“Development of a sustained release, high absorption and stable system based on microencapsulated green propolis standardized extract (EPP-AF®) – i-CAPs”

APIS FLORA®

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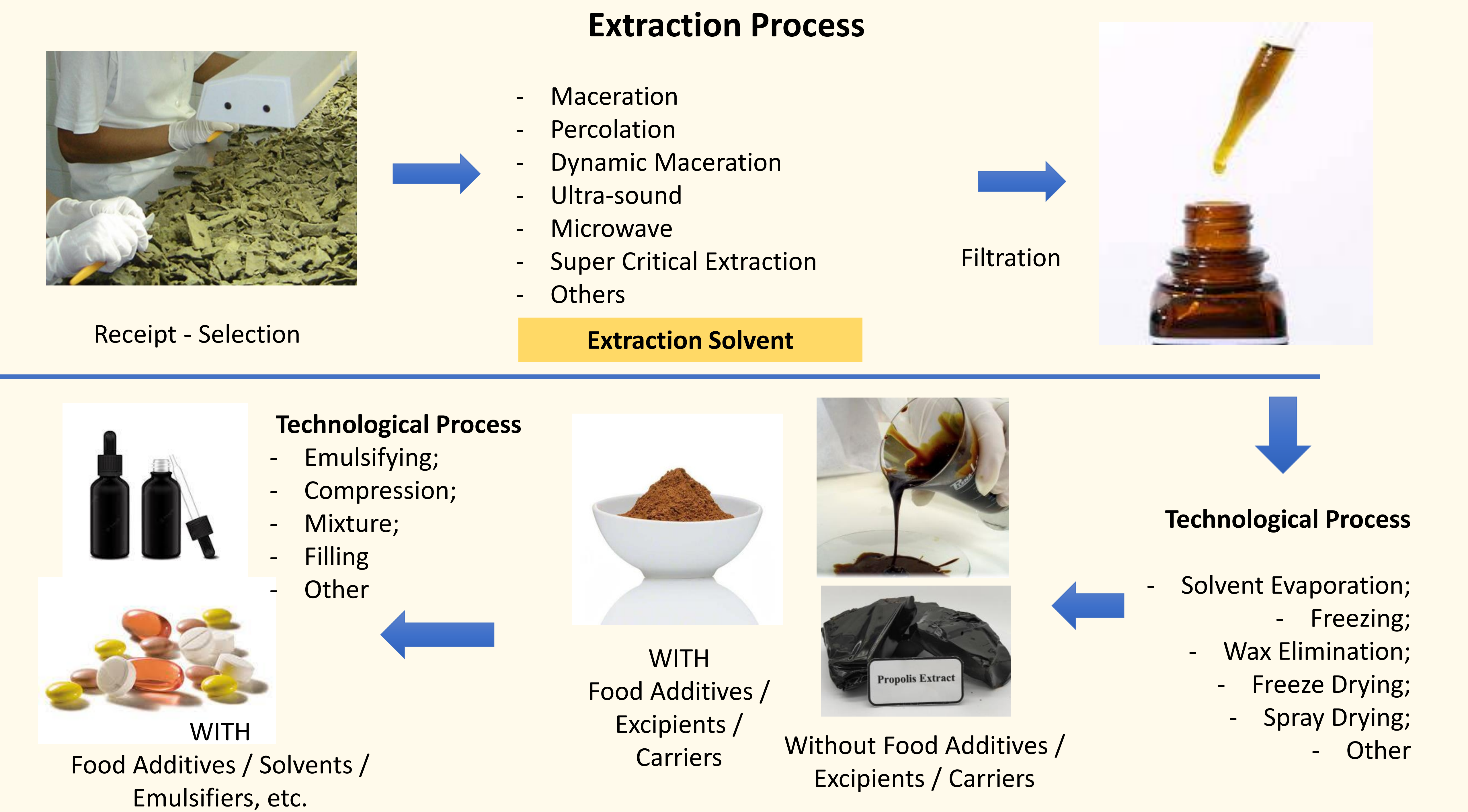
Propolis Raw Material

Propolis Extract - Liquid

Propolis Extract – Semi-Solid

Propolis Extract – Powder



Extraction Process
Receipt - Selection
- Maceration
- Percolation
- Dynamic Maceration
- Ultra-sound
- Microwave
- Super Critical Extraction
- Others
Extraction Solvent
Filtration
Technological Process
- Solvent Evaporation;
- Freezing;
- Wax Elimination;
- Freeze Drying;
- Spray Drying;
- Other
Propolis Extract
Without Food Additives / Excipients / Carriers
WITH Food Additives / Excipients / Carriers
Technological Process
- Emulsifying;
- Compression;
- Mixture;
- Filling
- Other
WITH Food Additives / Solvents / Emulsifiers, etc.

PROPOLIS extract

Propolis possess several functional activities
Alcoholic Extract is the most Traditional
Strong smell, taste and collor
Ethanol is undesirable by Consumers

Microencapsulation

Protect the actives from Propolis
Mask smell, taste and collor
Suitable for several different presentations
Improve Stability
Increase Absorption

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BRAZILIAN PROPOLIS EPP-AF

BRAZILIAN PROPOLIS

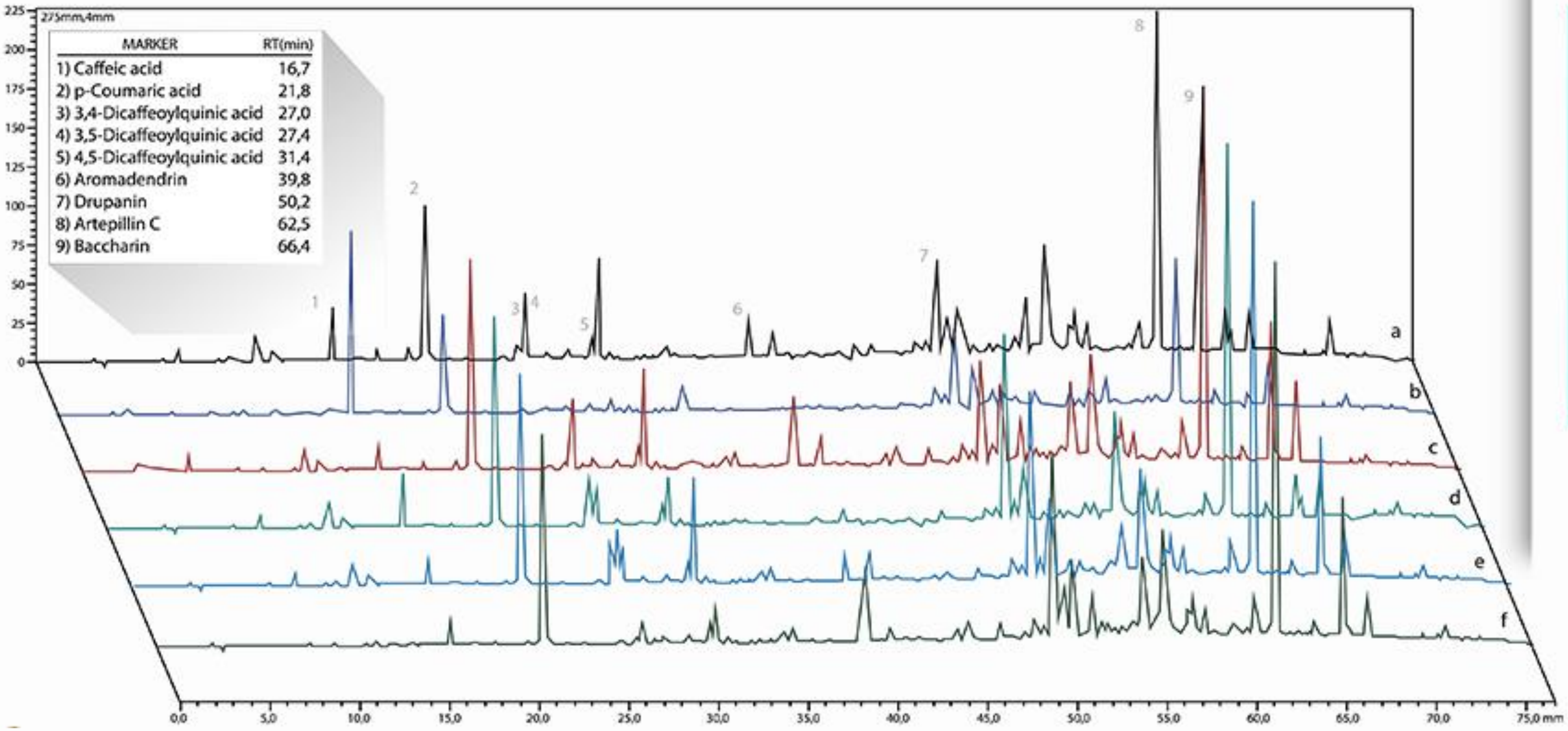
Bees & Biodiversity

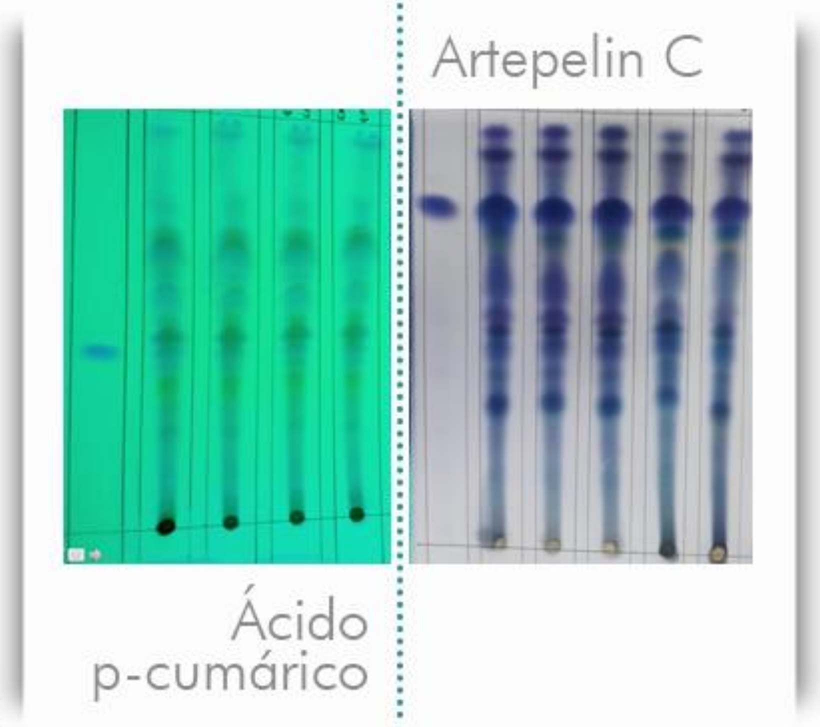
Bee *(Apis mellifera africanized)*

“In natura” propolis extracted from Salatino et al. 2011.

EPP-AF®

Batch to batch reproducibility





METHODOLOGY

METHODOLOGY

Development & Characterization

01

Propolis Microcapsules Development

02

Chemical and Physical Characterization

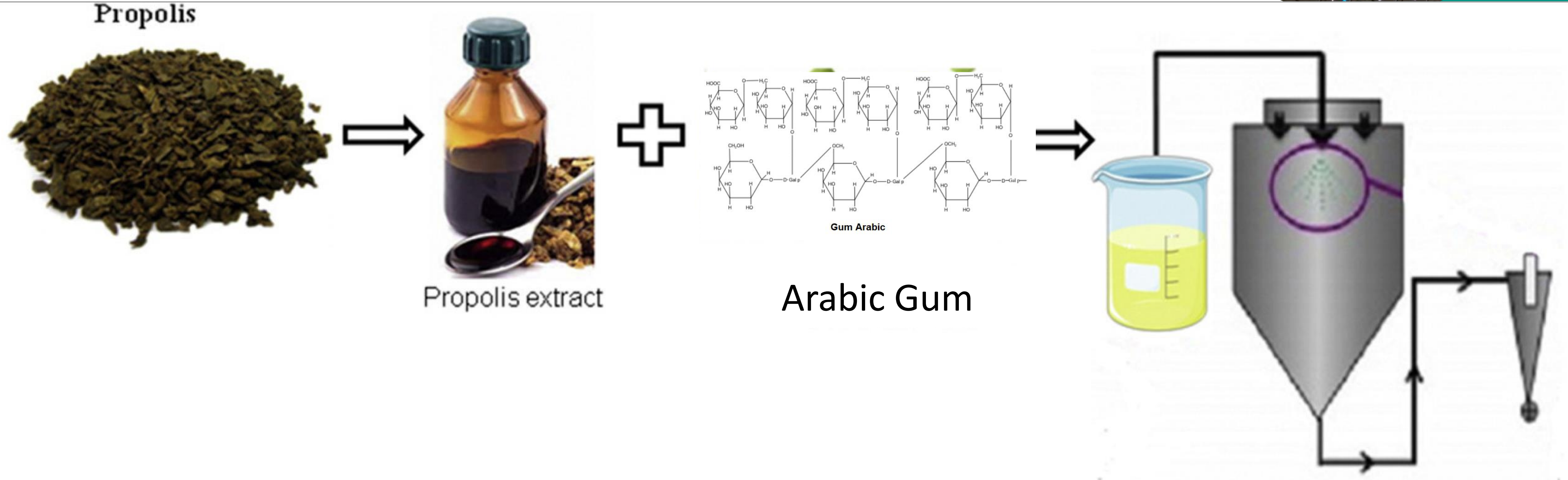
03

"In vitro" Safety and Efficacy Evaluation

EPP-AF(R) MICROCAPSULES

Development & Characterization

- EPP-AF(R) raw material *blend*
- EPP-AF Alcoholic Extraction and Emulsion Preparation with Arabic Gum;
- Dryness using Spray Dryer Technology (Marquiafável et al. 2015)

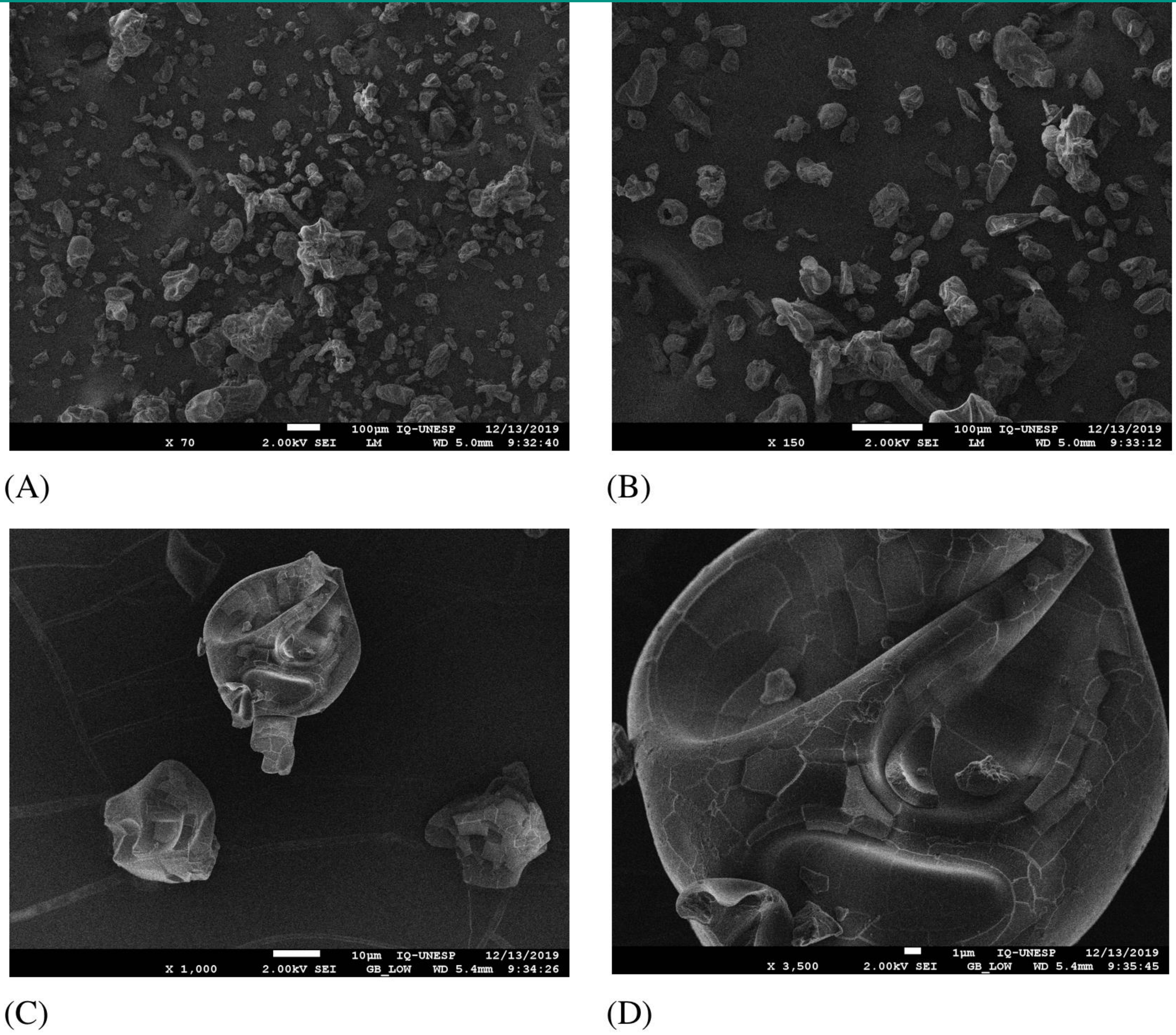


RESULTS

01. Chemical and Physical Characterization

02. *In vitro* Biological Properties

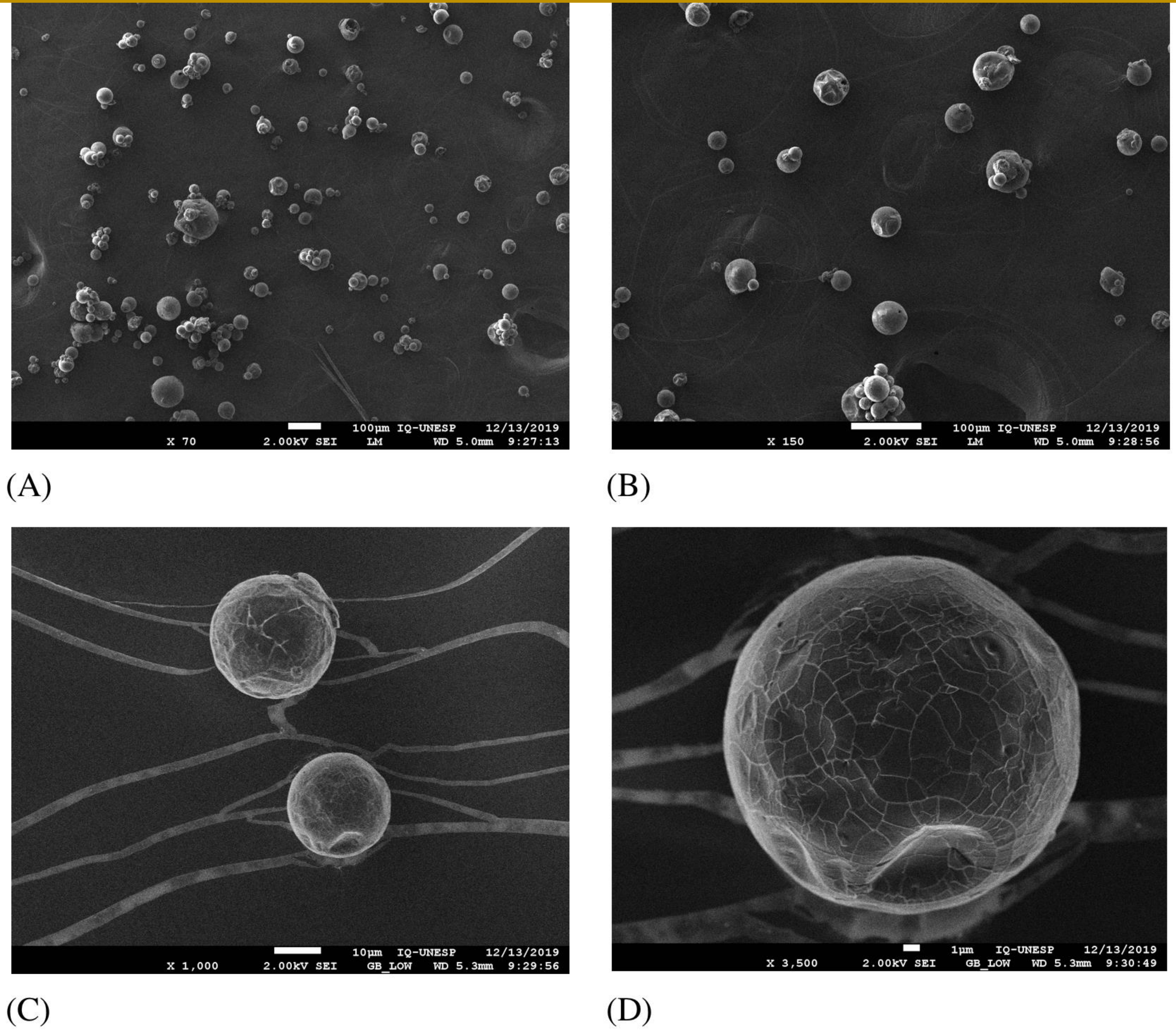
MEV ENCAPSULANT



Photomicrographs for Arabic gum (A) Magnified 70x; (B) Magnified 150x. (C) Magnified 1000x e (D) Magnified 3500x.

MEV
Propolis EPP-AF Microcapsules

Photomicrographs for Propolis standardized extract EPP-AF(R) loaded microcapsules (A) Magnified 70x; (B) Magnified 150x. (C) Magnified 1000x e (D) Magnified 3500x.



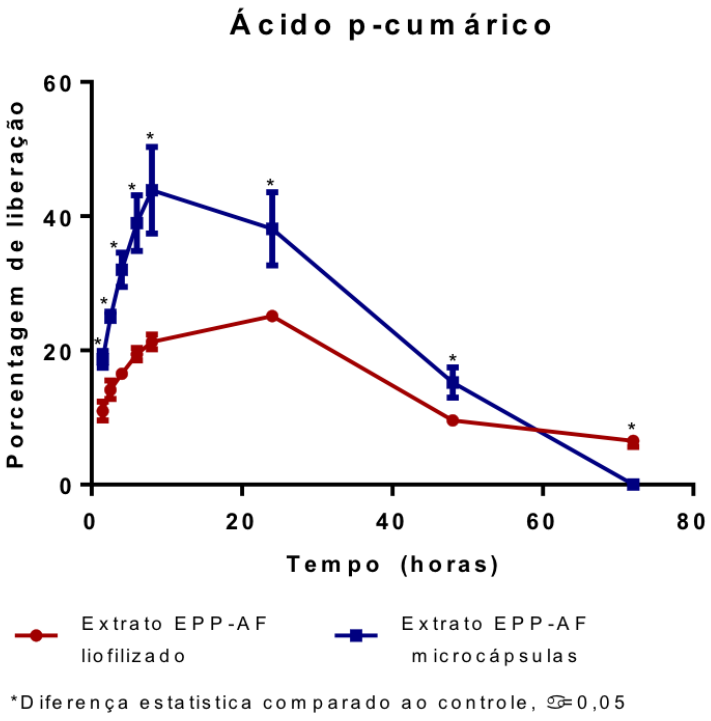
FREE LYOPHILIZED
EPP-AF(R) MICROCAPSULES

ADVANTAGES

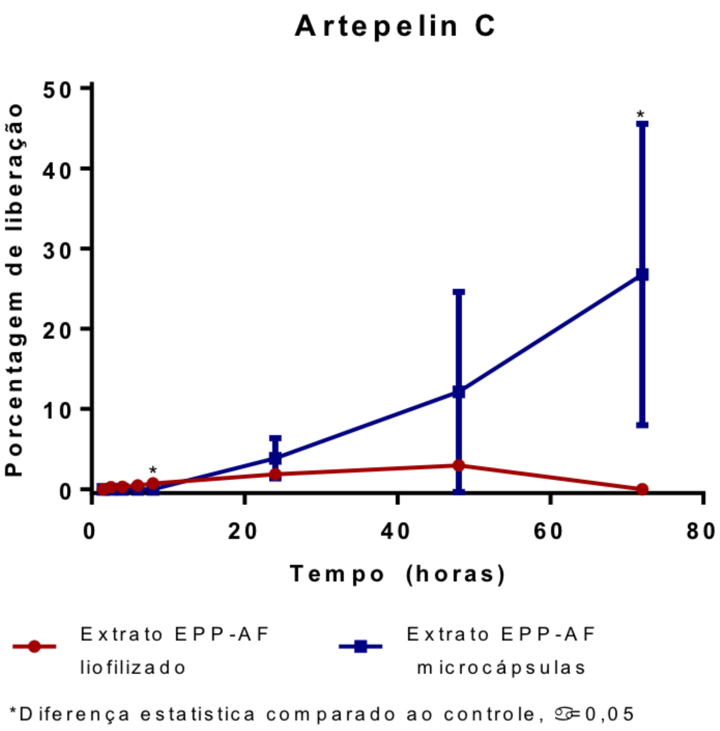
(A) Dispersion of EPP-AF extract free (lyophilized) and
(B) EPP-AF loaded microcapsules, in the concentration equivalent to 0.5% (w/v) of propolis dry matter in water.

%ME = 93.7 ± 0.7

SUSTAINED RELEASED SYSTEM



(A)



(B)

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Bactericidal Minimum Concentration (BMC) – results expressed as propolis dry matter (mg/mL) (n=3) - ***Dispersion of the sample in hydro alcoholic solution 40%v/v***

Samples Samples	Bactericidal Minimum Concentration ± Standard Deviation	
	E .coli	*S. aureus*
EPP-AF free	27.50 ± 0.00	3.44 ± 0.00
EPP-AF loaded Microcapsules	27.50 ± 0.00	3.44 ± 0.00

Bactericidal Minimum Concentration (BMC) – results expressed as propolis dry matter (mg/mL) (n=3) - ***Dispersion of the sample direct in the Müller Hinton Medium***

Samples	Bactericidal Minimum Concentration ± Standard Deviation					
	E .coli	*K. pneumarie*	*P. aruginosa*	*S. aureus*	*S. epidermidis*	MRSA
EPP-AF free	100.00 ± 0.00	91.67 ± 0.00	91.67 ± 0.00	55.00 ± 0.00	55.00 ± 0.99	110.00 ± 0.00
EPP-AF loaded Microcapsules	55.00 ± 0.00	27.50 ± 0.00	33.67 ± 0.00	1.72 ± 0.00	6.88 ± 1.98	5.73 ± 1.98

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ANTIOXIDANT ACTIVITIES

FRAP and DPPH

Antioxidant Activity of Propolis and Propolis microcapsules using FRAP and DPPH methods (n=3, average ± DP).

Samples	FRAP (μmol Fe^{II}/ mg propolis dry matter) ± SD	DPPH (IC50) (μg propolis dry matter /mL) ± SD
EPP-AF$^{(R)}$ Propolis Free	2,873± 0,045	6,500 ± 0,062
EPP-AF$^{(R)}$ Propolis Microcapsules	2,654 ± 0,062*	7,342 ± 0,058*

*Statistical different (p<0,05) – comparison of EPP-AF$^{(R)}$ loaded microcapsules with the correspondent EPP-AF$^{(R)}$ free.

Limitation of the System and the Methodology

Samples were all solubilized in ethanol 70% and US for 30 minutes;

Time of reaction around 60-90 minutes

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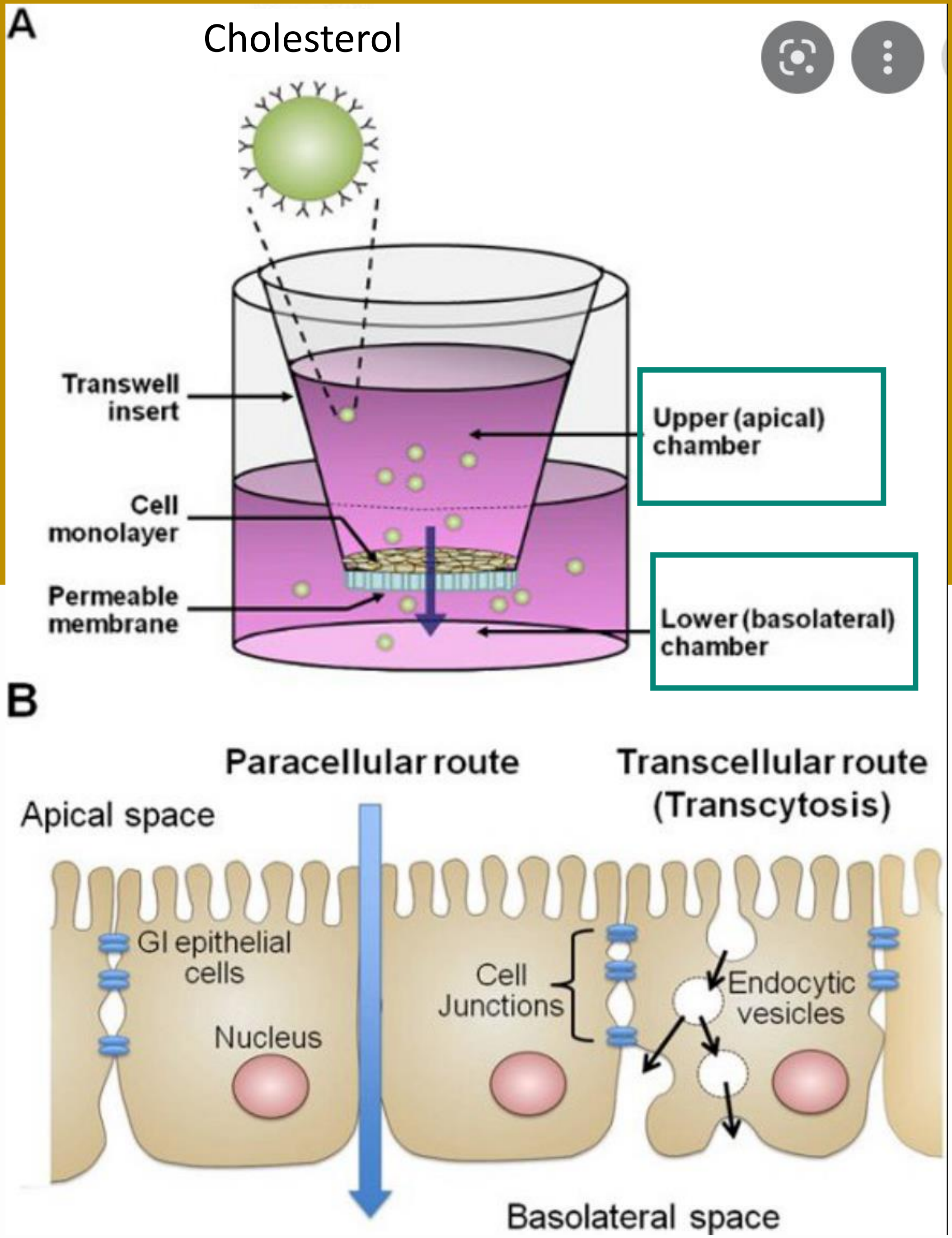
ANTITUMORAL AND ANTI-INFLAMMATORY EFFECTS

Cytotoxic and anti-inflammatory activity of EPP-AF® Propolis free extract and EPP-AF® microcapsules extract (n=3, average ± SD).

	Cytotoxic activity (GI_{50}, µg/mL)				Anti-inflammatory activity (IC_{50}, µg/mL)
	AGS	**Caco2**	**MCF-7**	**PLP2**	**RAW264.7**
EPP-AF® Free	184±2[a]	241±20[a]	296±23[a]	146±11[a]	86±3[a]
EPP-AF® Microcapsules	154±1[b]	117±1[b]	271±25[a]	156±4[a]	59±0.1[b]
Ellipticine	1.23±0.03[c]	1.21±0.02[c]	1.02±0.02[b]	1.4±0.1[b]	-
Dexametasone	-	-	-	-	6.3±0.4[c]

GI_{50} – Concentration that inhibited 50% of the net cell growth; IC_{50} – Sample concentration providing 50% of inhibition of NO production. **Different letters in each row represent statistically significant differences with a significance of 5%.**

HYPOCHOLESTEROLEMIC ACTIVITY



HYPOCHOLESTEROLEMIC ACTIVITY

Hypocholestorolemic activity of EPP-AF® Free extract and EPP-AF® Microcapsules through Caco2 cell monolayers transport assay.

		Cholesterol (µM)	**Cholesterol (%)**
Control	UC	26.86±0.80^{a}	53.7±1,60^{a}
	LC	21.82±1.03^{C}	43.6±2.05^{C}
EPP-AF® Free	UC	35.96±0.37^{b}	71.9±0.75^{b}
	LC	13.29±0.28^{B}	26.6±0.57^{B}
EPP-AF® Microcapsules	UC	39.06±0.40^{c}	78.1±0.79^{C}
	LC	10.10±0.29^{A}	20.2±0.58^{A}

UC – upper compartment; UL – lower compartment. **Different lowercase letters in each row represent statistically significant differences between the upper compartments, while different capital letters in each row represent statistically significant differences between the lower compartments, both of which with a significance of 5%.**

PERMEABILITY STUDIES

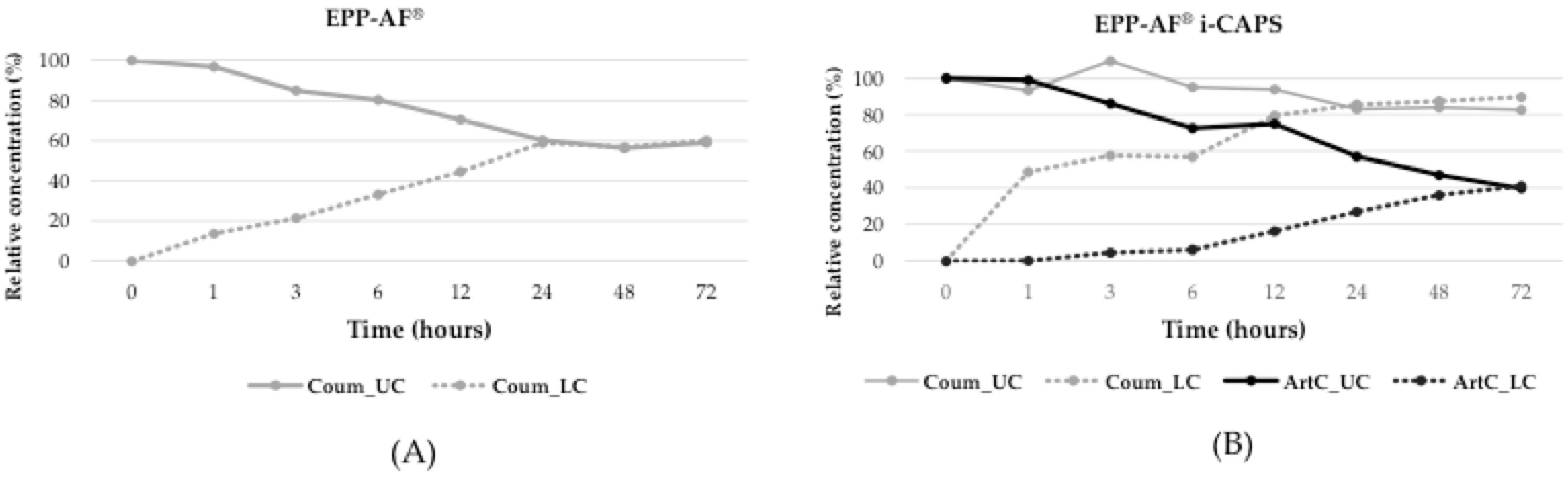


Figure 8 - Permeability Results of EPP-AF® and EPP-AF® i-CAPS through Caco-2 monolayer transport model (n=3, mean ± SD) for the two selected biomarkers *p*-coumaric acid (Coum) and artepillin C (ArtC). UC - UC – upper compartment; LC – lower compartment.

PERMEABILITY STUDIES

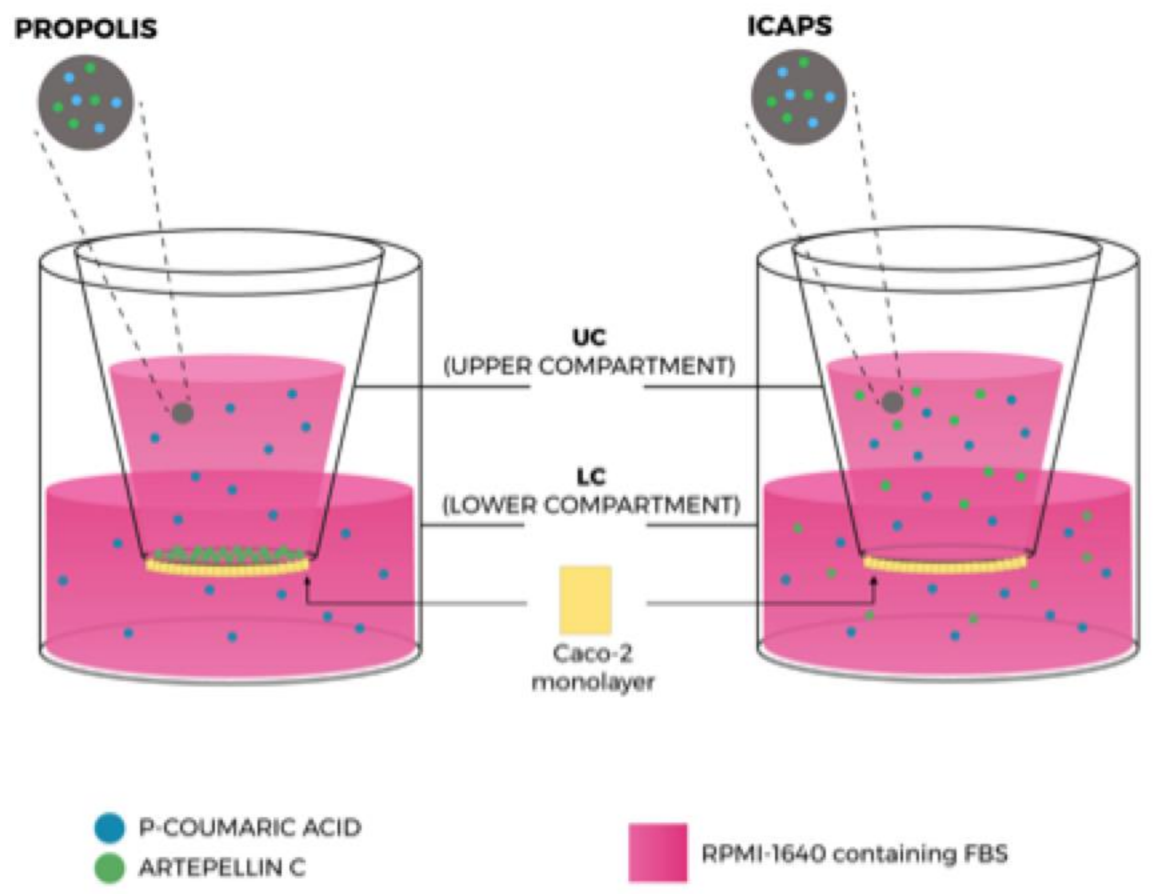


Figure 7 – Illustration of the Permeability Caco-2 monolayer transport model and results with EPP-AF® and EPP-AF® i-CAPS, including for the two selected biomarkers *p*-coumaric acid and artepillin C.

CONCLUSION

EPP-AF(R) Microcapsules succesfully obtained;
Spheric Shape and %ME of 93.7± 0.7;
FIR and TGA behavior of the Arabic Gum;
Both EPP-AF® Free and Microencapsulated offered antimicrobial and antioxidant activities;
Antitumor Effects were Antihypercholesterolemic Effects were better reached by EPP-AF Microcapsule
36 months of Shelf-life
Higher Absorption


ECOCERT
PRÓPO MAX
GÉLULES
IMMUNITÉ
FR-BIO-01
PROPOLIS
VERTE BRUNE BIO
BIOFLAVONOÏDES
ARTÉPILLINE C
40 GÉLULES
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LEHNING
DEPUIS 1935
PRÓPO MAX
GOUTTES IMMUNITÉ
PRÉPARE LES DÉFENSES
PROPOLIS VERTE BIO
BIOFLAVONOÏDES ARTEPILLINE C
PRÓPO MAX
GOUTTES IMMUNITÉ
PRÉPARE LES DÉFENSES
PROPOLIS VERTE BIO
LEHNING
DEPUIS 1935
PRÓPO MAX
GOUTTES IMMUNITÉ
DÉFENSES AFFAIBLIES
PROPOLIS VERTE BRUNE BIO
BIOFLAVONOÏDES ARTEPILLINE C
SANS ALCOOL
30ML
PRÓPO MAX
GOUTTES IMMUNITÉ
DÉFENSES AFFAIBLIES
PROPOLIS VERTE BRUNE BIO
LEHNING
DEPUIS 1935
PRÓPO MAX
SPRAY GORGE
DOUX / GRENADE
PROPOLIS VERTE BRUNE BIO
BIOFLAVONOÏDES ARTEPILLINE C
30ML
PRÓPO MAX
SPRAY GORGE
DOUX / GRENADE
PROPOLIS VERTE BRUNE BIO
LEHNING
DEPUIS 1935
PRÓPO MAX
SPRAY GORGE
FORT / HUILES ESSENTIELLES
PROPOLIS VERTE BRUNE BIO
BIOFLAVONOÏDES ARTEPILLINE C
30ML
PRÓPO MAX
SPRAY GORGE
FORT / HUILES ESSENTIELLES
PROPOLIS VERTE BRUNE BIO
LEHNING
DEPUIS 1935
PRÓPO MAX
SPRAY GORGE
SANS ALCOOL
PROPOLIS VERTE BRUNE BIO
BIOFLAVONOÏDES ARTEPILLINE C
30ML
PRÓPO MAX
SPRAY GORGE
SANS ALCOOL
PROPOLIS VERTE BRUNE BIO

APIS FLORA KIDS

iCaps Super Power

Apis Flora R&D Team

ANDRESA BERRETTA

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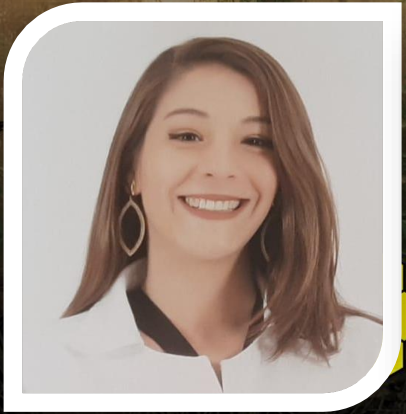
NATHALY ALCAZAR



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FINANCIADORA DE INOVAÇÃO E PESQUISA

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“Propolis International Standard Proposed by ISO – an important regulatory framework for authenticity and quality requirements of the international propolis market”

Andresa A. Berretta[1] & Miguel Vilas-Boas[2]

[1]Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil; ABEMEL (Brazilian Association Honey Exporters), Brazil;
[2]Instituto Politécnico de Bragança,Bragança, Portugal.

Mainly types of Propolis

Brown – Populus spp.
Green – Baccharis dracunculifolia
Red – Dalbergia and Clusia spp.

Brazilian Red propolis — Poplar propolis — Brazilian green propolis

https://healthywithhoney.com/composition-of-propolis/

Opportunities

The current global supply of propolis is estimated to be 700-800 ton/year, with an market value of US$ 700 million/year, forecasted to reach US$ 829 million by 2027.

Challenges

Lack of Standardized Methodologies and Technical Specification
Lack of Regulation in most of the countries

De Oliveira et al. 2021

G. W. Fernandes et al. (eds.), *Baccharis*,
https://doi.org/10.1007/978-3-030-83511-8_23

Bee Propolis Specification – ISO-NP 24.381

This project was constructed by experts from 17 countries around the globe.

TThe document specified quality requirements, analytical methods, packaging, marking, labelling, as well as storage and transportation conditions for propolis produced by *Apis mellifera* bees.

De Oliveira et al. 2021

G. W. Fernandes et al. (eds.), *Baccharis*,
https://doi.org/10.1007/978-3-030-83511-8_23

Bee Propolis Specification – ISO-NP 24.381

SAMPLES

- Sample 1 – Poplar type (China)
- Sample 2 – Poplar type (Portugal)
- Sample 3 – Red type (Brazil)
- Sample 4 – Poplar type (Romania)
- Sample 5 – Green type (Brazil)
- Sample 6 – Poplar type (Italy)
- Sample 7 – Poplar type (Turkey)

Laboratories Involved: 14 applied 13 participated*

Members of Inter Laboratory Proficiency**

- Belgium – CARI ASBL
- Brazil – APIS FLORA
- Brazil – CNPLab
- China – Jiangsu Collaborative Innovation Centre of Chinese Medical Industrialization
- China – Joint Laboratory of Propolis Professional Committee of the China Bee Products Association
- China - Technology Center of Qinhuangdao Customs
- France - CTCPA
- German – Intertek
- Italy – Laboratory of Biochemistry and Glycobiology of Department of Life Sciences, UNIMORE
- Portugal – Institute Polytechnic of Bragança
- Romania – Laboratory for Quality Control of Bee Products and Bee Diseases
- Spain – Laboratorios Apinevada SL
- Turkey – Altiparmak Gida Sanayi Ve Ticaret A.S.
- Turkey – Beeo Propolis Research and Development Center

*All were approved for be part. The laboratory declined for internal problems;
**Laboratories identified by alphabetic order. The identification of the laboratories were not done neither, in the technical report or in the presentation in order to avoid any kind discomfort.

Methodologies Evaluated – Annex A - K

Recommended – 11 Parameters (Annex A – K)

• Ethanol Extractable (dry matter);
• Loss on Drying;
• Ash Content;
• Wax Content;
• Total Phenolic Content;
• Total Flavonoid Content (total flavone/flavonol Content);
• Total Flavonoid Content (Polyamide method);
• Chemical Characterization and Quantification of
  • Polyphenols in Poplar Propolis (LC/MS);
  • Polyphenols in Green Propolis (HPLC/DAD);
  • Polyphenols in Red Propolis (HPLC/DAD and HPLC/MS);
• Antioxidant Capacity (DPPH).

Time Line of Inter Laboratory Project

2021, Aug/Sept

ILCP Proposal

Laboratories Application

Donators of Samples

2021, October

BIPEA received and Delivered the Samples

2021, November

Laboratories received the samples and Started the execution of the protocols;

Except China because Customs troubles.

2021, Dec - Deadline

Deadline for Closing the Results and Up-load in the BIPEA plataform, 2021, Dec 20th (*)

First Meeting to discuss methodologies doubts.

2022, Jan - Deadline

Second Meeting to clarify methodologies doubts, with adjustments in the WD draft text;

Deadline to upload results at BIPEA platform

2022, Feb/Mar – Closing

Technical Report from BIPEA scheduled for Feb, 14th;

Technical Report received on Feb, 24th;

Closing Meeting, March 3-4th.

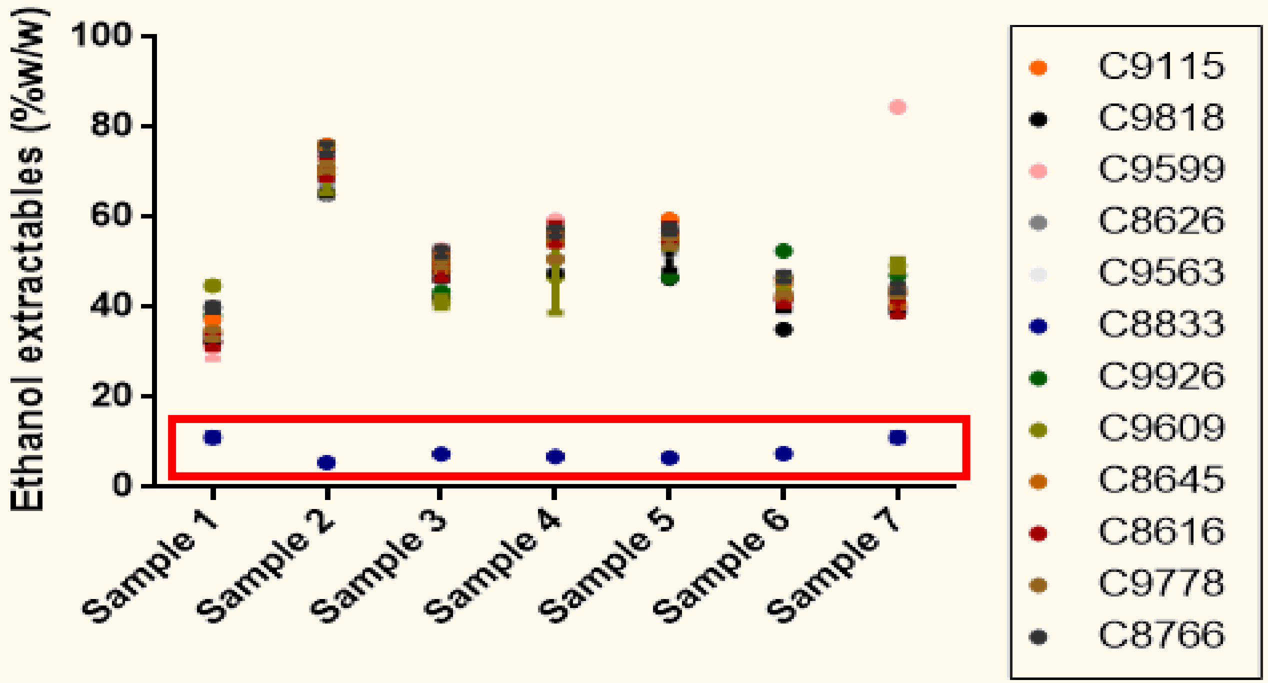
(*) With the consensus of the members, the deadline line to present the results to BIPEA was postponed in 30 days, since the 3 Chinese labs not received the samples on time. Considering the method of Annex G, the lack of these 3 laboratories could potentially affect substantially the results.

Results

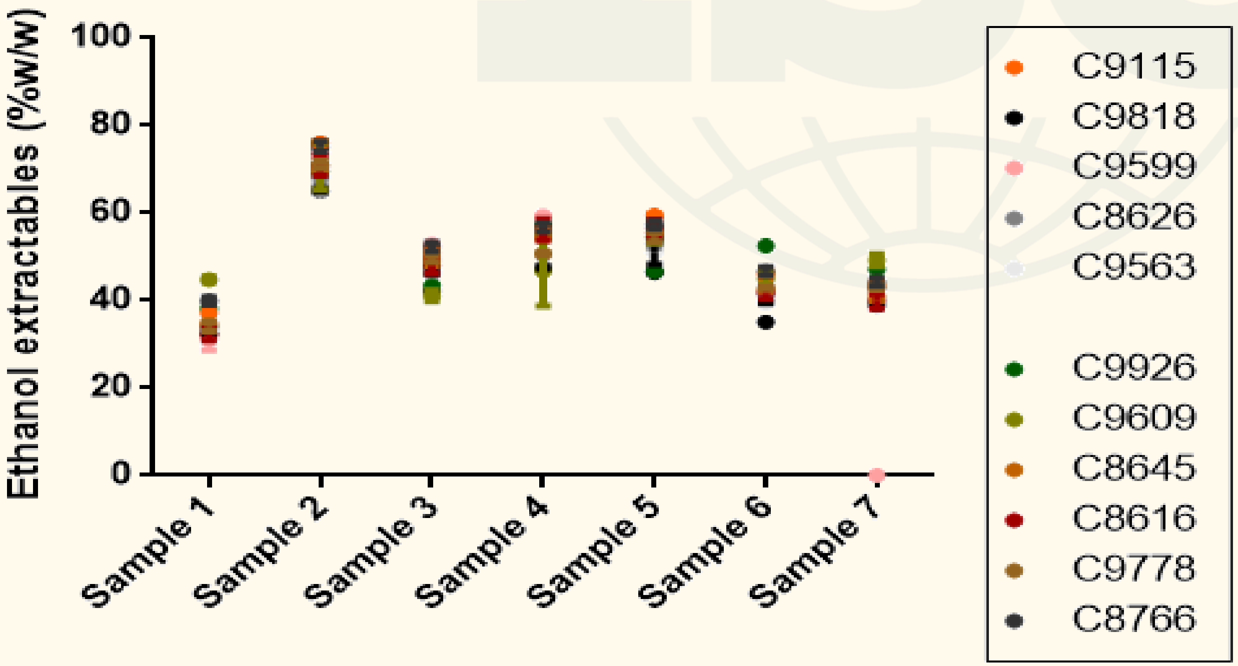
Bee Propolis Specification – ISO-NP 24.381

Inter Laboratory Proficiency Results

Annex A – Ethanol Extractable

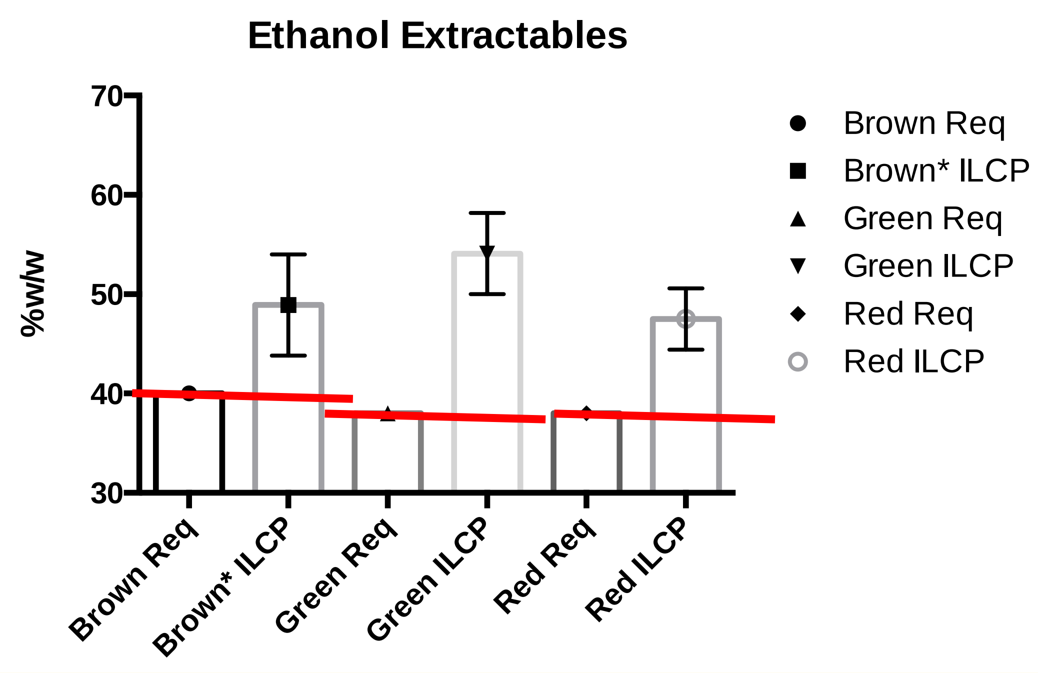


Full Results

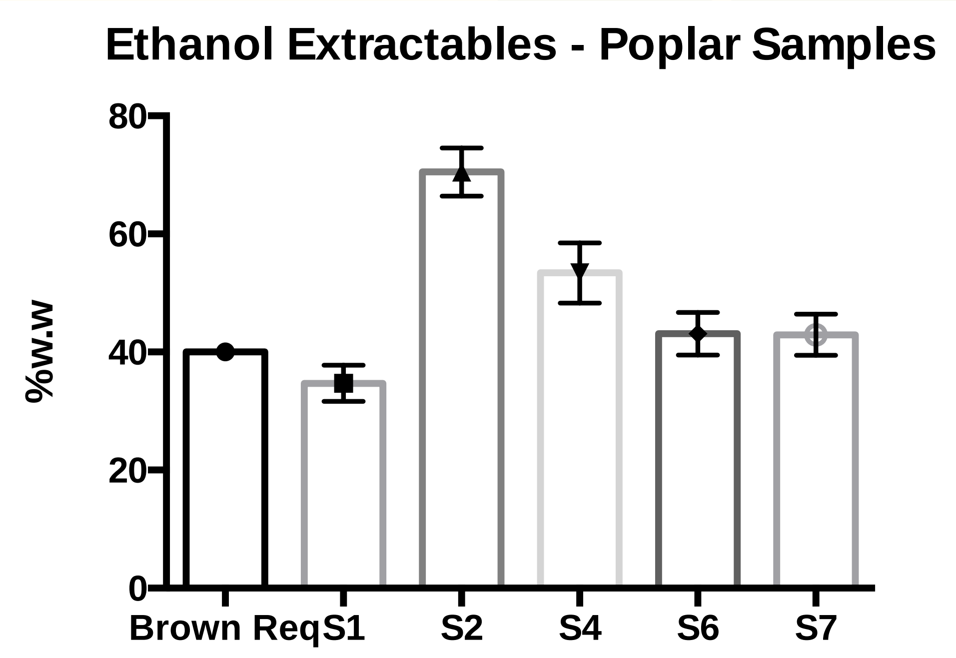


Excluding Outliers

Annex A – Ethanol Extractable – Average/SD



Presentation of the Results [averages (robust SD)] x Limits previously established



Presentation of the Results (Poplar) [averages (robust SD)] x Limits previously established

Table 4.2 Requirements x Results

Parameters and Limits specified in the WD
Average and Standard Deviation of the Results (S)

Summary – Inter laboratorial Results x Table Requirements

	Brow Propolis	Proficiency Samples		Green Propolis	Proficiency Samples		Red Propolis	Proficiency Samples	
Method	Req	Mean	S (Xpt)	Req	Mean	S (Xpt)	Req	Mean	S (Xpt)
Ethanol extractables (as dry matter) min	**40**	48,9	5,1	**38**	54,1	4,1	**38**	47,5	3,1
Loss of drying (%w/w) - max	**8**	2,8	0,7	**8**	9,5	0,9	**8**	4,2	0,2
Ash Content (%w/w) - max	**5**	0,9	0,1	**5,4**	3,2	0,3	**5,4**	1,1	0,1
Wax Content (%w/w) - max	**50**	46,0	4,2	**27,2**	22,9	3,8	**38**	59,0	3,0
Total phenolic compounds (Folin) – min (%w/w) as gallic acid	**10**	13,2	4,5	**7,6**	10,9	2,4	**6,5**	7,9	2,4
Total phenolic compounds (Folin) – min (%w/w) as galangin	**20**	24,1	5,3	**15,2**	19,5	1,9	**13**	14,5	3,6
Total Flavonoids (or flavone/flavonol) - %w/w (min) as quercetin	**3**	5,0	1,0	**1,1**	3,3	0,6	**0,5**	4,4	0,6
Total flavonoids (Polyamide Method) - % w/w (min) as rutin	**8**	13,4	1,8	**2,2**	6,5	0,7	**1,1**	5,5	1,5
Total antioxidant capacity (DPPH) – EC50 – maximum value (ug/mL)	**25**	12,3	2,3	**40**	14,0	2,5	**50**	20,7	2,2

New Proposal for the Table of Requirements

Table 1 — Physical and chemical requirements for bee propolis and test methods for each characteristic

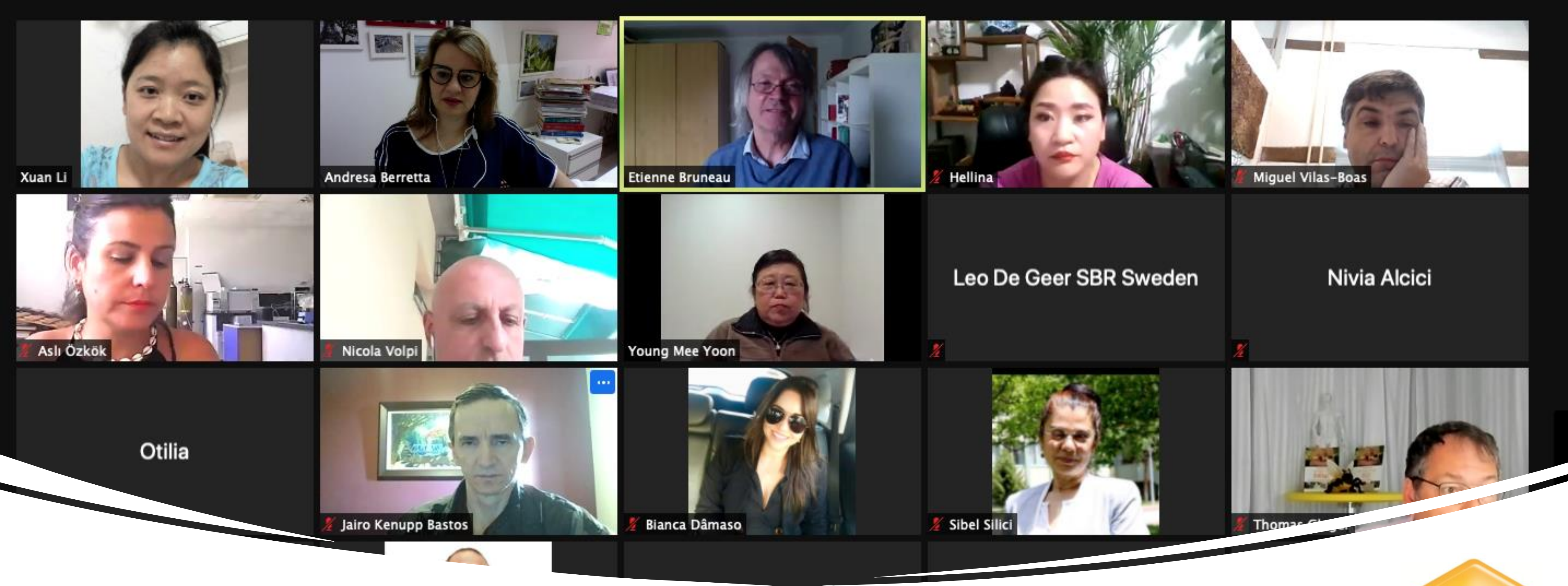
Characteristic	Min. or max.	Requirements (on a dry basis)			Test method
		Brown propolis (4.1.1)	Green propolis (4.1.2)	Red propolis (4.1.3)	
Ethanol extractables of propolis (as dry matter), in % mass fraction	min.	30,0	30,0	30,0	Annex A
Loss of drying, in % mass fraction	max.	10,0	10,0	10,0	Annex B
Ash content, in % mass fraction	max.	5,0	5,0	5,0	Annex C
Petroleum ether extractables of propolis (as dry matter), in % mass fraction	max.	65,0	30,0	60,0	Annex D
Total phenolic compounds (Folin), in % mass fraction, as gallic acid[a]	min.	10,0	7,0	7,0	Annex E
Total phenolic compounds (Folin), in % mass fraction, as galangin[a]	min.	17,0	12,0	12,0	Annex E
Total flavonoids ($AlCl_3$), in % mass fraction, as quercetin	min.	3,0	1,0	0,5	Annex F
Total flavonoids (polyamide method), in % mass fraction, as rutin	min.	6,0	2,0	1,0	Annex G
Total polyphenolics by high-performance liquid chromatography (HPLC) (poplar, green and red propolis)	—	Presence of: apigenin, caffeic acid, CAPE, p-coumaric acid, chrysin, ferulic acid, galangin, pinobanksin and pinocembrin	Presence of: caffeic acid, p-coumaric acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, cinnamic acid, drupanin, artepellin C and baccharin	Presence of: calycosin, isoliquiritigenin, formononetin and biochanin	Annex H Annex I Annex J
Total antioxidant capacity (DPPH) – EC50, in µg/ml	max.	25,0	40,0	50,0	Annex K

[a] Total phenolic can be expressed as gallic acid or galangin equivalents. To convert from gallic acid to galangin, multiply the value obtained using the conversion factor of 1,7. To convert from galangin to gallic acid, multiply the value by 0,59.

Project Leader
Prof. Zetian Lv

General Secretary
TC34/SC19
Bee Products
Mrs. Xuan Li

Convenor
Dra. Andresa Berretta



Thank You !

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- atian580@163.com

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