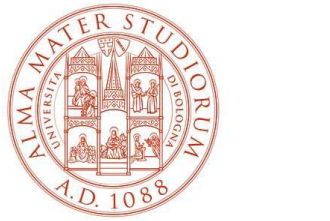
Alma Mater Studiorum
Università di Bologna



Dipartimento di Scienze Mediche Veterinarie

PRODUZIONI ANIMALI E SICUREZZA ALIMENTARE

Development of qualitative and quantitative methods for the determination of bioactive molecules for animal feeds

DOTTORATO IN SCIENZE VETERINARIE XXXVIII CICLO AA 23-24

PNRR DM 352, Borsa cofinanziata da Vetagro S.p.A

Dott.ssa Maria Federica Marchesi

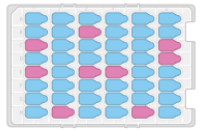
Supervisore: Prof.ssa Teresa Gazzotti

Co-Supervisore: Dr.ssa Roberta Majer

Anno accademico 2023/2024

RESEARCH ACTIVITY

SAPONINS



Gravimetric determination in micorencapsulated

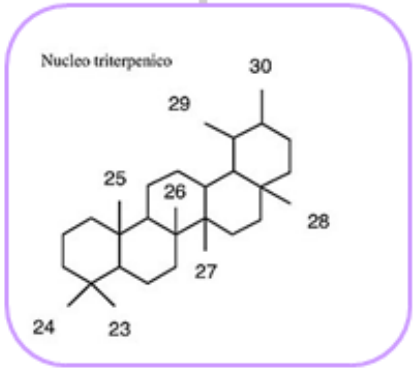
Uv–Vis determination in microencapsulated

Bioactivity assay

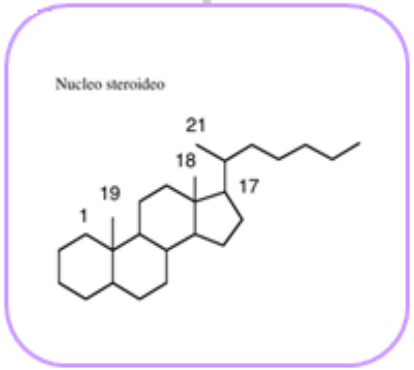
BIBLIOGRAPHIC STUDY

Saponins

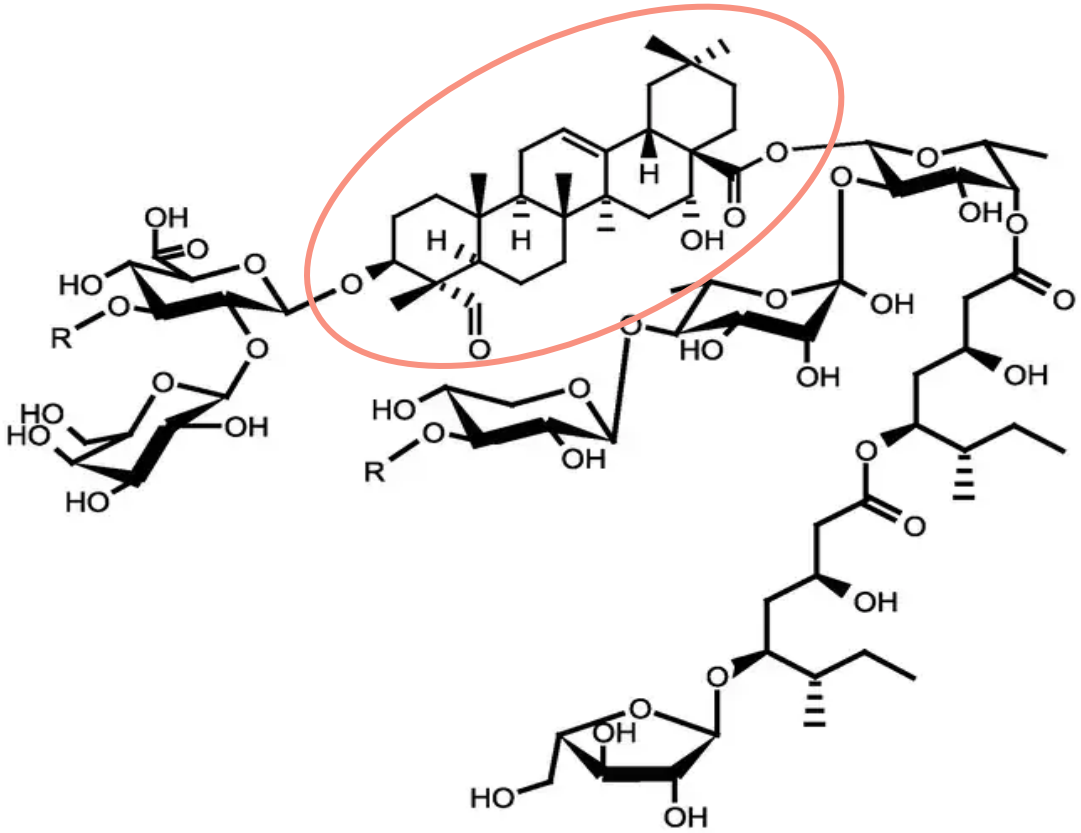
Triterpenoid



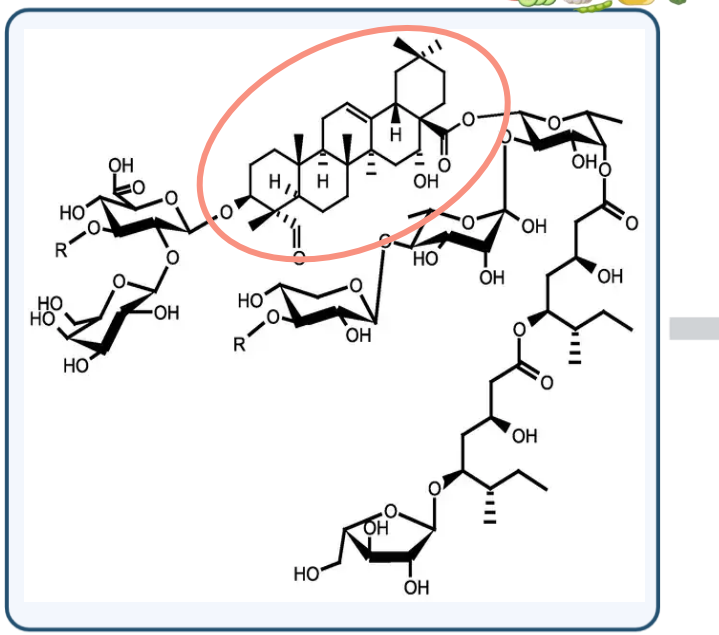
Steroidal



Aglycone



Saponins



Chemical properties

- Aglycone responsible for biological activities
- Variable type and numbers of sugars
- Instability of the aglycone during transit: protect it to ensure it reaches the site of action unaltered

Biological impact

- Antiviral, antimicrobial, anti-inflammatory
- Helps nutrient absorption in poultry
- Helps to reduce methane production in cows

Introduction

BIBLIOGRAPHIC STUDY

- **Saponins**
  - **Triterpenoid**
    - Nucleo triterpenico
  - **Steroidal**
    - Nucleo steroideo

Plant containing saponins

Dryer

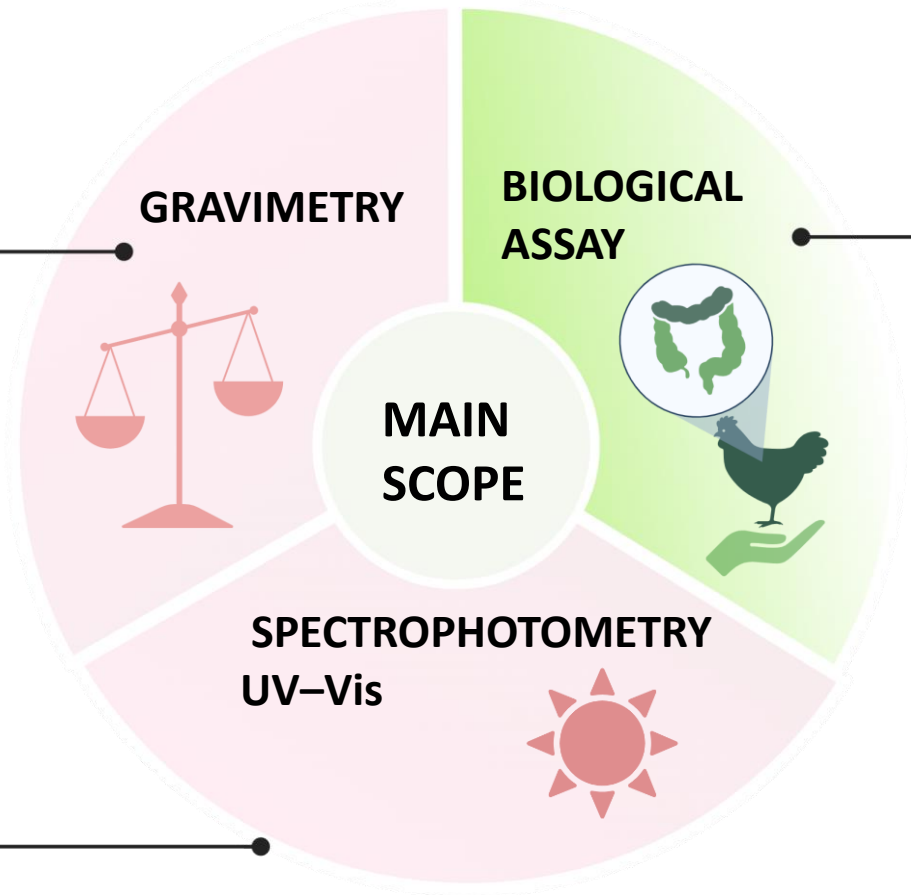
Natural extract rich in saponins (powder)

Natural extract rich in saponins microencapsulated

AIM

1. To develop a method for quantifying saponins
2. Evaluation of the biological activity of saponins

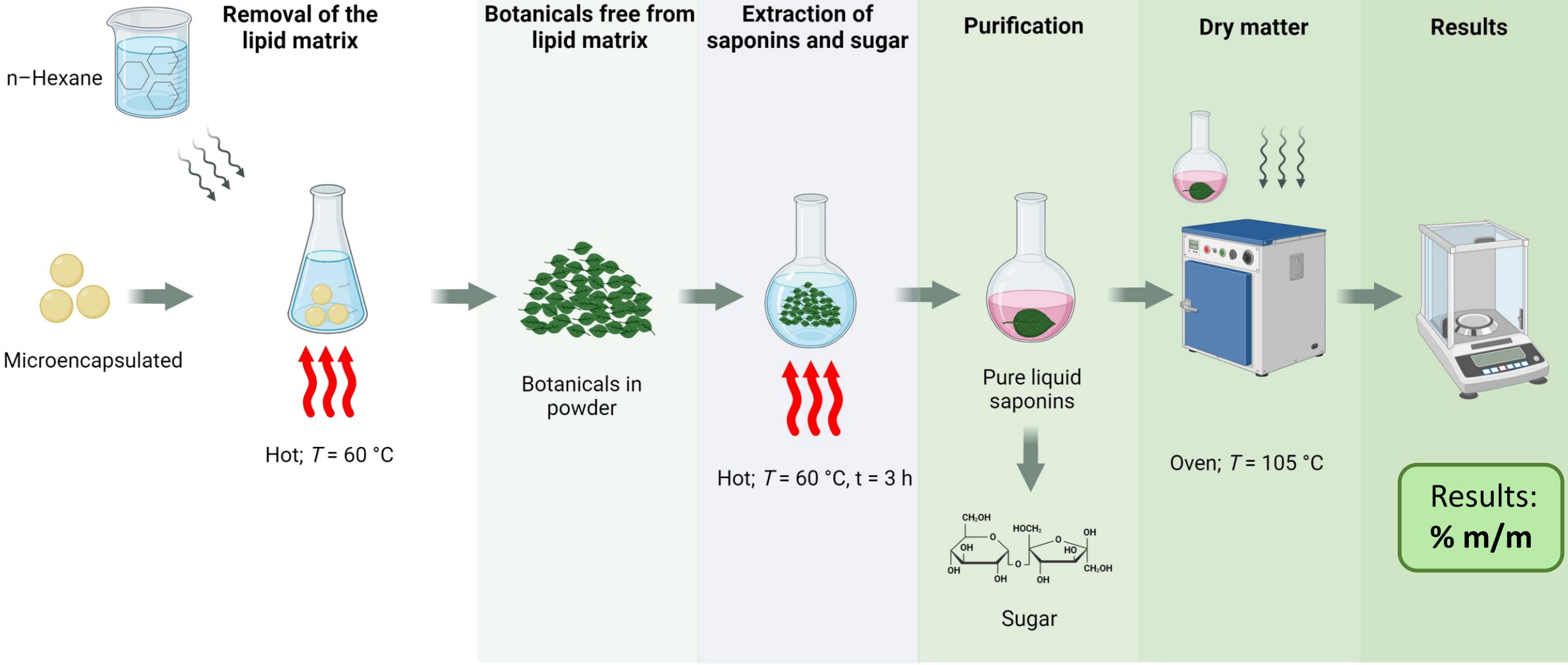
Quantification method for microencapsulated saponins: simple, rapid, and **applicable across different labs**. The chosen lipid matrix is due to a **lack of existing methods in the literature**



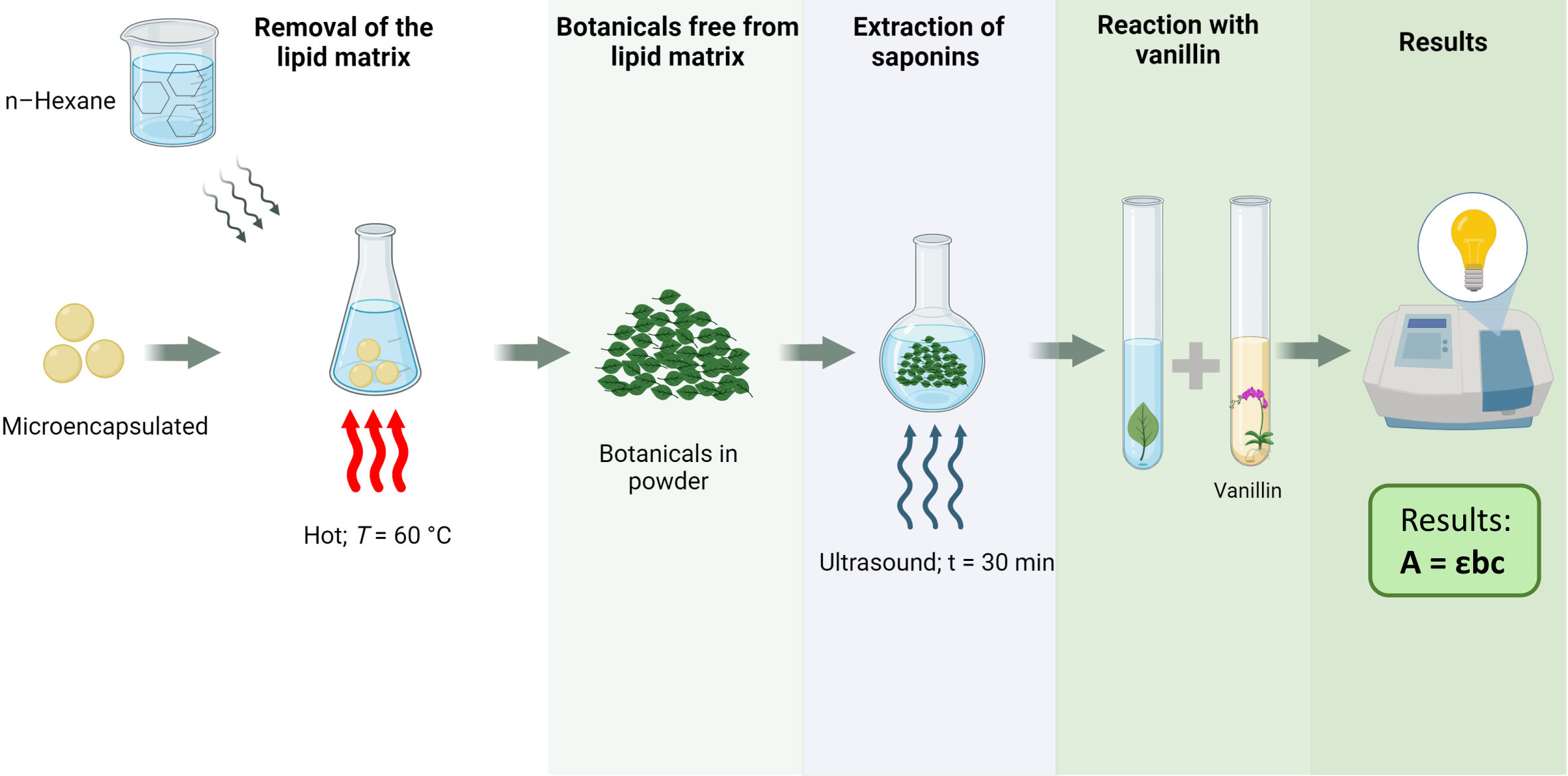
study the antimicrobial effect of saponins on chicken pathogens

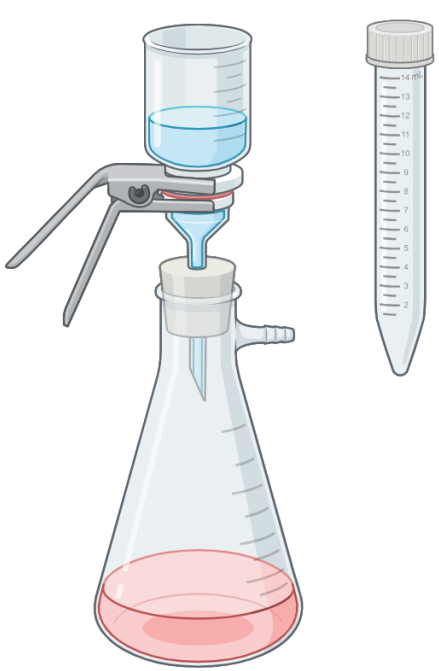
Quantification method for microencapsulated saponins: simple, rapid, and **applicable across different labs**. The chosen lipid matrix is due to a **lack of existing methods in the literature**

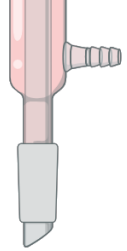
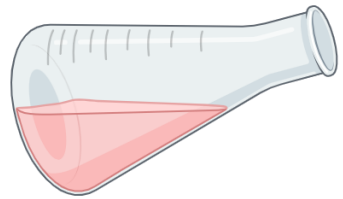
First study:
analytical methodologies


Removal of the lipid matrix
Botanicals free from lipid matrix
Extraction of saponins and sugar
Purification
Dry matter
Results
n−Hexane
Microencapsulated
Hot; T = 60 °C
Botanicals in powder
Hot; T = 60 °C, t = 3 h
Pure liquid saponins
Sugar
Oven; T = 105 °C
Results:
% m/m

Spectophotomery Uv–Vis

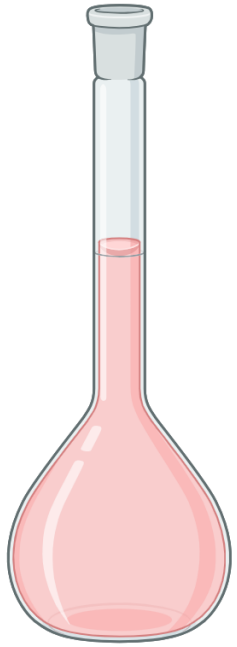
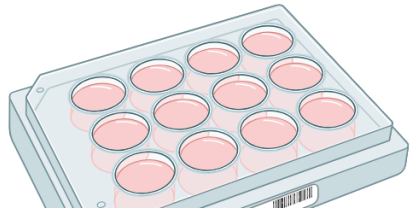


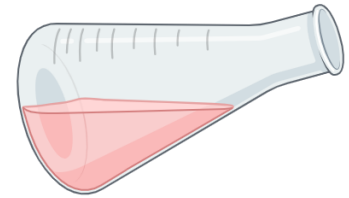





First study: results

	Saponins percentage: Theoretical	Saponins percentage: Experimental	Standard deviation	Pro
Gravimetry	0.029 %	0.026 % (recovery = 88.45 %)	0.13	• Simple, rapid, and robust method • No method reported in the literature
Spectrophotometry UV–Vis	1.47 %	1.46 % (recovery = 99.57 %)	0.10	• Simple, rapid, and robust method • No method reported in the literature

Second study:
biological assay

Biological study

Plant collection and drying

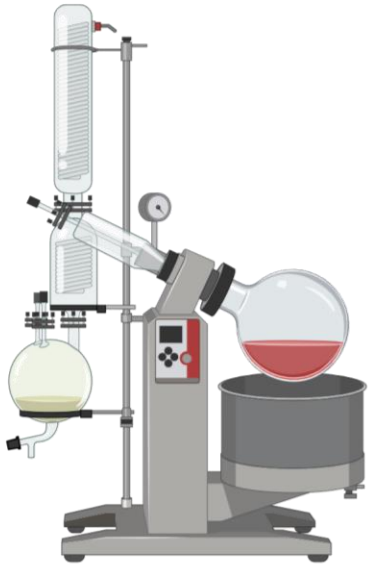
Grinding

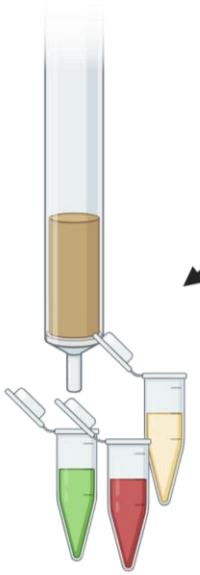
Plant powder

Hot extraction

Filtration

Rotary evaporation

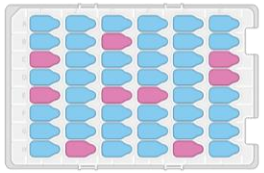




Different fraction collected

Chromatography

Biological assay

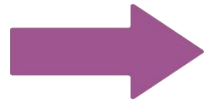


Three different solvents are tested:

- MeOH 100 %
- MeOH 50 %
- H_2O

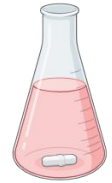
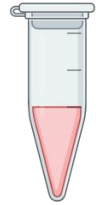


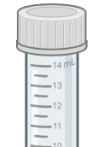

Second study: results



In progress






Future steps

Future steps

Saponins

- **Gravimetry**
  - Recovery : 88.45 %
    - *Std. Dev* : 0.13
- **Uv–Vis**
  - Recovery : 99.57 %
    - *Std. Dev* : 0.10
- **Biological assay**
  - In progress

Future steps

- Biological assay
- Comparison of the predictied biological activity with the theoretical one
- Characterization of single saponins with MS–TOF

Thanks for the attention