mL

Disc diffusion tests before and after chemical derivatization of pentacyclic triterpenes



Unpublished data

Conclusion and discussion

- **Fraction III**, abundant in pentacyclic triterpenes with a hydroxyl functional group at carbon 3, **eliminated up to 89% of biofilm formation of *P. aeruginosa*** at 3 mg/mL and reduced the production of pyocyanin, a pigment and virulence factor.
- **Pentacyclic triterpenes** from *M. beecheii* propolis, when **chemically derivatized** by modifying the **hydroxyl group** at carbon 3, **lost or notably reduced their antibacterial activity**.

Antibiofilm activity of Pentacyclic triterpenes against *P. aeruginosa*

Triterpene (Fraction)	Concentration	% of biofilm reduction	Biological source	Reference
β-amyrin acetate (I)	0.5 mg/mL	85 %	<i>Vernonia auriculifera</i>	Kiplimo et al., 2011
Lupeol and Lupenone (II)	0.015 mg/mL	79.70 %	<i>Crataeva nurvala</i> in Silver nanopartilces	Ali S. et al, 2017
α-amyrin (IV)	0.043 mg/mL	38 ± 2 %	<i>Platostoma rotundifolium</i>	Rasamiravaka, T et al. 2017
α-amyrin and glutinol mixture (IV)	3 mg/mL	81.94 ± 9.7 %	<i>M. beecheii propolis</i>	This work

Conclusion and discussion

- **Fraction III**, abundant in pentacyclic triterpenes with a hydroxyl functional group at carbon 3, **eliminated up to 89% of biofilm formation of *P. aeruginosa*** at 3 mg/mL and reduced the production of pyocyanin, a pigment and virulence factor.
- **Pentacyclic triterpenes** from *M. beecheii* propolis, when **chemically derivatized** by modifying the **hydroxyl group** at carbon 3, **lost or notably reduced their antibacterial activity**.

Minimum inhibitory concentration against *E. coli*, *S. aureus* and *P. aeruginosa* of different biological sources

Triterpene (Fraction)	Minimum inhibitory concentration (MIC) (mg/mL)			Biological source	Reference
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>		
β-amyrin acetate (I)	0.09	0.09	0.078	Semisynthetic	Hichri F. et al 2003
Lupenone (II)	2.8	2.8	-	<i>Acacia cochliacantha</i>	Manríquez-Torres 2017
β-amyrin (IV)	10	2.5	2.5	<i>Dolichos Pachyrhizus</i>	Musa, N. et al. 2024
Fraction IV	-	-*	-	<i>M. beecheii propolis</i>	This work

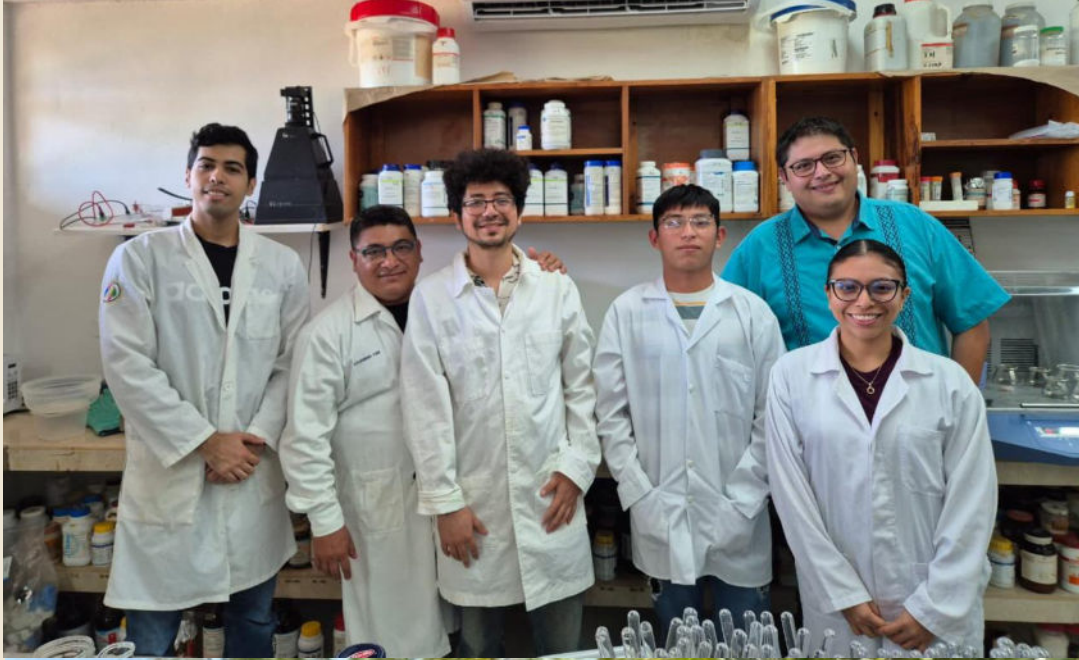
* It was observed only an 77 % of inhibition of *S. aureus* with 1.5 mg/mL of fraction **IV**, a mixture of α-amyrin and β-amyrin

Conclusion and discussion

- **Fraction III**, abundant in pentacyclic triterpenes with a hydroxyl functional group at carbon 3, **eliminated up to 89% of biofilm formation of *P. aeruginosa*** at 3 mg/mL and reduced the production of pyocyanin, a pigment and virulence factor.
- **Pentacyclic triterpenes** from *M. beecheii* propolis, when **chemically derivatized** by modifying the **hydroxyl group** at carbon 3, **lost or notably reduced their antibacterial activity**.

Minimum inhibitory concentration (MIC) against different bacteria of stingless bees propolis ethanolic extract

Stingless bee that harvested the propolis	MIC (mg/mL)	Bacteria tested	Reference
<i>Melipona beecheii</i>	0.5	<i>E. coli</i>	This work
	0.04	<i>P. aeruginosa</i>	Fonte-Carballo et al. 2016
	0.07	<i>E. coli</i>	
	0.07	<i>S. aureus</i>	
<i>Melipona solani</i>	3	<i>S. aureus</i>	Grajales-Conesa et al. 2021
<i>Scaptotrigona mexicana</i>	4	<i>S. aureus</i>	
<i>Tetragonisca angustula</i>	9.9	<i>S. aureus</i>	Farnesi, A. P. (2007).



email: aaron3012@hotmail.com

email 2: DD14081648merida.tecnm.mx

Cell: +52 9992419499

Ln: [linkedin.com/in/aar3n-santana](https://www.linkedin.com/in/aar3n-santana)



My hive



Thank you very much

References

- Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., ... & Dekker, D. M. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *The Lancet*, 404(10459), 1199-1226.
- Nasreen, M., Sarker, A., Malek, M. A., Ansaruzzaman, M. D., & Rahman, M. (2015). Prevalence and resistance pattern of *Pseudomonas aeruginosa* isolated from surface water. *Advances in Microbiology*, 5(1), 74-81.
- Ochieng'Oduor, J. M., Onkoba, N., Nyachieo, A., & Maloba, F. (2016). Experimental phage therapy against haematogenous multi-drug resistant *Staphylococcus aureus* pneumonia in mice. *African Journal of Laboratory Medicine*, 5(1), 1-7.
- Yin, R., Cheng, J., Wang, J., Li, P., & Lin, J. (2022). Treatment of *Pseudomonas aeruginosa* infectious biofilms: Challenges and strategies. *Frontiers in Microbiology*, 13, 955286.
- Joshi, S., Lahiri, D., Ray, R. R., & Davoodbasha, M. (Eds.). (2023). Microbial Biofilms: Challenges and Advances in Metabolomic Study.
- Ray, R. R., Nag, M., & Lahiri, D. (Eds.). (2021). *Biofilm-mediated diseases: causes and controls*. Springer Singapore.
- Rabbee, M. F., Begum, M. K., Islam, M. J., Chowdhury, P. A., Chowdhury, O. A., Zohora, F., ... & Azad, A. K. (2016). Multidrug resistance phenotype and plasmid profiling of *Escherichia coli* isolates causing urinary tract infections in north east part of Bangladesh. *Br Microbiol Res J*, 15(6), 1-11.

References

- Peršurić, Ž., & Pavelić, S. K. (2021). Bioactives from bee products and accompanying extracellular vesicles as novel bioactive components for wound healing. *Molecules*, 26(12), 3770.
- Kelly, J. B., Nolan, A. C., & Zeden, M. S. (2024). How can we escape the ESKAPEs: Antimicrobial resistance mechanisms and what lies ahead?. *PLoS pathogens*, 20(6), e1012270.
- Okeke, I. N., de Kraker, M. E., Van Boeckel, T. P., Kumar, C. K., Schmitt, H., Gales, A. C., ... & Laxminarayan, R. (2024). The scope of the antimicrobial resistance challenge. *The Lancet*, 403(10442), 2426-2438.
- Muñoz P. G. [Gustavo Muñoz Pallas]. (13 de junio de 2025). Abejas Meliponas Mayas, "Xunan Cab", y su miel medicinal en Maní, Yucatán [Archivo de Video]. Youtube.
<https://www.youtube.com/watch?v=Tw5kly4oqCQ&t=803s>
- Kiplimo, J. J., Koorbanally, N. A., & Chenia, H. (2011). Triterpenoids from *Vernonia auriculifera* Hiern exhibit antimicrobial activity. *African Journal of Pharmacy and Pharmacology*, 5(8), 1150-1156.
- Ali, S. G., Ansari, M. A., Khan, H. M., Jalal, M., Mahdi, A. A., & Cameotra, S. S. (2017). *Crataeva nurvala* nanoparticles inhibit virulence factors and biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. *Journal of Basic Microbiology*, 57(3), 193-203.
- Rasamiravaka, T., Ngezahayo, J., Pottier, L., Oliveira Ribeiro, S., Souard, F., Hari, L., ... & Duez, P. (2017). Terpenoids from *Platostoma rotundifolium* (Briq.) AJ Paton alter the expression of quorum sensing-related virulence factors and the formation of biofilm in *Pseudomonas aeruginosa* PAO1. *International Journal of Molecular Sciences*, 18(6), 1270.

References

Hichri, F., Jannet, H. B., Cheriaa, J., Jegham, S., & Mighri, Z. (2003). Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds. *Comptes rendus. Chimie*, 6(4), 473-483.

Manríquez-Torres, J. J., Zúñiga-Estrada, A., González-Ledesma, M., & Torres-Valencia, J. M. (2007). The antibacterial metabolites and proacacipetalin from *Acacia cochliacantha*. *Journal of the Mexican Chemical Society*, 51(4), 228-231.

Musa, N. M., Sallau, M. S., Oyewale, A. O., & Ali, T. (2024). Antimicrobial activity of lupeol and β -amyirin (triterpenoids) isolated from the rhizome of *Dolichos pachyrhizus* harm. *Advanced Journal of Chemistry, Section A*, 7(1), 1-14.