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STUDIUM GENERALE CIVITATIS PERUSII • A.D. MCCCVIII •

III SIMPÓSIO INTERNACIONAL EM INVESTIGAÇÕES QUÍMICO-FARMACÊUTICAS

I ENCONTRO IBERO-AMERICANO DE PLANTAS MEDICINAIS DR. MAHABIR GUPTA

I CONGRESSO LUSO-BRASILEIRO DE CIÊNCIAS E TECNOLOGIAS EM SAÚDE

2022

UNIVALI

BIOWASTE-*to*-BIOFUTUTURE:

A challenging approach for new sustainable medicines and cosmetics

WASTE or OPPORTUNITY??

Prof.Maurizio Ricci

Key-points

1

- The increase of global population, raises several concerns about the global consumption of biomass, fossil fuels, metals, and minerals

2

- 840 million people suffering from hunger and malnutrition
- while others are dealing with food over-consumption and **related diseases**, together with **INCREASING FOOD WASTE PRODUCTION**

3

- Global food lost and waste has a cost of USD 2.6 trillion annually, which is enough to feed the millions of undernourished people in the world

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RETHINK.....

This worring scenatio calls us to an act of social responsibility!!!!

«rethinking economic models»

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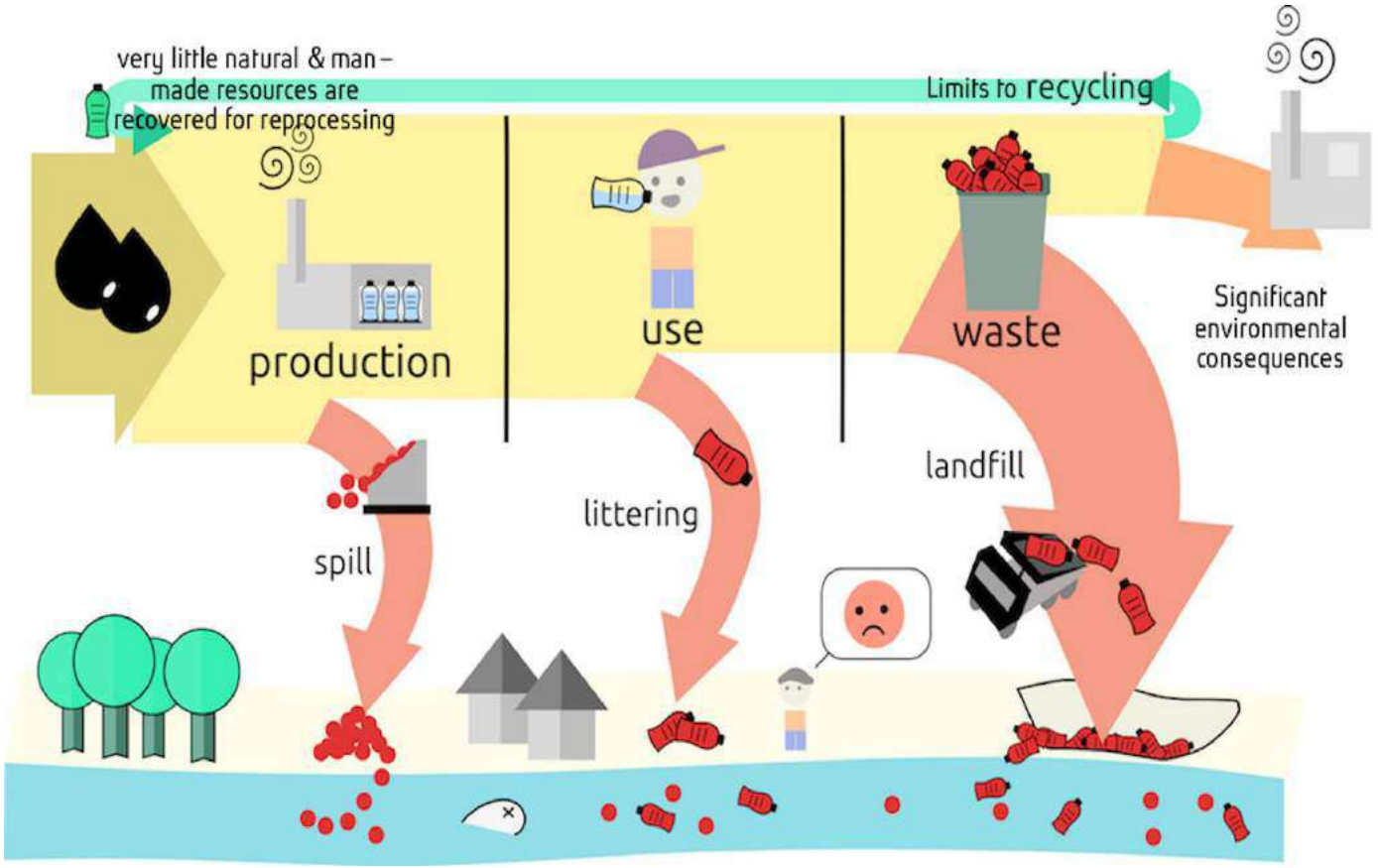
From Linear to Circular economy

Food is similar to other products that people consume in a **LINEAR MANNER**, with little recycling of unused or discarded materials taxing on resources and the environment.



From Linear to Circular economy

"circular economy"

New model based principally on
- **Reducing**
- **Reuse**
- **Recycling**

create a **closed-loop system**

To minimize
- the use of resource
- the generation of waste

- To use its content for making different and safe goods, incentivizing and increasing the food systems' transformation

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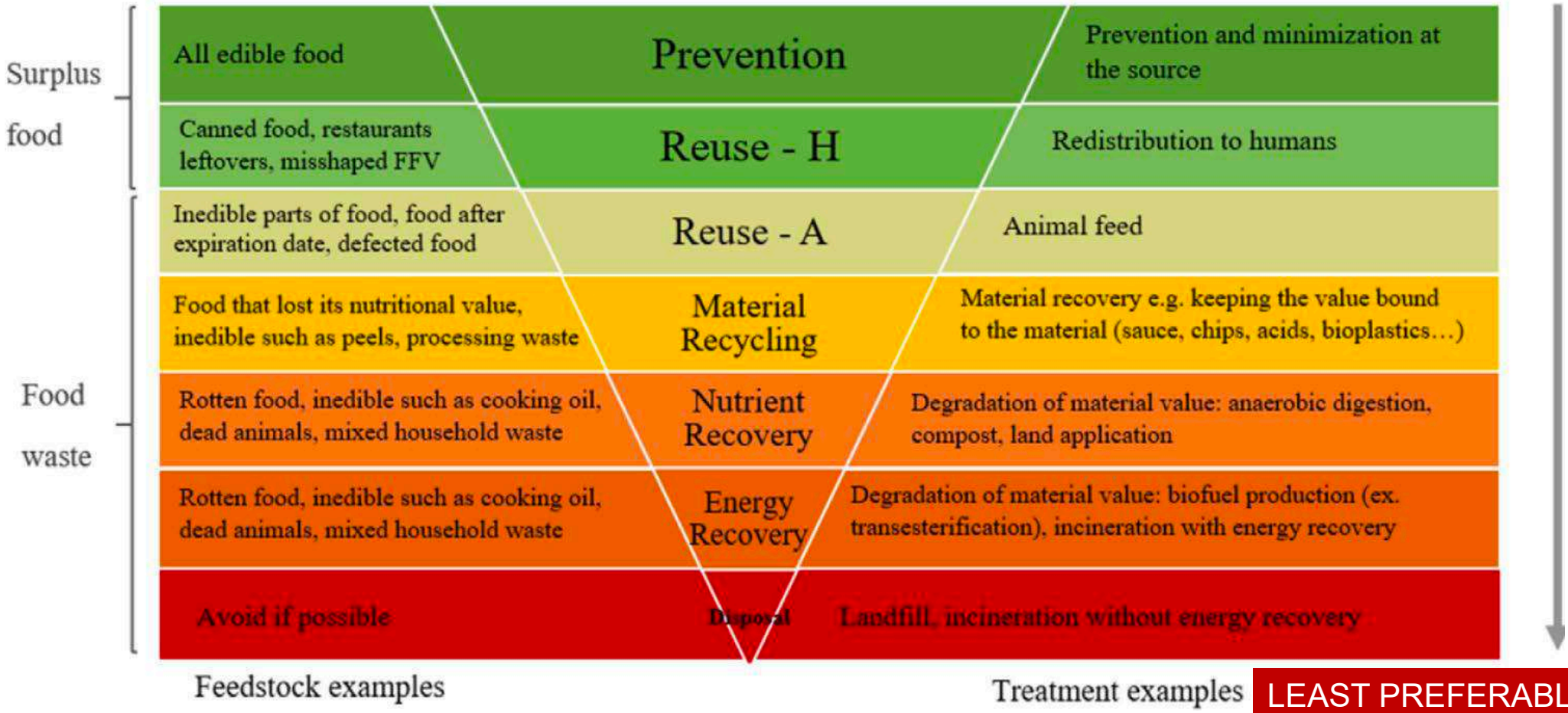
What we ca do in Academia?

New Paradigm

"waste hierarchy"

MOST PREFERABLE



LEAST PREFERABLE

New Paradigm

RECYCLE: “any recovery operation by which waste materials are transformed into products, materials or substances for original or other purposes”.

REUSE: "any operation by which products or components that are not waste are reused for the same purpose for which they were designed"

REDUCE: "minimize the contribution of **primary energy**, raw materials and waste by improving efficiency in production and consumption processes"

Aim of the Project

Optimizing the use of local natural resources, focusing our attention on wastes of some typical italian plants in particular :

ONION

SAFFRON

- Develop new sustainable products, in the field of medicines and cosmetics

Transform waste into a potential source of wealth

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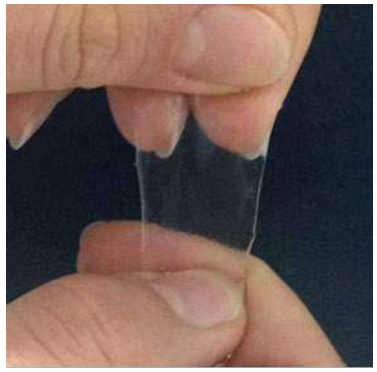
Wounds treatment → Circular health

NATURAL SOURCES

FORMULATION

Onion skins →

Bioadhesive film



Saffron petals →

Hydrophilic gel

Topic #1

ONION SKINS

Topic #1 - red onion skin

molecules

MDPI

Article

Bioadhesive Polymeric Films Based on Red Onion Skins Extract for Wound Treatment: An Innovative and Eco-Friendly Formulation

Cinzia Pagano [1,†], Maura Marinozzi [1,†], Claudio Baiocchi [2], Tommaso Beccari [1], Paola Calarco [1], Maria Rachele Ceccarini [1], Michela Chielli [1], Ciriana Orabona [3], Elena Orecchini [3], Roberta Ortenzi [4], Maurizio Ricci [1], Stefania Scuota [4], Maria Cristina Tiralti [1] and Luana Perioli [1,*]

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Topic #1- red onion skin

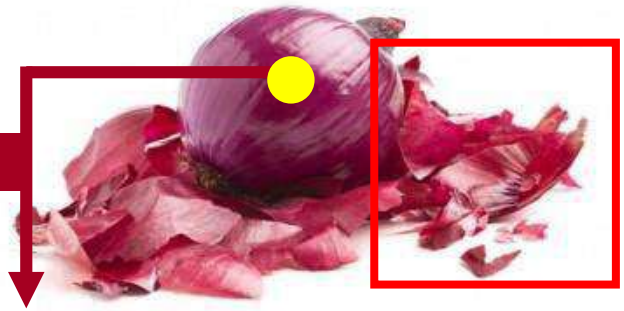
Onion is worldwide cultivated vegetable for

- food,
- flavoring

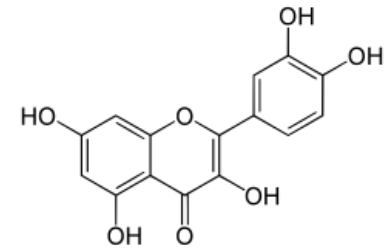
Pulp
is endowed with
many properties

- antioxidant
- lipid- and glucose-lowering
- antiinflammatory
- antimicrobial
- **EUDERMIC, ANTI-KEILOSIS**

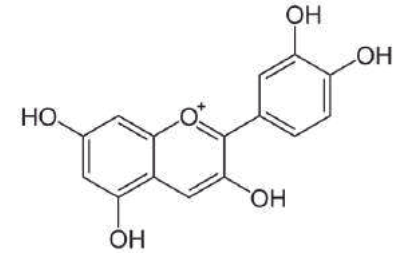
Red onion, variety *Rojo duro*



FLAVONOIDS



quercetin

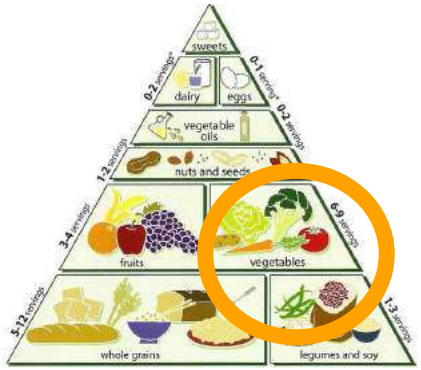


cianidin

RECYCLE

REUSE

REDUCE



POPULAR FOOD

SEPARATE WASTE COLLECTION

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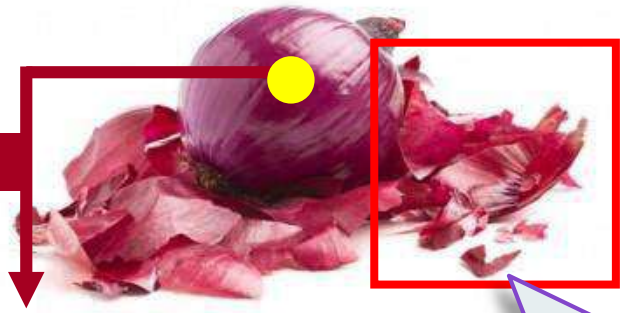
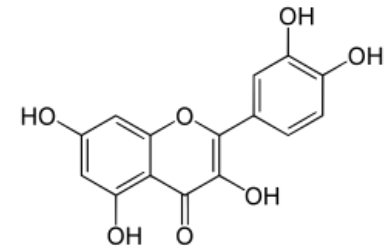
Red onion, variety *Rojo duro*

PULP

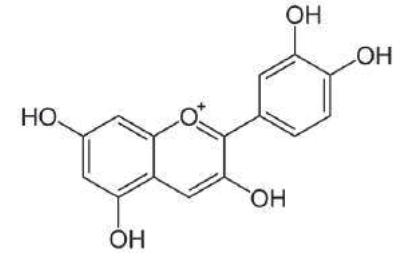
SKINS



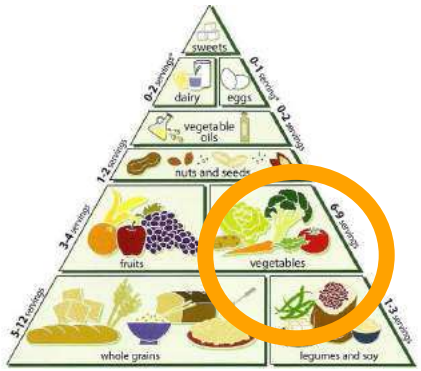
FLAVONOIDS



quercetin



cianidin



the non-edible outside layers, are the main waste material obtained from onion processing and consumption.
Since they are rich in flavonoids, odorless and can be stored for a long time after the harvesting, we saw in them an interesting source to be investigate

POPULAR FOOD

SEPARATE WASTE COLLECTION

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Aim of the work

Development of novel **POLYMERIC FILMS** loaded with dry skins extract of Rojo duro onion, farmed in Cannara (Umbria, Italy) for **skin desease treatment**

biological cultivation (without fertilizers and pesticides)

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Prof. Maurizio Ricci

WORK PROJECT

1

a) D.S. extraction according to green chemistry principles
b) evaluation of the activity of the obtained **extract** in terms of

- antioxidant, radical scavenging
- Antibacterial
- antiinflammatory
- effect on cells viability

2

formulation of the extract as **HYDROGEL BIOADHESIVE FILM** for skin treatment and its valuation in terms of

- activity
- safety

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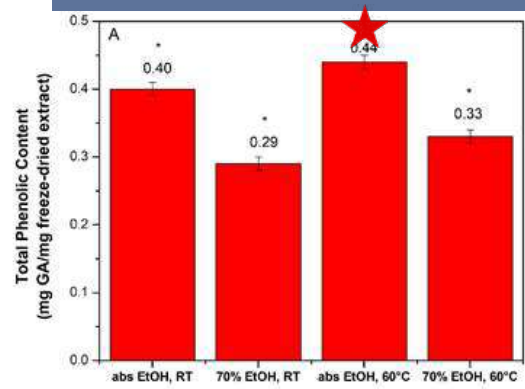
what were the best extraction conditions?

recovery material

Extraction Conditions: solvent, temperature	Recovery yield (%)[a]
abs EtOH, RT	6.4
70% EtOH, RT	8.7
abs EtOH, 60°C	7.9
70% EtOH, 60°C	9.7

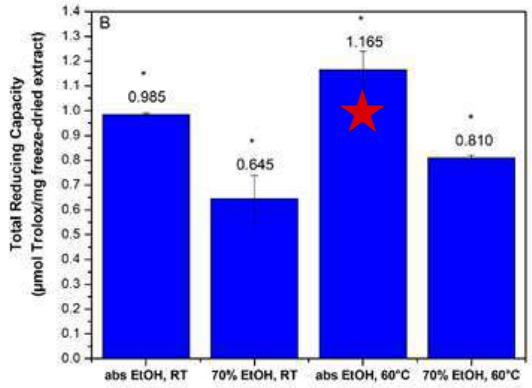
total phenol content (TPC)



Folin-Ciocalteu assay

antioxidant activity

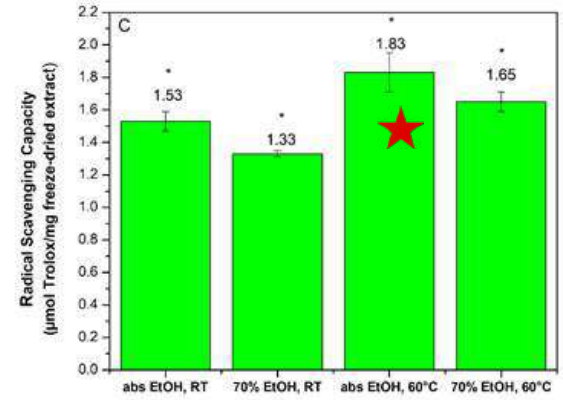
total reducing capacity (TRC)



FRAP (ferric ion reducing antioxidant power) assay

★ these results are in accordance with TPC results,

radical scavenging capacity (RSC)



DPPH (2,2-diphenyl -1-picrylhydrazyl) assay

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Antibacterial Activity Assay

- *S. epidermidis*
- *S. aureus*
- *L. innocua*
- *E. faecalis*

Minimum Inhibitory Concentration (MIC)
and
Minimum Bactericidal Concentration (MBC)

MHB (Muller Hilton Broth) suspension $1x10^5$ CFU/mL
tested concentrations 30, 15, 7.50, 3.75, 1.88, 0.94, 0.47 mg/mL

positive control: MHB + bacterial suspension;
negative control: MHB + onion extract (solution at the tested scalar concentrations)
antibiotic control: MHB + ampicillin

Table 2. Minimum inhibitory (MIC) and minimum bactericidal (MBC) values of OLE and the reference antibiotic ampicillin expressed as mg/mL ± SD (n = 3).

	S. epidermidis	*S. aureus*	*L. innocu*
OLE MIC	0.47 ± 0.00	0.94 ± 0.00	3.75 ± 0.0
OLE MBC	0.94 ± 0.00	1.88 ± 0.00	7.50 ± 0.0
Ampicillin MIC	0.13 ± 0.00	0.13 ± 0.00	0.50 ± 0.0
Ampicillin MBC	0.50 ± 0.00	0.25 ± 0.00	1.00 ± 0.0

DS extract suitable for wound treatment

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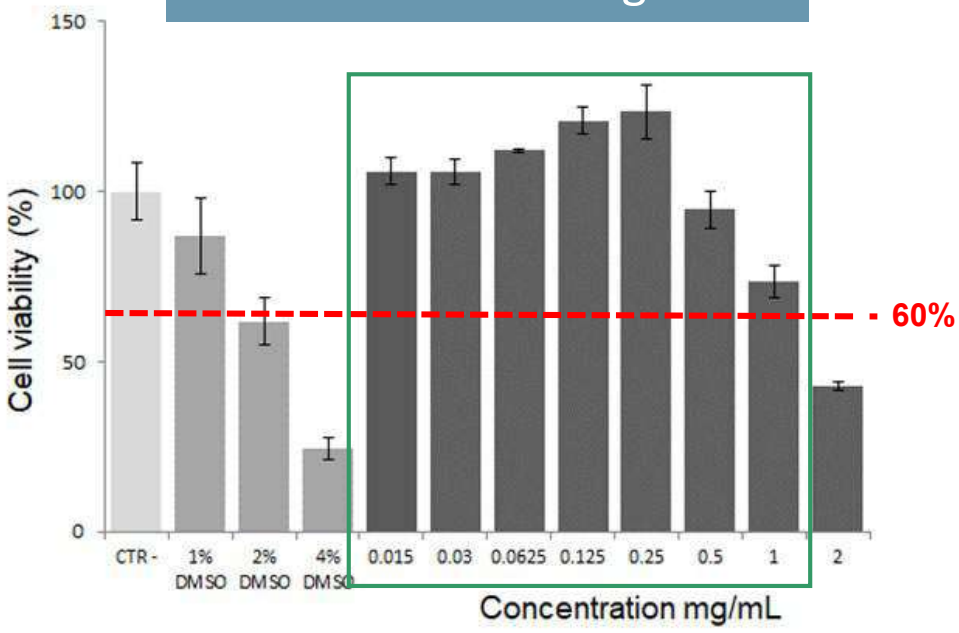
Citotoxicity studies

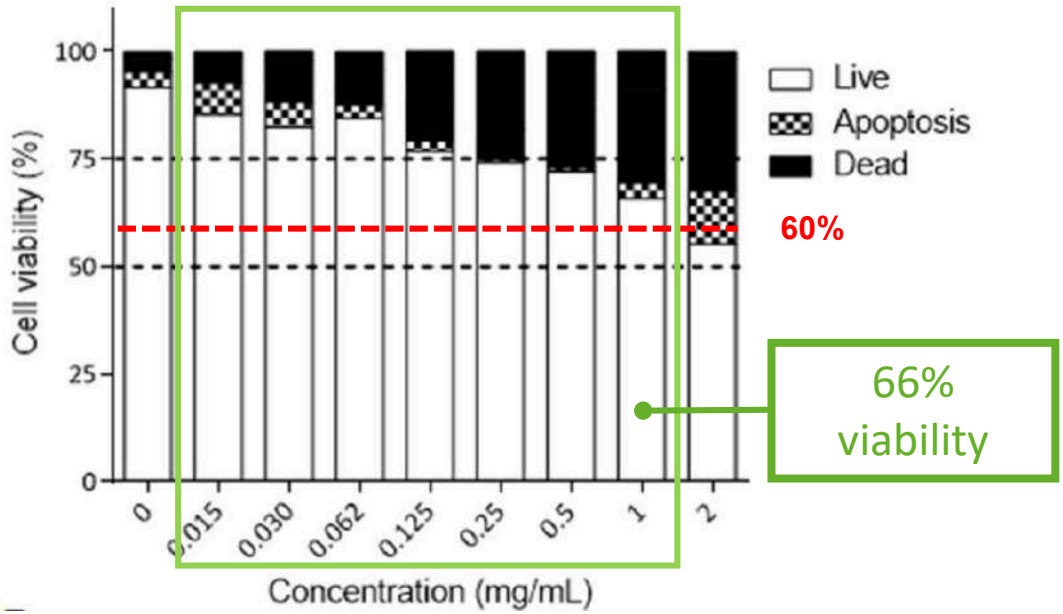
Were performed in order to identify OLE safe concentrations

Two cell lines were assayed

HaCat: keratinocytes for wound healing



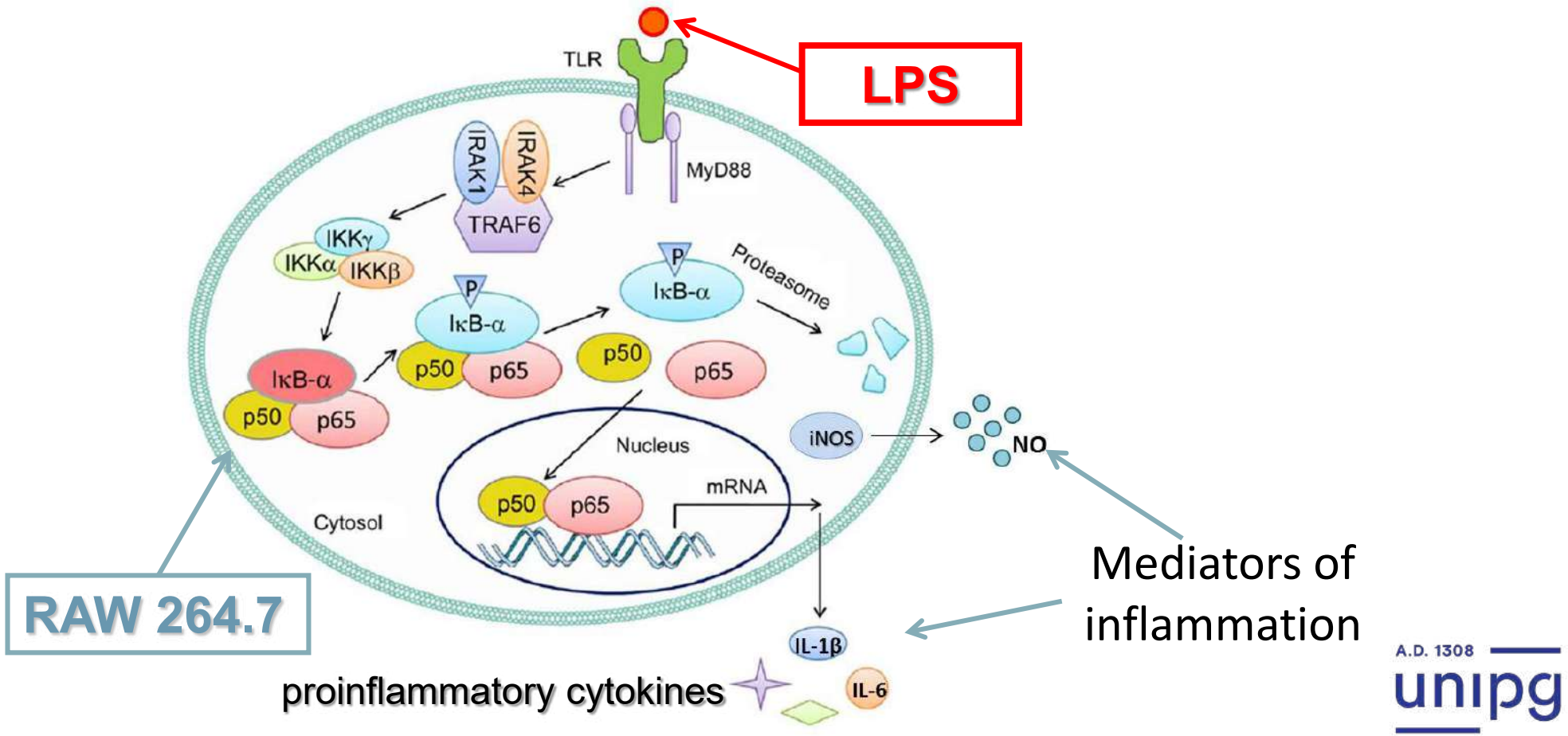
RAW 247: macrophages used to evaluate the anti-inflammatory activity



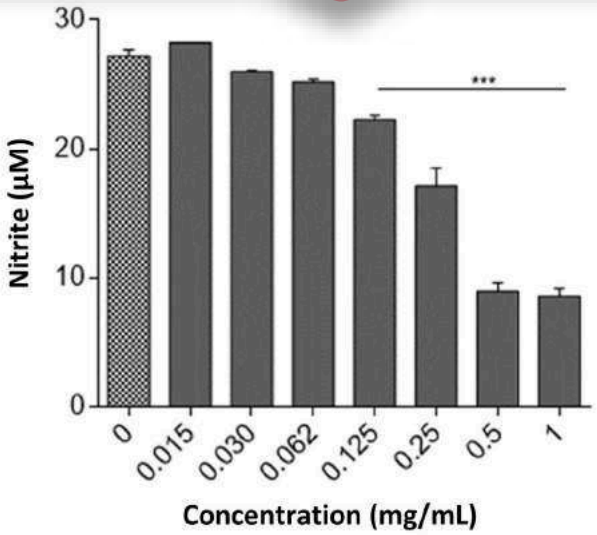
The extract is safe for both cell lines in the concentration range **0.015 - 1 mg/mL (viability > 60%)**

Anti-inflammatory activity

Lipopolysaccharide (*Bacterial endotoxin able to stimulate the production of inflammation mediators*) -activated RAW 264.7 cells were incubated with different safe concentrations of the extract

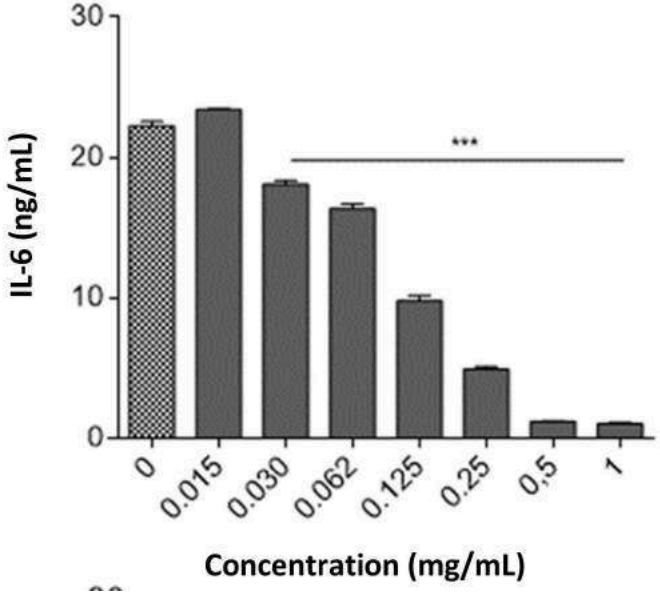


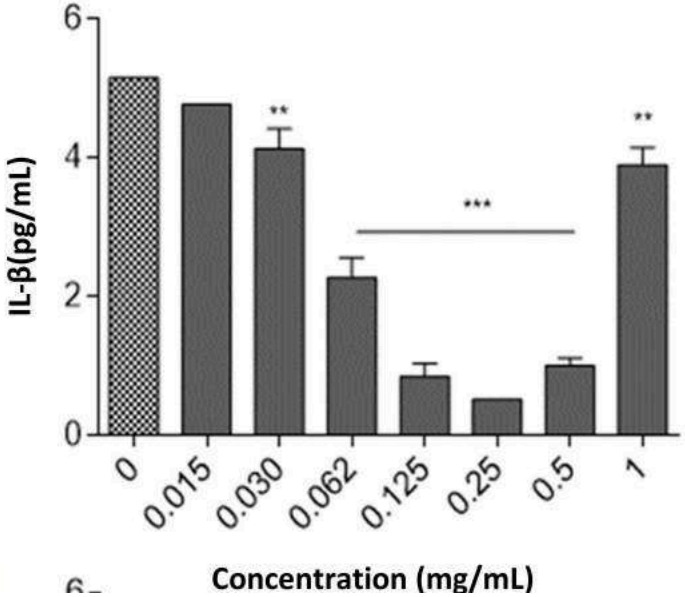
Anti-inflammatory activity



- **Down regulation of NO release** (IC_{50}= 0.230 ± 0.022 mg/mL)
- **IL-6 cytokine release inhibition** (IC_{50} = 0.090 ± 0.008 mg/mL)
- **IL-1β cytokine release inhibition** (IC_{50} = 0.054 ± 0.002 mg/mL)

OLE inhibited the production of inflammatory mediators with IC_{50} lower than the highest cytotoxic concentrations (i.e., 0.5-1 mg/mL).





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Adhesive hydrogel film characteristics

The antioxidant, anti-inflammatory and antibacterial activities observed for pure OE suggested that it could be a suitable active ingredient for wounds treatment to be incorporated in a suitable formulation overcoming some limits of conventional formulations such as:

- **Limited and incomplete release**
- **Limited amount at the action site**
- **Low residence time**

HYDROGEL FILM

- Auto-adhere to skin (without the aid of glue),
- Protect the wound from mechanical solicitations,
- Avoid occlusion and pain
- Be easily removable and able to promote a sustained OE release

SOLVENT CASTING METHOD

Gel preparation

mechanical stirring (600 rpm)

Degassing

Conditioning Planetary Mixer
(10 minutes, 2000 rpm)

Casting

3.5 g gel

silicon molds (ø = 3.5 cm)

Drying

oven at 37.0° C ± 0.1 for 24 h

stored at R.T. and 40% relative humidity until use

Pagano, C. Ceccarini, M.R.; Calarco, P.; Scuota, S.; Conte, C.; Primavilla, S.; Ricci, M.; Perioli, L. Bioadhesive polymeric films based on usnic acid for burn wound treatment: Antibacterial and cytotoxicity studies. Colloids Surf. B Biointerfaces 2019, 178. stored at RT and 40% relative humidity (RH) until use

HYDROGEL COMPOSITION

DS EXTRACT

- Treatment of the infection
- Antiinflammatory activity

POLYMERS

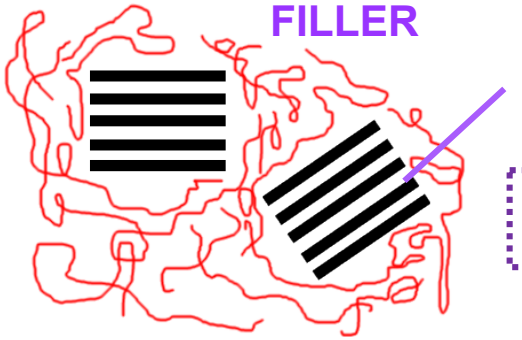
Sodium carboxymethylcellulose (NaCMC)

- Adhesion
- Film forming

Polyvinylpyrrolidone K 90 (PVP)

- Plasticity
- Film forming
- Adhesion

FILLER



bentonite nanoclay

Macrocomposite:
Two separated phases

- Improvement of mechanical properties

HYDROGEL COMPOSITION

hydrogel	NaCMC %	PVP K90 %	bentonite %	glycerol %	water %
1	2	0.1	1	10	86.9
2	2	0.1	2	10	85.9
3	2	0.1	3	10	84.9
4	2	0.1	4	10	83.9
5	3	0.1	4	10	82.9
6	3	0.1	1	10	85.9

- hydrogel aspect (homogeneity and consistency)
- physical stability,
- easy casting (difficult for very viscous gels)
- final film appearance (detection of visible imperfection under visual inspection)

Film preparation & loading

Starting from the selected hydrogel n. 6 composition, three different hydrogel films were prepared, loaded with 1.0 (B1), 3.0 (B2) and 5.0% (B3) w/w of OLE

	GEL composition						CONTENT in the corresponding FILM after casting
Hydrogels	**DS extract (w/w %)**	**NaCMC (w/w %)**	**PVP K90 (w/w %)**	**bentonite (w/w %)**	**glicerol (w/w %)**	**ultrapure water (w/w %)**	**DS extract mg /cm²**
B1	1.0	3.0	0.1	1.0	10.0	84.9	3.64
B2	3.0	3.0	0.1	1.0	10.0	82.9	10.92
B3	5.0	3.0	0.1	1.0	10.0	80.9	18.20

ø = 3.5 cm

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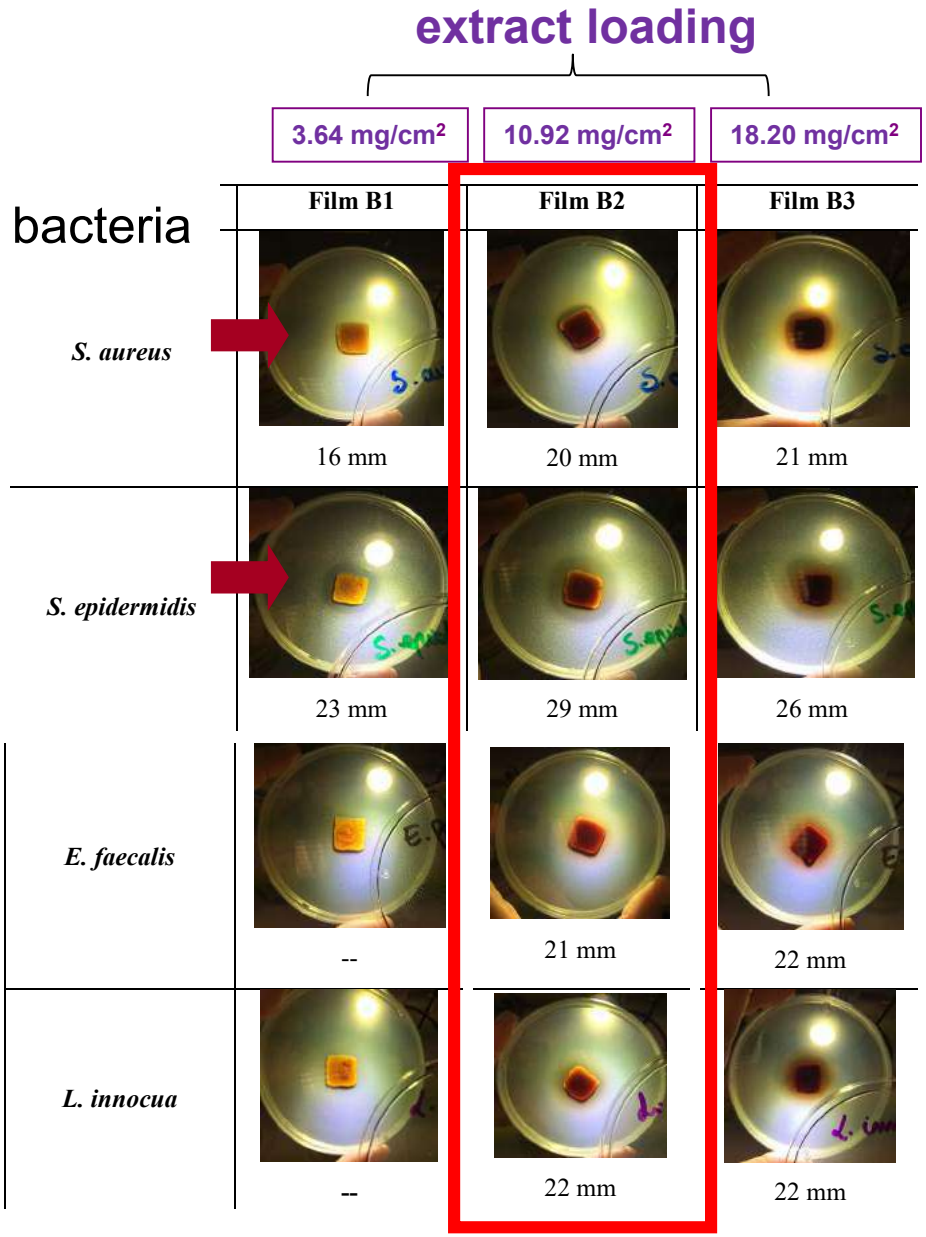
microbiological tests on D.S. extract loaded films



B1 produces inhibition zone only for S. aureus and S. epidermidis

B2 and **B3**, containing D.S. extract in different amount, produced **similar inhibition (halos)** for all the tested bacterial strains.

S. epidermidis resulted **the most sensitive strain**, analogously to the results obtained for the unformulated D.S. extract

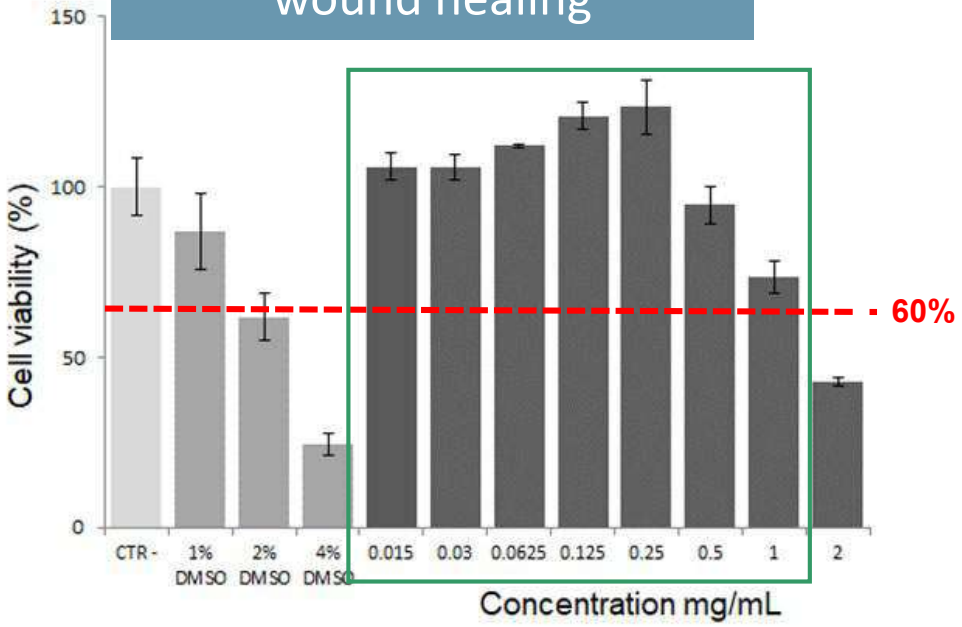
B2, containing **10.92 mg/cm² of D.S.** extract, could be the most suitable formulation for wounds application.

For this reason, it was further characterized

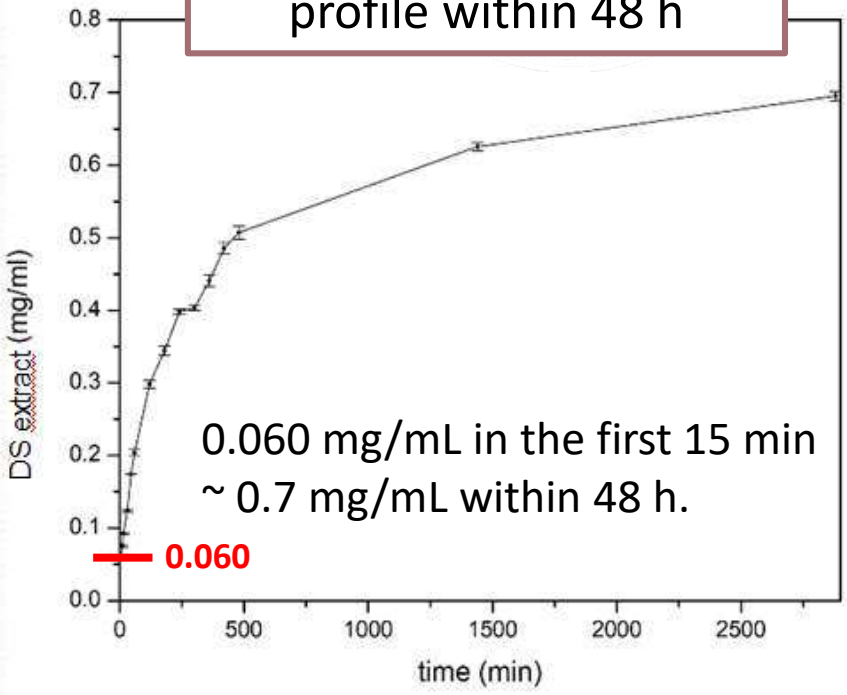
OLE Release profile

hydrogel film B2

HaCat: keratinocytes for wound healing



Extract sustained release profile within 48 h



0.060 mg/mL in the first 15 min
~ 0.7 mg/mL within 48 h.

1.the developed formulation allowed to reach in a short time, after the application, the effective concentration necessary for the anti-inflammatory activity 0,06 mg/mL.

2. **within 48 h** the released concentration remained well **below 2 mg/mL**, value at which the viability of both macrophage and keratinocyte cell lines was impaired

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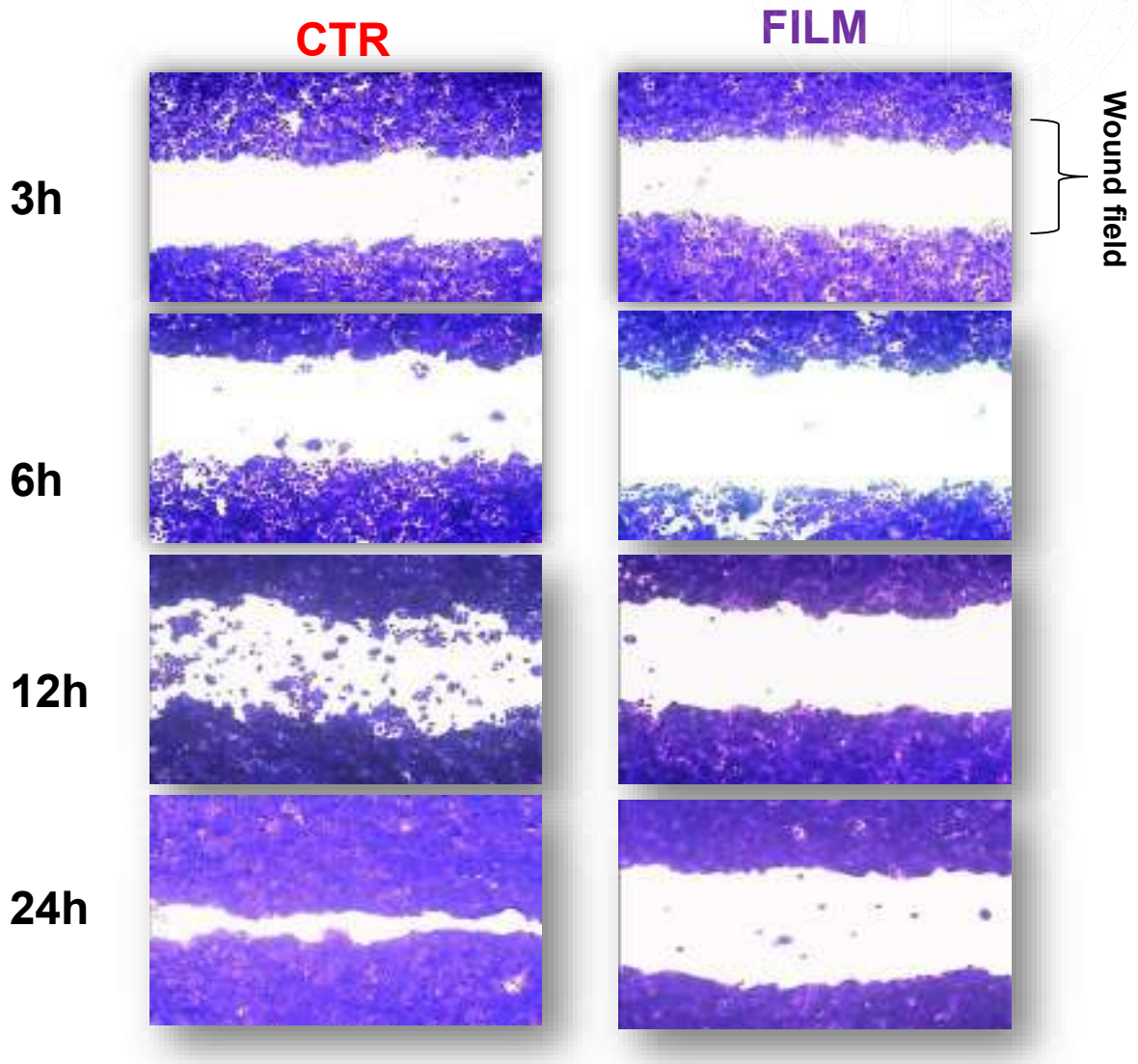
Wound healing assay

HaCat cells

The amount of extract released from the film after 24 h (~ 0.5 mg/ml) was incubated with cells and the effect on wound field closure was observed.



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scars and keloids

Formulation: medical device **GEL**
Use: **repeated daily** administration for **at least 6 months**.
Composition: purified water, allantoin, pullulan, EDTA, sodium benzoate, propylene glycol, phenoxyethanol, carbomer, triethanolamine, **alcholic onion extract**, glycosaminoglycans, benzoic acid, dehydroacetic acid, sodiun jaluronate.

disadvantages

- Strong onion odour
- Repeated daily administration for log times

Formulation: medical device **HYDROACTIVE PATCH**

Composition: polyurethane + **pulp onion extract**
Removal: Dab the skin around the plaster with a sponge (or cotton wool pad) soaked in warm water and lift one flap while continuing to wet the affected area.
Remove it slowly.

disadvantages

- Painful removal
- Special disposal of exhausted patch
- Possible Allergy problems due to the adhesive

- Easy removal by washing,
- odourless,
- self adhesive
- anallergic

N.B.

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Conclusion

why onion skins?

Comparative analyses between the edible portion (**PULP**) and the outer dry skins (**DS**) of the Rojo Duro onion showed that

1. **total antioxidant** content is clearly **higher for DS** than for PULP (77 folds)!!!!
2. **radical scavenging capacity** is **higher for DS** than for PULP (20 folds)
3. **D.S. extract** resulted particularly **active** against bacteria
   - *Staphylococcus epidermidis*
   - *Staphylococcus aureus*
   - *Streptococcus pyogenes,*
4. skins are **odorless**
5. skins can be **stored for a long time** after harvesting
6. skin use respect the environment
7. skin use avoids food biomass consumption

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Topic #1

CROCUS SATIVUS LINNAEUS

Topic #1 - Crocus sativus L.

International Journal of Pharmaceutics 625 (2022) 122067

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Starch-based sustainable hydrogel loaded with *Crocus sativus* petals extract: A new product for wound care

Cinzia Pagano [a,1,*], Maria Rachele Ceccarini [a,1], Marco Faieta [b], Alessandro di Michele [c], Francesca Blasi [a], Lina Cossignani [a], Tommaso Beccari [a], Eleonora Oliva [b], Paola Pittia [b], Manuel Sergi [b], Sara Primavilla [d], Domiziana Serafini [a], Lucia Benedetti [a], Maurizio Ricci [a], Luana Perioli [a]

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Cultivation in Italy

Città della Pieve

Gubbio

Spoleto

Cascia

Topic #1 - Crocus sativus L.

Stigmas represent the noble and gentle part of saffron plant

dried and used as a spice to flavour foods and as a dye to color foods and other products

DIETARY SUPPLEMENTS

Not just a food……..

COSMETICS

as anti-age, lenitive, anti-UV agent as well as in perfumery

110,000–160,000 flowers are needed to obtain 1 kg of spice

Topic #1 - Crocus sativus L.

But……What about the petals?
Do we throw them away?

Saffron petals….
a source of active ingredients

ACTIVE INGREDIENTS
Gallic Acid
Chlorogenic Acid
Caffeic Acid
EGCG
Isoquercetin
Quercetin
Kaempferol

Aim of the work

suitable extraction method able to preserve as much as possible their content integrity

Extraction

Characterization

Formulation

HEALTH

STARCH GEL

formulation based on extract for indicated topical application

Dynamic Maceration

Best Extraction method

Freeze dried petals
(2.46 g)

EtOH 70%

(200 ml), 1500 rpm, 45°C, 90 min

filtration under
vacuum

Drying
(rotary evaporator)

Freeze-drying

EtOH 70% **SE-A_1**

Dynamic Maceration

Best Extraction method

Freeze dried petals
(2.46 g)

EtOH 70%

(200 ml), 15

filtration under
vacuum

The project was carried out considering the "one-health" concept. Ethanol (EtOH) was choosen as extraction solvent because its environmental impact is less than other possible solvents as acetone, chloroform, ecc. moreover it can be by re-used for many extraction cycles after proper distillation.

(rotary evaporator)

Freeze-drying

EtOH 70% **SE-A_1**

Characterization of extracts

SE-A1 extract characteristics in term of yield and water solubility as well as the TPC,

Sample	Yeld (g of extract /100 g freeze-dried petals)	Water solubility*	TPC** (mg GAE/g extracts)
SE-A_1	58.94 $\pm$ 0.05	236.6	46.97 ± 0.02

*maximum amount of extract solubilized in a fixed amount of bidistilled water

**The results were expressed as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g).

Characterization of extracts

Phenolic compounds identified and quantified in saffron petal extracts by HPLC . Data are reported as µg/g ± SD

Gallic Acid	Chlorogenic Acid	Caffeic Acid	EGCG	Syringic Acid	Orientin	Rutin	Isoquercetin	3-OH-Benzoic Acid
58.86 ± 8.95	54.67 ± 10.22	0.55 ± 0.11	5.29 ± 0.87	0.24 ± 0.04	0.15 ± 0.02	<LOQ	3.37 ± 0.53	<LOQ

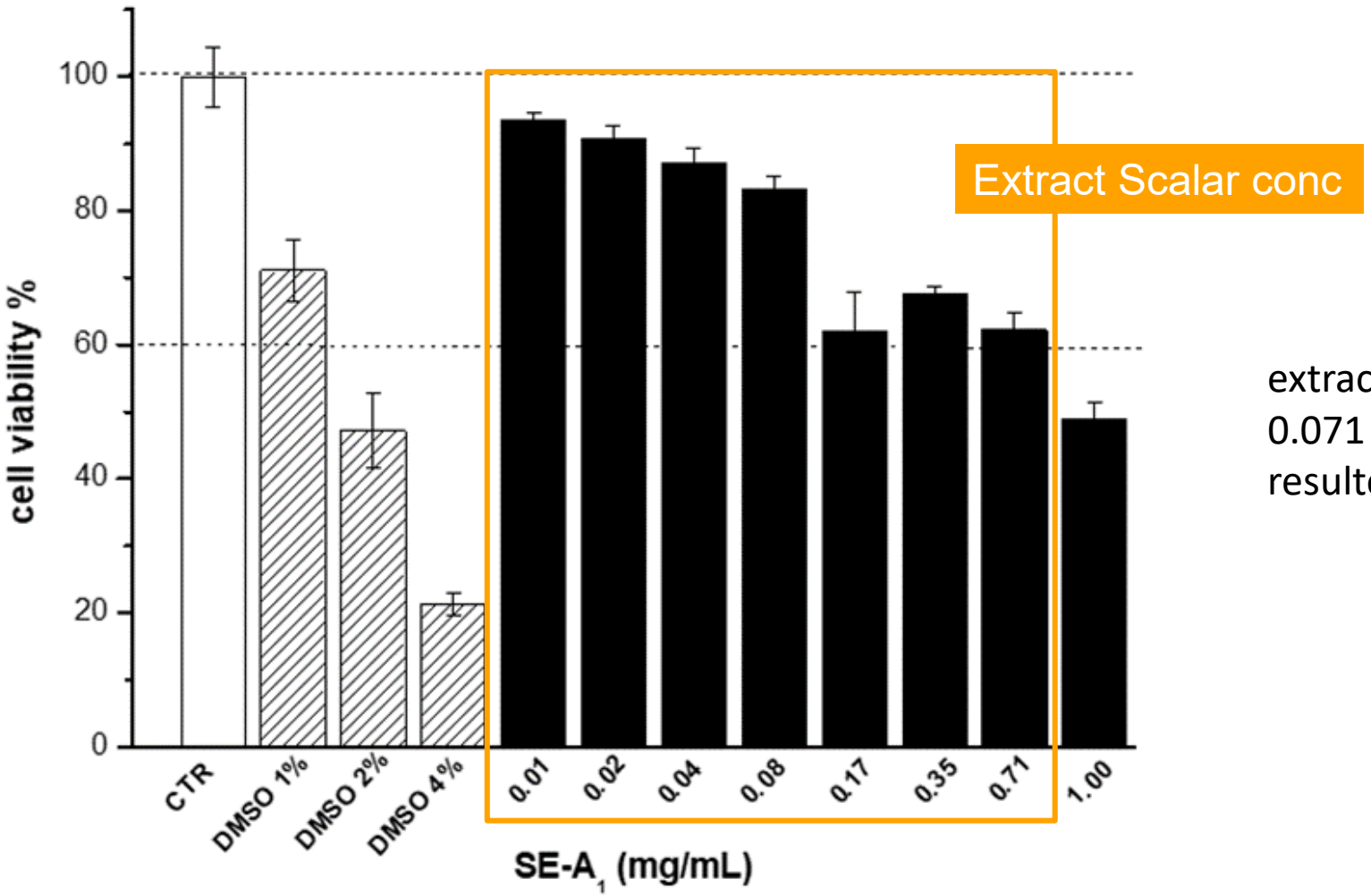
p-Coumaric Acid	Ferulic Acid	Myricetin	Luteolin	Quercetin	Apigenin	Kaempferol	Naringenin	Isoxhanthoumol
1.86 ± 0.31	0.32 ± 0.06	3.88 ± 0.69	0.35 ± 0.06	23.09 ± 3.46	0.02 ± 0.01	8.77 ± 1.46	5.09 ± 0.92	2.43 ± 0.36

TPC and antioxidant activity values (mean value ± SD), determined by ABTS*$^{+}$, DPPH* and FRAP assays, of different SE extracts.

	TPC (mg GAE/g extracts)	ABTS*$^{+}$ (mg TE/g extracts)	DPPH* (mg TE/g extracts)	FRAP (mg TE/g extracts)
SE-A_1	46.97 ± 0.02	89.24 ± 0.74	23.99 ± 0.12	3.01 ± 0.00

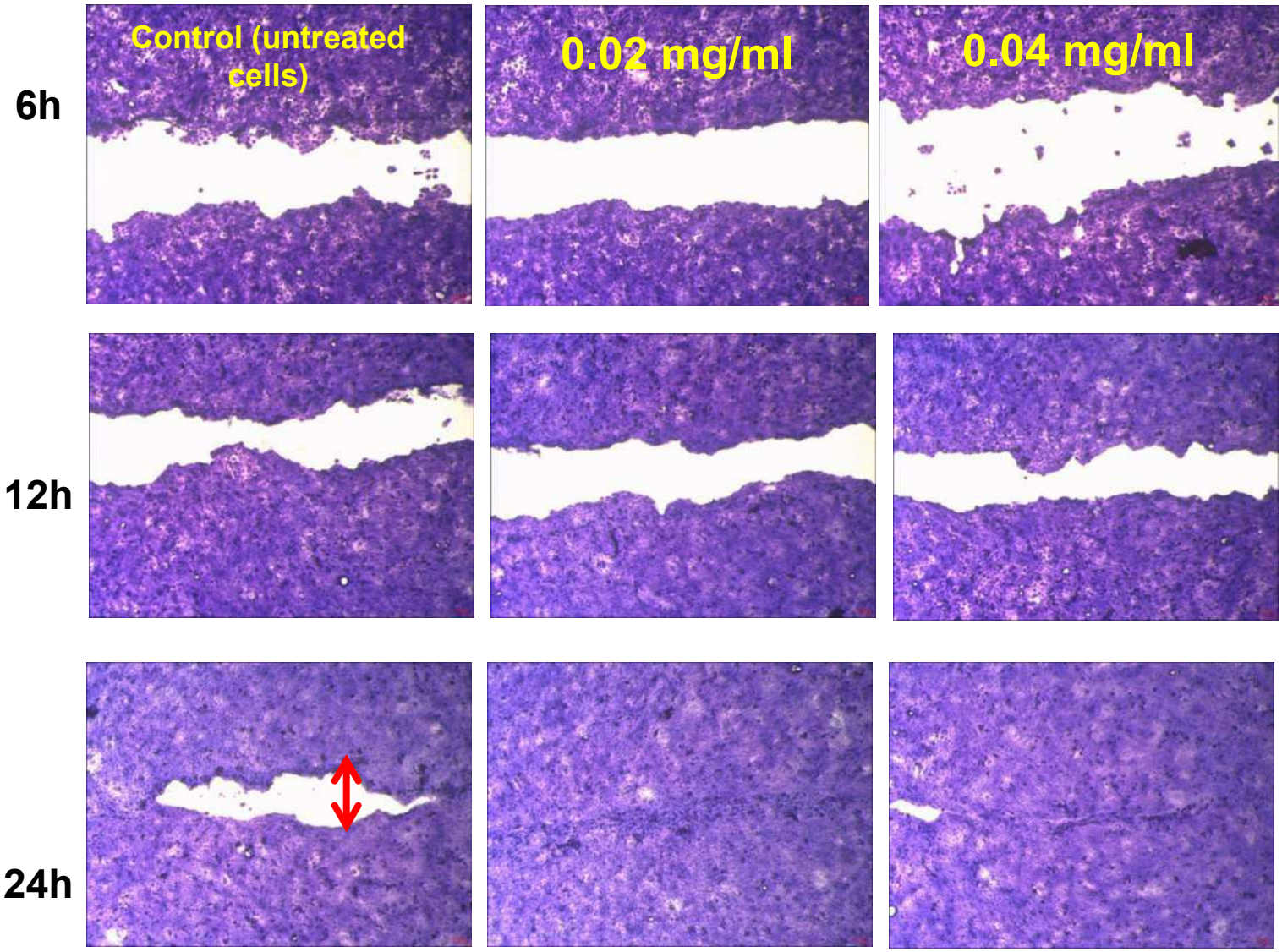
Characterization of extracts

CITOTOXICITY: HaCaT (immortalized human keratinocytes) model cell system representative of epidermidis



extract SE-A1 in the range 0.01-0.071 mg/mL (viability > 80 %) resulted safe for HaCat cells

Wound healingin vitro assay



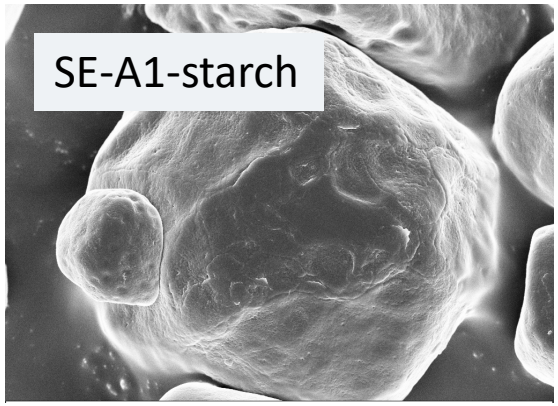
The petal saffron extract, showed suitable antioxidant activity as well as ability to stimulate keratinocytes growth → useful to be introduced in a topical formulation to treat skin diseases such as superficial wounds.

Formulation - flowability

Poor flow properties making very difficult all the phases for the development of any type of formulation as well as weighing operations

→ Support SE-A1 on starch powder with high flowability

PREPARATION: The solvent, separated from exhausted petals after the extraction, was concentrated by a rotary evaporator and then diluted with bidistilled water until 25 mL. Starch powder was suspended in this solution (1:2 SE/starch w/w ratio) and then freeze-dried.



According to the flowability scale (Ph. Eur. 10th Ed.) the powder shows **good flow** character (C.I. 16.43 %; H.R. 533 1.17)

Flow Character	Hausner Ratio	CI (%)
Excellent/very free flow	1.00-1.11	≤10
Good/free flow	1.12-1.18	11-15
Fair	1.19-1.25	16-20
Passable	1.26-1.34	21-25
Poor/cohesive	1.35-1.45	26-31
Very Poor/very cohesive	1.46-1.59	32-37
Very, very poor/ approx. non-flow	>1.60	>38

Formulation

SE-A_1-starch based hydrogel

WHY STARCH?

Because Corn starch powder is largely employed in many health products as excipient and is classified as G.R.A.S. from FDA. Moreover, its use is consistent with the recent European Chemicals Agency (ECHA) purpose to restrict the use of intentionally added microplastic particles (this category includes many excipients commonly used in formulations)

Optimization of the recipe of "starch gel" reported in Ph. Eur. 10th Ed.

- corn starch powder 9.960 %.
- SE-A_1-CS 0.045 %.
- glycerol 70.000 %.
- water 20.000 %.

Self-preserving capacity

» Generally, water-based formulations require the addition of preservatives for a microbiological stabilization of the formulation.

» in vitro studies evaluating its ability to prevent microorganism growth during the storage conditions

	Days				
	0	7	14	21	28
Aerobic Colony Count 30 °C (CFU/g)	<10	<10	<10	<10	<10
Total Molds Count (CFU/g)	<10	<10	<10	<10	<10

The formulation does not require the addition of preservatives for the period considered for the test.
This is important because preservatives in topical formulations are often responsible for allergic reactions, sensitization making the formulation less tolerable from the patient.

In summary

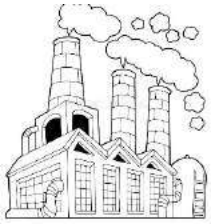
- The best water-soluble extract was prepared by maceration in ethanol 70 %
- The extract, showed suitable antioxidant activity as well as ability to stimulate keratinocytes growth useful for topical formulations in superficial wound treatment
- the extract formulated in a starch-based hydrogel useful for an atraumatic application of damages skin demonstrated antimicrobial activity toward S. epidermidis and self-preserving capacity
- The obtained results suggest that saffron petals are a precious source that must be exploited for therapeutic uses transforming waste in a valuable product.

FINAL CONCULUSIONS AND ADVANTAGES

- **Patient**: the extracts, properly formulated, represent useful and promising alternatives to conventional treatments of skin disease

- **Environment:** onion skins and saffron petals are wastes. Their use is an eco-friendly solution, supporting the food supply chain, able to recycling and valorizing waste material, without using further biomass in accordance with the principles of food ethics and circular economy.

- **Industry:** dry skin extract production is performed by green, scalable and low cost methods, without production of special wastes. This approach could lead to the development of a parallel economy able to create new activities and jobs



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DIPARTIMENTO 2018 di ECCELLENZA 2022 progetto DELPHI

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Istituto Zooprofilattico Sperimentale Umbria Marche

pharmacology

Ciriana Orabona

Department of Experimental Medicine, Sect. Pharmacology

Thank you for your "sustainabile" attention

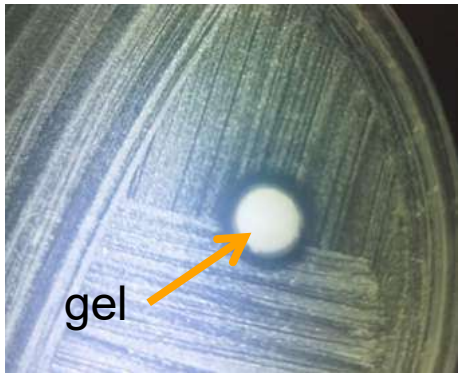
Grottaglie (TA), Italy, murales

circular knowledge

Prelimirary Antibacterial activity

The developed starch gel showed activity against *S. epidermidis*, as demonstrated by the inhibition halo measured (9.5 mm)

This activity is attributable to phenolic compounds, especially gallic and chlorogenic acids the most abundant in the extract.



The obtained result is very interesting considering that S. epidermidis, belonging to human skin microbiota, could become pathogenic in some cases such as wounds and could develop resistance toward conventional antibiotics

- However it must be underlined that the agar diffusion method does not allow to evaluate deeply this activity.
- In fact, the extract diffusion from the gel (seeded in the center of the petri plate) to the solidified agar medium, could be reduced due to the viscosity of the latter.
- For this reason further studies will be performed by different experiments

Recurring Key-words

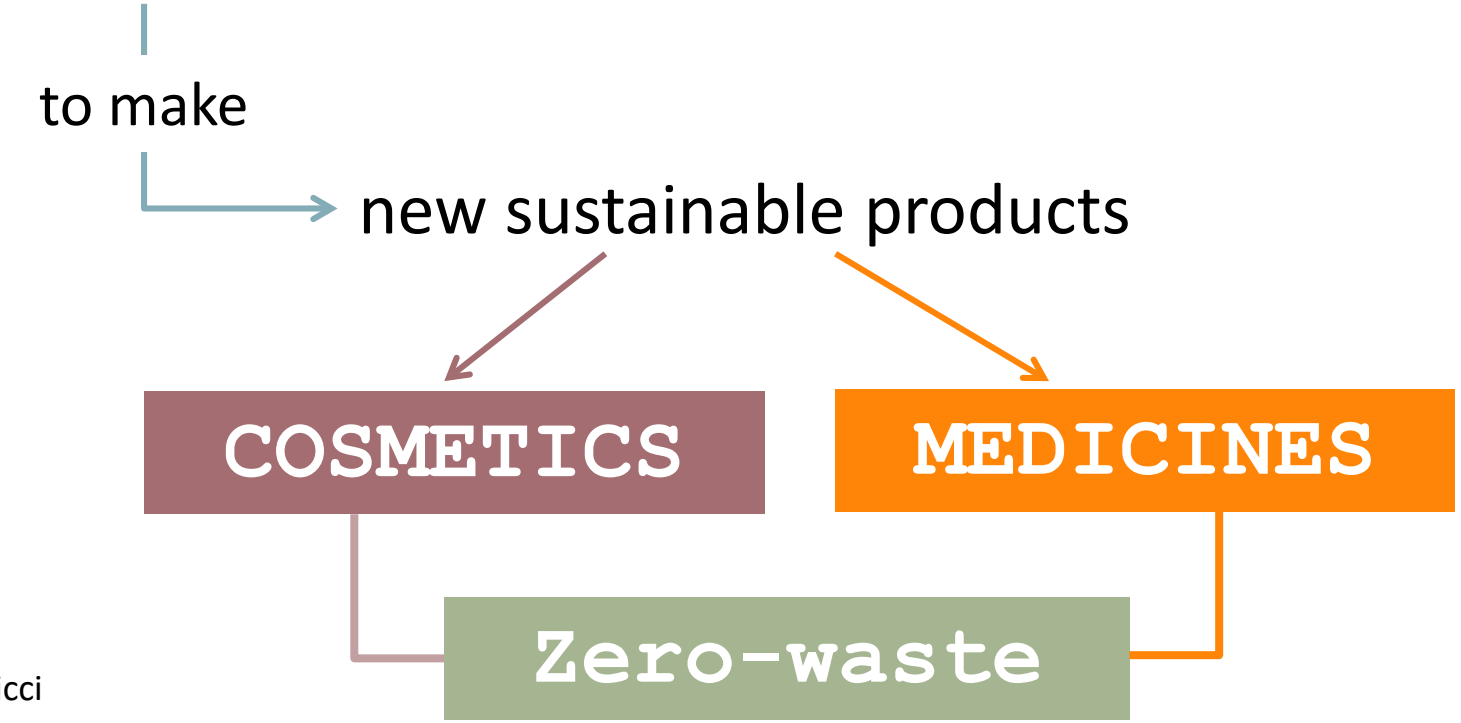
"circular economy"

The circular economy is a new concept of economy able of operating in a "closed-loop" **NORMALLY FAVORING SUSTAINABILITY** (especially in terms of energy)

"sustainability"

New Paradigms

- It is essential to leave behind the old models of linear economy to follow the **CIRCULAR ECONOMY** as a virtuous system that allows to transform food/plant bio-waste **into richness**, extracting and using their natural ingredients and biopolymers



Skin disease treatment

HEALTH CARE PROBLEM

- More than 250 million acute wound cases, approximately 50 million traumatic wound (such as abrasions, punctures, lacerations, and incision) cases, and more than 5 million burn cases are recorded and treated every year globally.
- **Chronic wounds** (as venous ulcers, diabetic ulcers, pressure ulcers) are estimated to affect 20–60 million people worldwide by 2026.
- Total medicare spending estimates for all wound types ranged from $28.1 - $96.8 billion (data 2018).
- The global wound closure products market is expected to exceed more than US$ 16.50 billion by 2024.

Global Wound Dressings Market 2018-2022

MedMarket Diligence. 2015. *Worldwide Wound Management, Forecast to 2026: Established and Emerging Products, Technologies and Markets in the Americas, Europe, Asia/Pacific and Rest of World*. Biddeford, ME: MedMarket Diligence

Wound Closure Products Market By Product type Analysis (Sutures, Adhesives and Tissue Sealants, Hemostats, Surgical Staples, Wound Closure Strips) and By Regional Analysis – Global Forecast by 2018 – 2024

S.R.Nussbaum et al., Value in Health 21 (2018) 27-32.

A.D. 1308 unipg

Prof. Maurizio Ricci

Limits of conventional formulation

Conventional formulations

gels

creams

LIMITED EFFICACY

- Limited and incomplete release
- Low residence time
- Limited amount at the action site

Best Extraction conditions

dry onion skins (4 g)
water washed
dried with cotton cloth

about five months after the onion harvesting.

1

absolute EtOH (160 ml) 60°C, 90 min.

2

a. skin decantation
b. supernatant recovery

solution from 3 extractions
on the same skins

3

rotary evaporator

solvent
evaporation
37°C

4

1. water hydration
2. centrifugation
(4000 rpm, 20 min **at 20° C**)

3 times

5

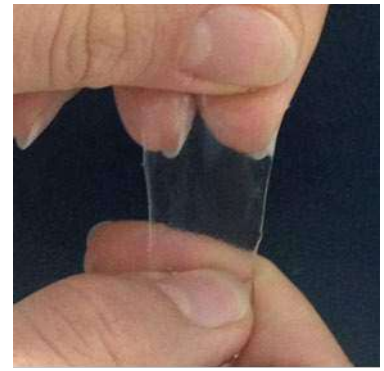
freeze drying

onion dry skin extract
(water soluble powder)

AIM of the work: ***development of novel polymeric films loaded with dry skins extract of Rojo duro onion, farmed in Cannara (Umbria, Italy) for burn wounds treatment***

characteristics of suitable polymeric adhesive films:

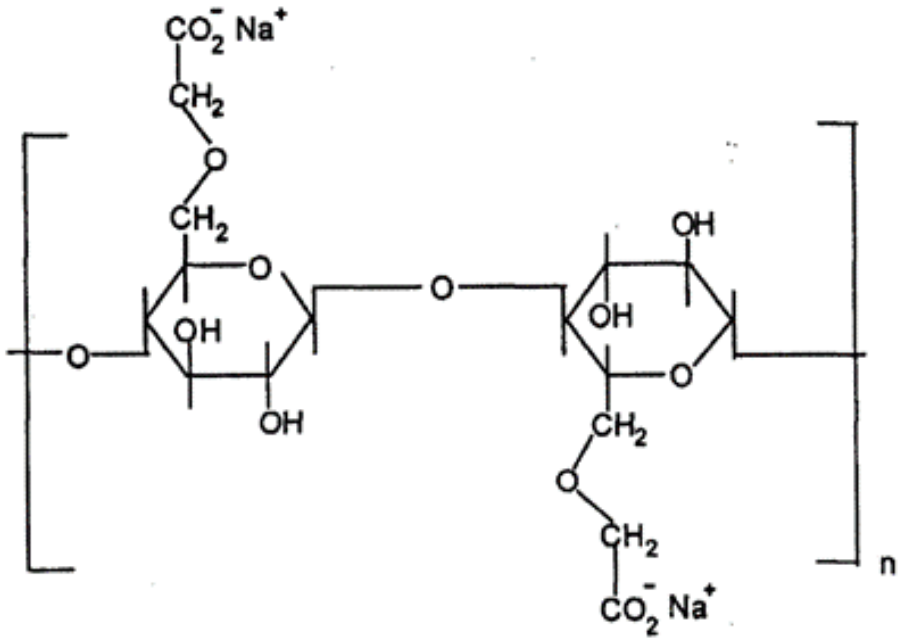
- Flexibility
- Adhesivity
- Mechanical resistance
- Easy removal
- High residence time



Polymeric adhesive film composition

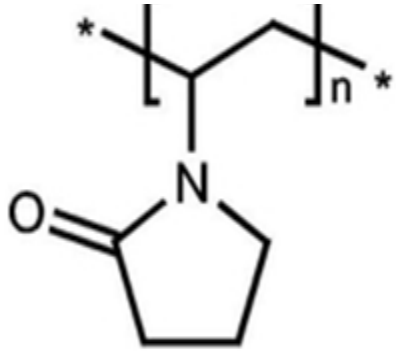
Sodium carboxymethylcellulose (NaCMC)

POLYMERS



- Adhesion
- Film forming

Polyvinylpyrrolidone K 90 (PVP)



- Plasticity
- Film forming
- Adhesion

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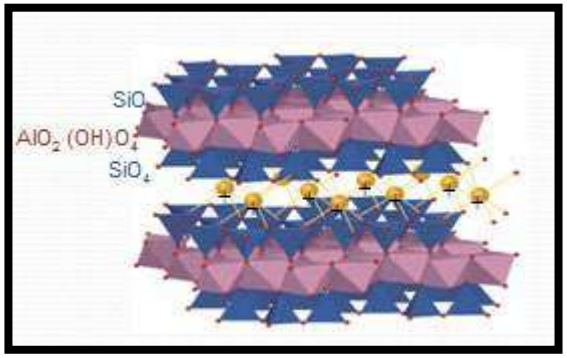
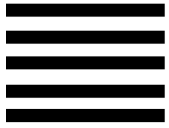
Topic #1- red onion skin

FILLER + POLYMER



bentonite nanoclay



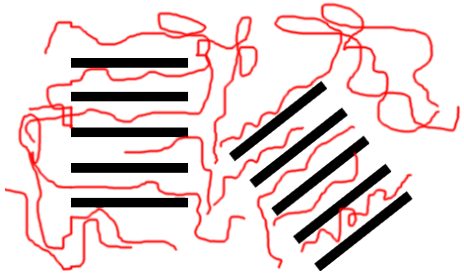
+

Polymer

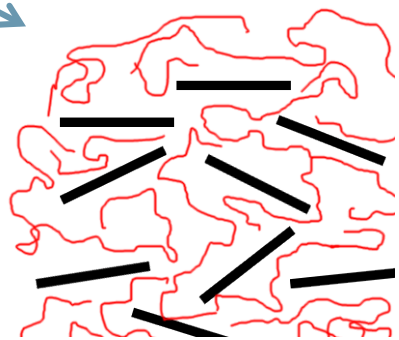
INTERACTIONS

Macrocomposite:
Two separated phases

improvement of mechanical properties
modification of gas permeability



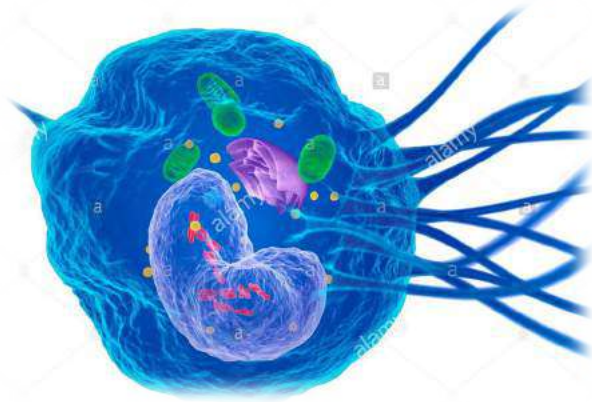
Intercalated Nanocomposite:
The polymer is intercalated in the interlamellar region

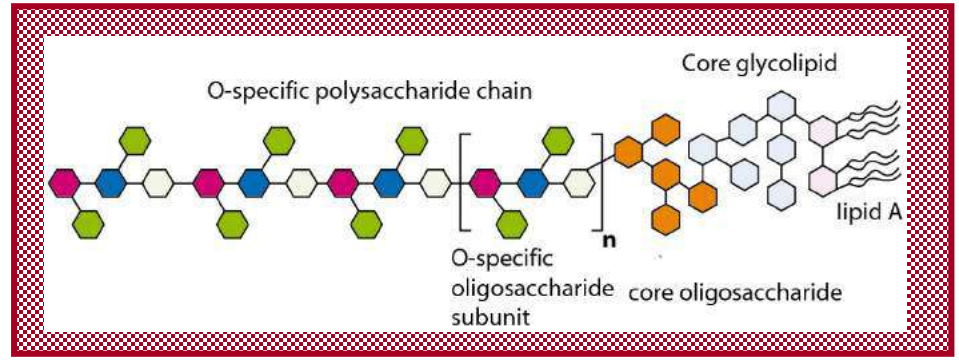


Nanocomposite:
Exfoliated lamellae and dispersed in the polymeric network

antiinflammatory tests: cellular model

- citotoxicity study
- antiinflammatory activity





RAW 264.7

Murine macrophage cells
very important role in immune responses

+

LPS

Bacterial endotoxin able to stimulate a response from the immune system

Used to simulate a generic inflammatory stimulus

cytokine secretion with
pro-inflammatory activity

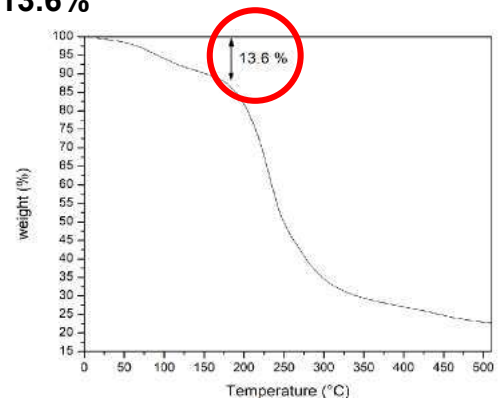
J ZHOU, Y-Y SUN, H ZHANG, et al., Prim-O-glucosylcimifugin Attenuates Lipopolysaccharideinduced Inflammatory Response in RAW 264.7 Macrophages, in *Pharmacognosy Magazine*, **13**(51):378–84 (2017)

H CHUNG, W KOH, W KYUNG KIM, et al., The Anti-Inflammatory Effects of Shinbaro3 Is Mediated by Downregulation of the TLR4 Signalling Pathway in LPS-Stimulated RAW 264.7 Macrophages, in *Mediators of Inflammation*, (2018)

film **B2** characteristics:

1. D.S. **extract** film **content** = **10.92 mg/cm²**
2. Film **water content** = **13.6%**

TGA analysis



3. **Film thickness:** dry condition, 0.43 mm (± 0.05), wet conditions 2.50 mm (± 0.04)

dry conditions

0.43 mm

very thin, so imperceptible after the application.

gauge

incubation in 10 mL of SWF,

thermostated at 32.0°C ± 0.5 for 8 h

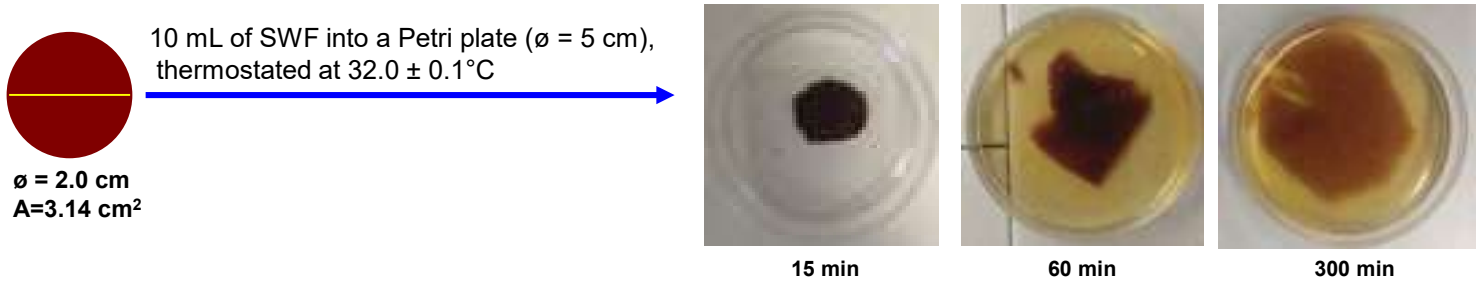
after contact with the Simulate Wound Fluid

2.50 mm

no excessive increase of the dimensions, good patient's acceptability during the use

swelling behavior and matrix erosion

When a polymeric film is immersed in water (or SWF) it swells and can lose its integrity.



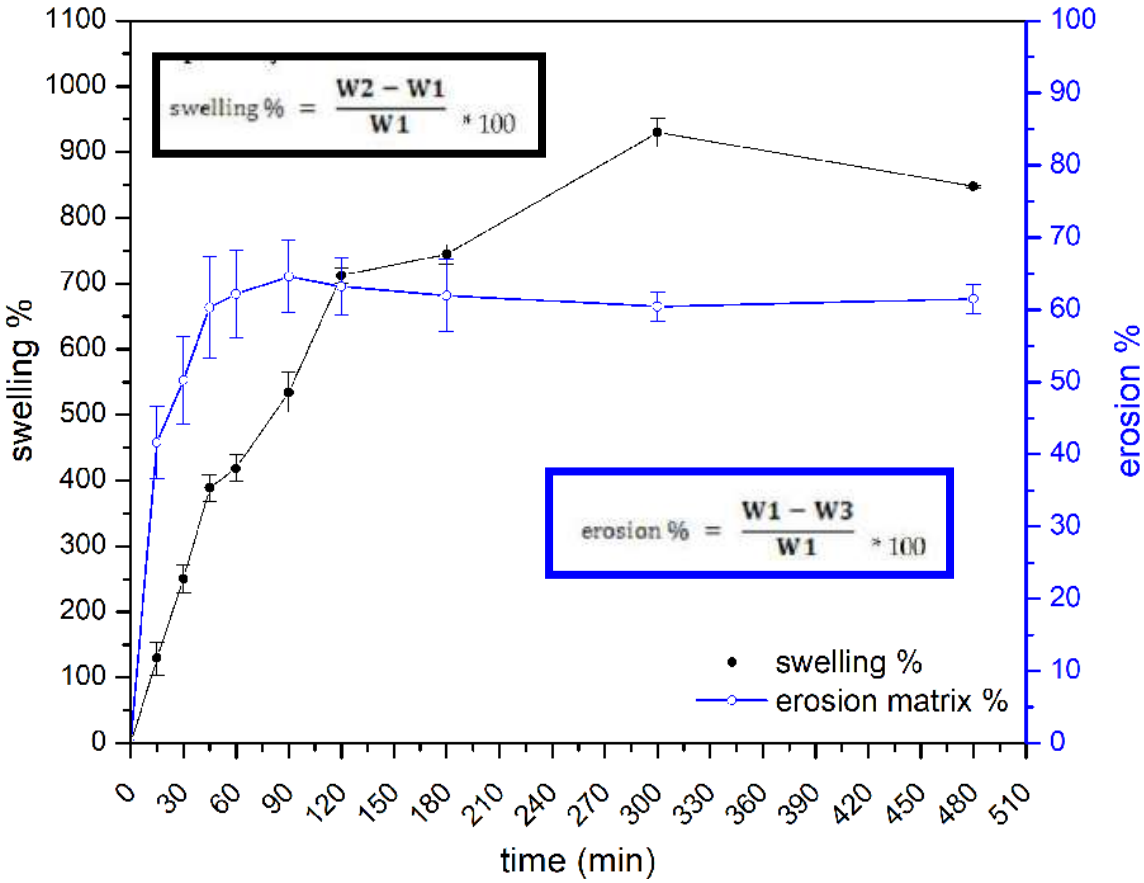
It is important to know **this behavior** because it affects

1. the **absorption** of exudate from the wound
2. the **release of** the active ingredient
3. the easy **removal** from the wound

Perioli, L.; Ambrogi, V.; Angelici, F.; Ricci, M.; Giovagnoli, S.; Capuccella, M.; Rossi, C. Development of mucoadhesive patches for buccal administration of ibuprofen. *J. Control. Release* **2004**, *99*, 73–82.

swelling behavior and matrix erosion

W1 = initial weight
W2 = swelled and wiped off
W3 = swelled, wiped off and dried over $CaCl_2$ (40% RH) for 48 h



- swelling is high capacity to absorb fluids
  - after 15 min = 2 fold its original weight
  - after 8 h = nine fold

high affinity for biological fluid

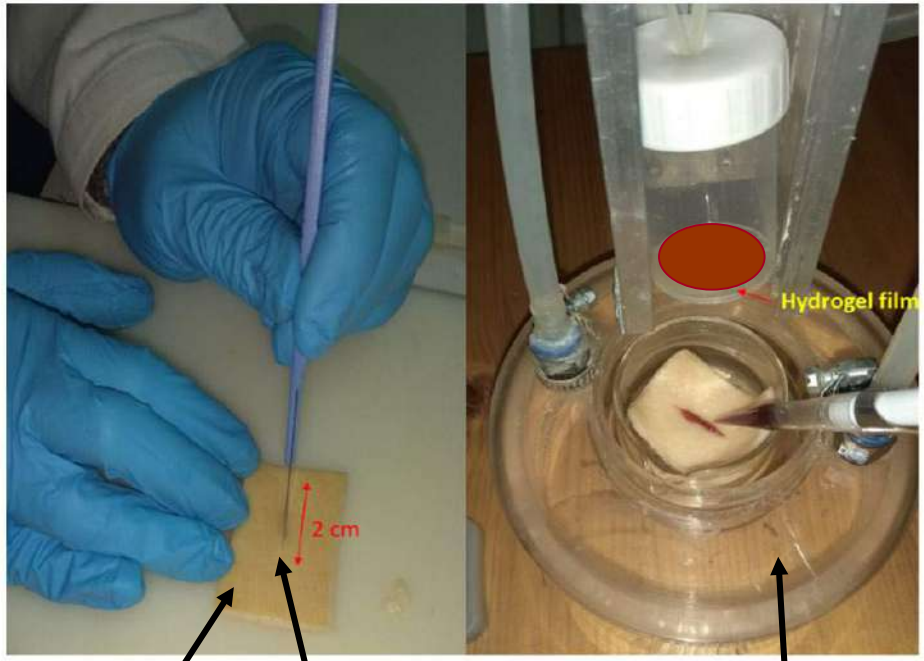
- weight loss (erosion matrix %) due to the gradual dissolution in the medium
  - ~40% after 15 min
  - 60% after 60 min, until the 8th h
  - after 8 h the polymeric network resulted completely relaxed

film can be easily removed by washing (atraumatic and pain free removal)

Perioli, L.; Ambrogi, V.; Angelici, F.; Ricci, M.; Giovagnoli, S.; Capuccella, M.; Rossi, C. Development of mucoadhesive patches for buccal administration of ibuprofen. *J. Control. Release* **2004**, *99*, 73–82.

Ex vivo adhesion studies

dinamometer



PROCEDURE

- contact between hydrogel film and skin sample by applying a light force (0.5 N) for 60 sec.
- contact with skin for further 60 sec
- traction for film detachment (triplicate) by system connected to the dynamometer

detachment force 0.4 N ± 0.06
detachment time 13.00 sec ± 0.57.

pig skin samples
(from shoulder region)

thermostatic bath at 32.0°C ± 0.5

skin incision, filled with 500 µL of SWF to simulate a wound,

interactions

hydrophobic

hydrophilic

binding of the hydrogel film to **the stratum corneum** of the peri-wound area.

binding of the **damaged area** characterized by the presence of exudate

Pagano, C.; Ceccarini, M.R.; Calarco, P.; Scuota, S.; Conte, C.; Primavilla, S.; Ricci, M.; Perioli, L.
Bioadhesive polymeric films based on usnic acid for burn wound treatment: Antibacterial and cytotoxicity studies. *Colloids Surf. B Biointerfaces* **2019**, *178*.

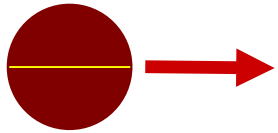
Ex vivo adhesion studies

the developed hydrogel film is able to

1. bind wounded skin
2. interact with the exudate and to swell
3. establish interactions (mainly hydrogen bonds) with the subcutaneous tissues surrounding the wound.
4. adhere to the skin surface avoiding the use of adhesives
5. be easy removed by washing

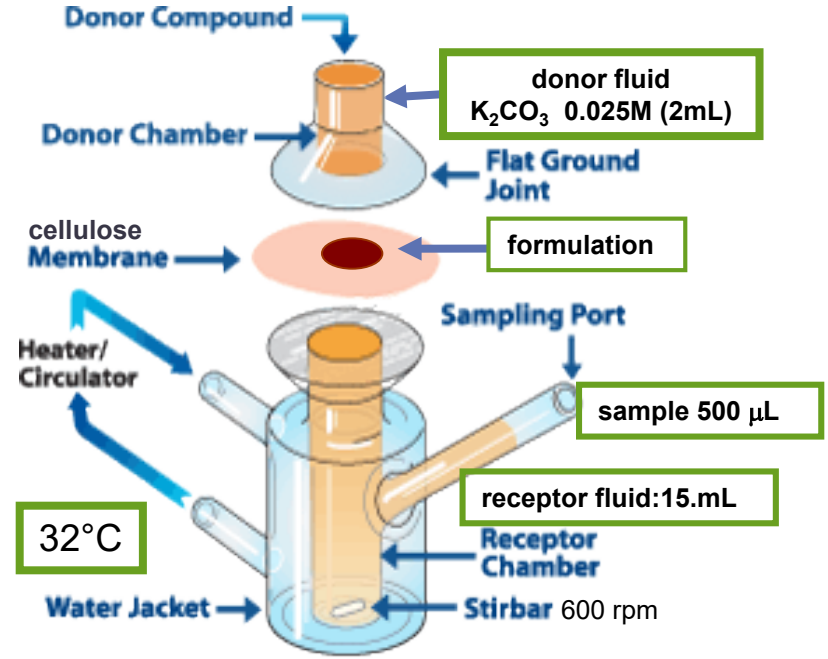
hydrophilicity and swelling can promote the release of the active ingredient?

in vitro release studies

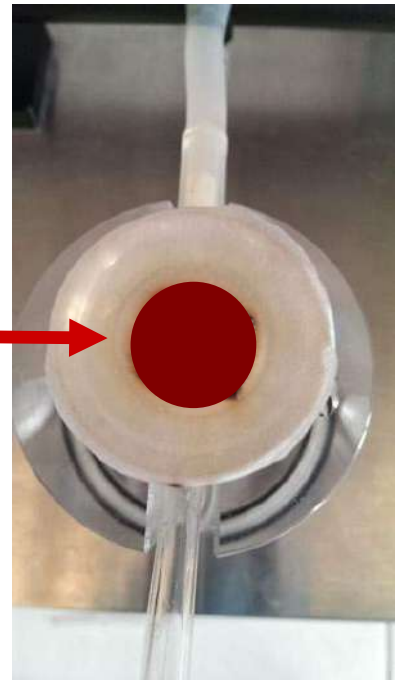


ø = 2.0 cm
A=3.14 cm^2

DS extract total content 34.29 mg

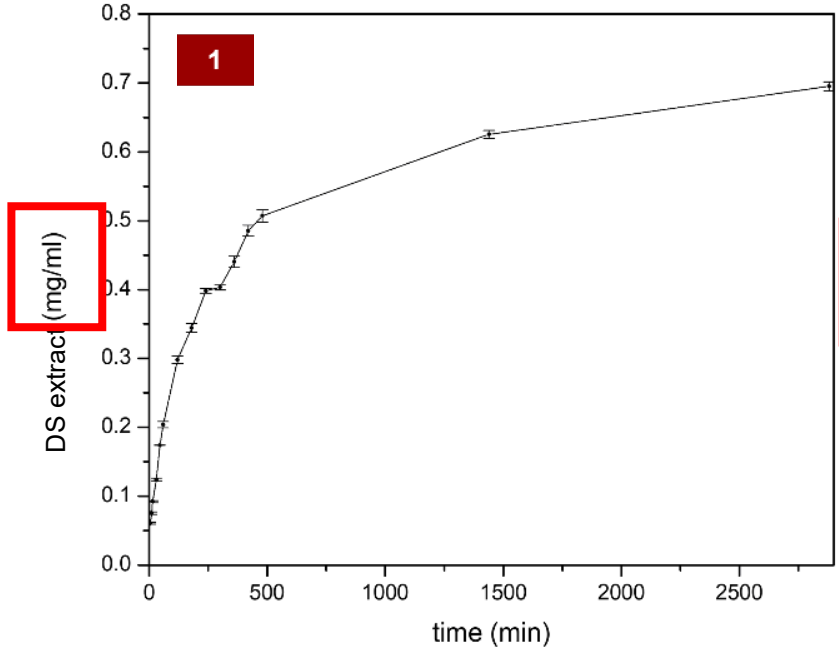


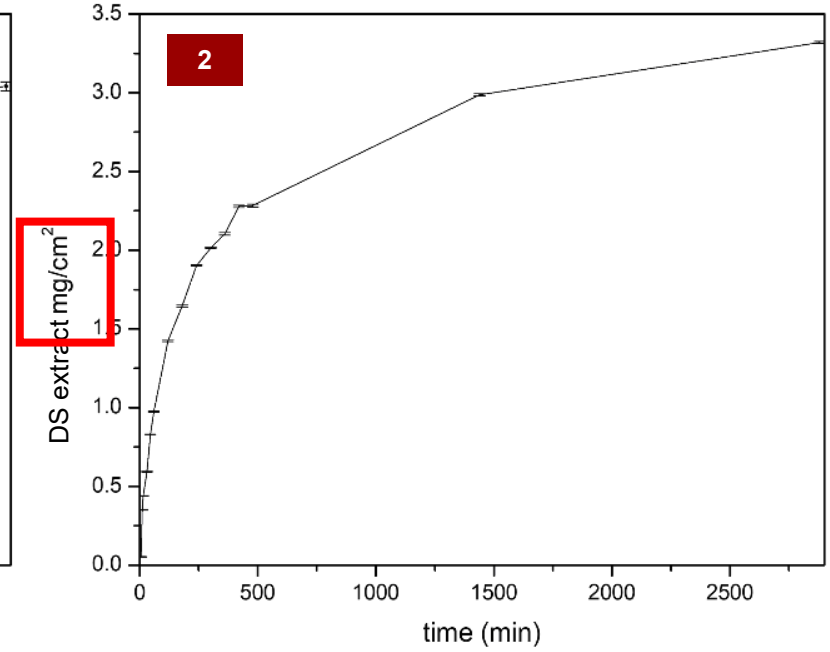
previously hydrated
100 µl SWF



Franz cell

in vitro release studies





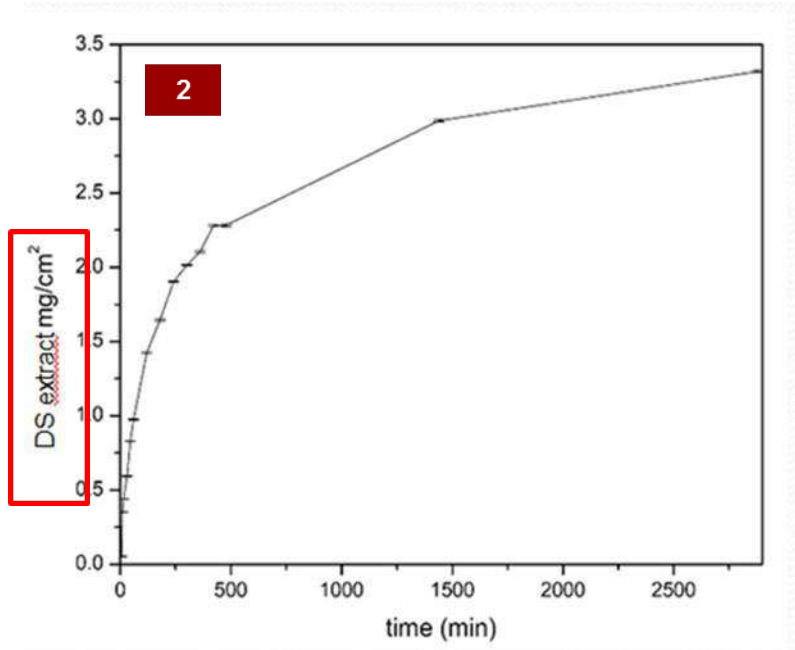
Test duration: 48 hours
Sampling 5,10,15, 30, 45, 60, 120, 240, 300, 360, 420, 480, 1440 and 2880 min
Receptor fluid: SWF (Simulated Wound Fluid) pH 6,5

NaCl 8.30 g
$CaCl_2$ 0.28 g
ultrapure water 1000 mL

Detection

UV-vis spectrophotometry (UV-Visible Agilent model 8453)
standard curve in SWF (λmax=280.0 nm, r = 0.9998)
average of three measurements (± SD)

Pagano, C.; Ceccarini, M.R.; Calarco, P.; Scuota, S.; Conte, C.; Primavilla, S.; Ricci, M.; Perioli, L.
Bioadhesive polymeric films based on usnic acid for burn wound treatment: Antibacterial and cytotoxicity studies. *Colloids Surf. B Biointerfaces* **2019**, *178*.

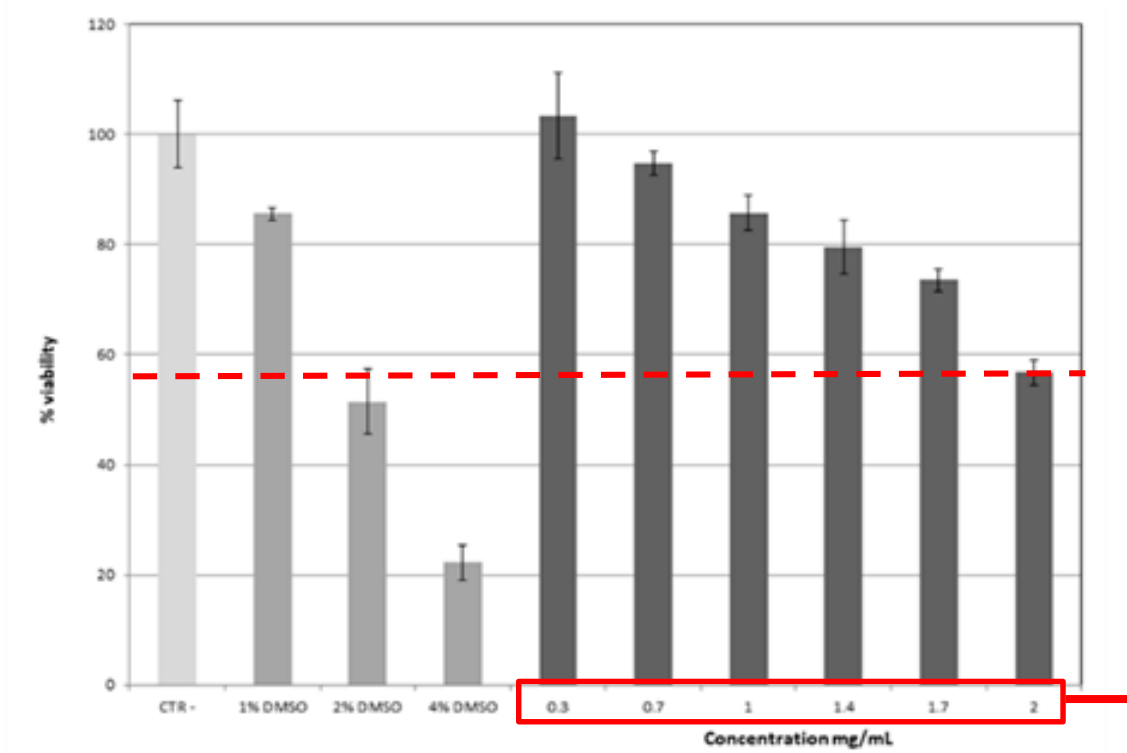


It is interesting to evaluate the amount of DS extract released per **unit area** (mg/cm^2) as hydrogel **film of different dimensions** can be prepared in order to be used for wounds of different size.

the amount of **DS extract per cm^2** was enough to obtain effective concentrations for both the antibacterial and anti-inflammatory activities.

These observations that the formulated hydrogel film B2 is a **suitable delivery system for DS extract for dermal applications in wounds treatment.**

In vitro safety studies of hydrogel film on HaCaT cell line



Evaluation of hydrogel film B2 cytotoxicity and safety on HaCaT cell line by MTT assay

MTT assay on HaCaT cell line

concentrations (0.25, 0.5, 1, 1.5, 2.0 mg/mL) obtained from hydrogel **film B2** (circle 3.14 cm^2) **incubated 24 h at 37°C in DMEM** complete medium and then used to treat HaCaT cells for 24 h.

MTT assay performed with the formulated DS extract showed resultsvery similar to the unformulated one.

D.S. extract alone and formulated in the hydrogel film became cytotoxic at the concentration of **2 mg/mL** that was above the maximum concentration obtained from the hydrogel film.

Both D.S. extract alone and hydrogel film B2 were safe on an *in vitro* skin model.

Topic #1- red onion skin

EUROPEAN MARKET

Albania

Switzerland

Poland

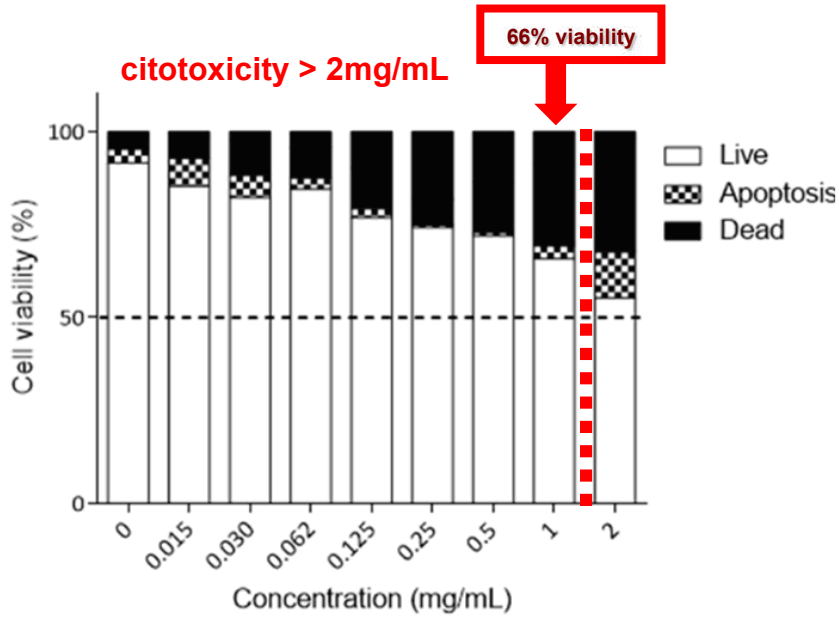
therapeutic indications:

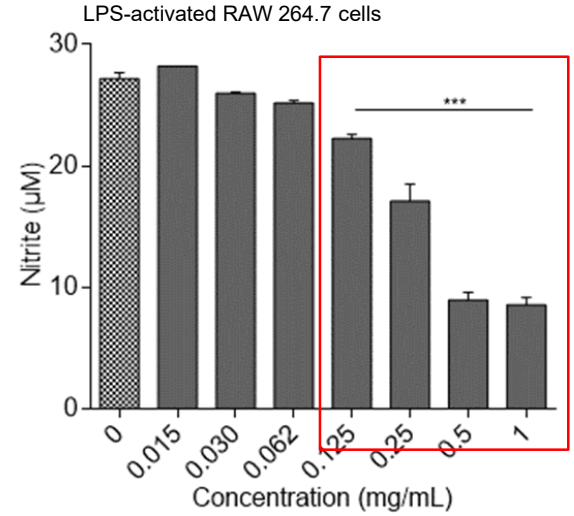
eudermic, skin softener,
anti redness,
treatment of wound, hypertrophic scars, keloids

only from the pulp

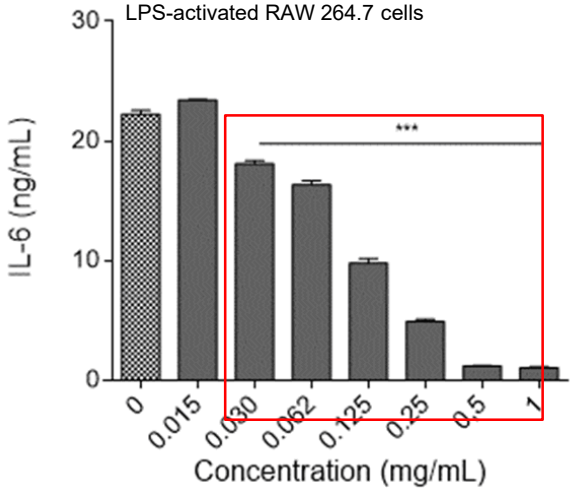
A.D. 1308 unipg

antiinflammatory tests

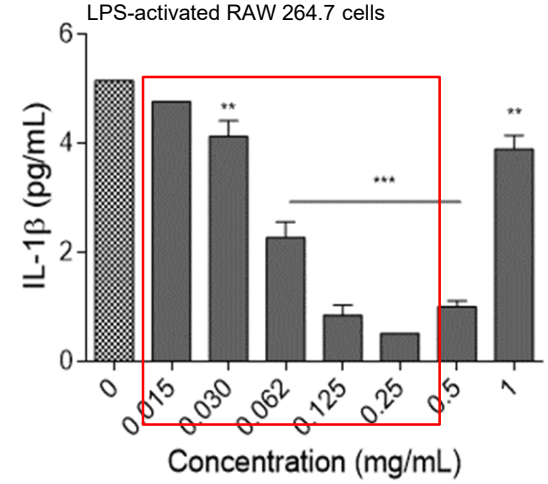




Significant reduction in the NO concentration proportional to the increase in the concentration of the extract



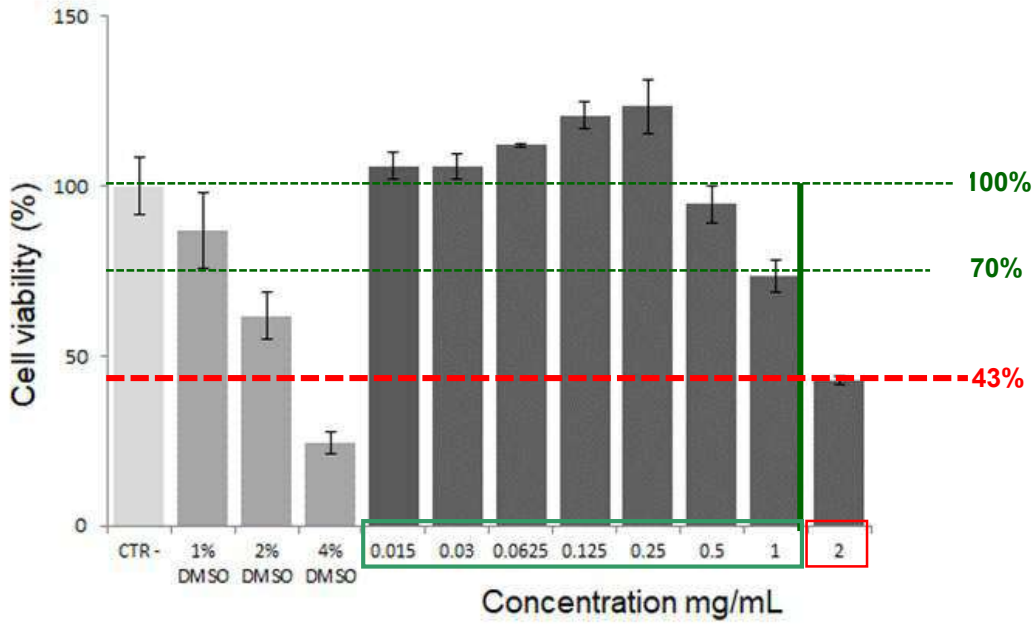
inhibitory effect of the extract on IL-6 production



Inhibitory effect in the concentration range 0.015 - 0.25 mg / mL of extract

An, Z.; Su, J. Acinetobacter baumannii outer membrane protein 34 elicits NLRP3 inflammasome activation via mitochondria-derived reactive oxygen species in RAW264.7 macrophages. Microbes Infect. 2018, 3-4, 143-153.
Escandell, J. et al., Bcl-2 is a negative regulator of interleukin-1β secretion in murine macrophages in pharmacological-induced apoptosis. Br. J. Pharmacol. 2010, 160, 1844–1856.

safety of the extract: citotoxicity studies



24 h incubation, DS extract concentation 0.015-2 mg/mL

- up to 1 mg/mL cell viability higher than 70%
- higher than 2 mg/mL cell viability value reaches the value of 43%

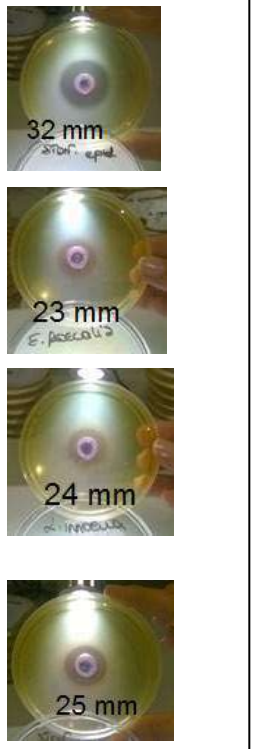
DS extract is safe for both cell lines (RAW 247 – HaCat) in the concentration range 0.015 - 1 mg/mL, while at 2 mg/mL the viability of both cell lines decreased around 50%.

The antioxidant, anti-inflammatory and antibacterial activities observed for pure OLE suggested that it could be a suitable active ingredient for wounds treatment

MICROBIOLOGICAL TESTS

Agar diffusion method: the Petri dishes were prepared by adding to the previously dissolved agar 1 ml of a bacterial suspension containing 10^5 UFC of the chosen microorganism, cooled and then used to seed 100 µl of solubilized extract in water.

	Bacterial strains	Inhibition halos (mm) DS extract 30 mg/mL
GRAM +	*Staphylococcus epidermidis* WDCM 00036	32
GRAM +	*Enterococcus faecalis* WDCM 00087	23
GRAM +	*Lactobacillus sakei* WDCM 00015	24
GRAM +	*Bacillus cereus* WDCM 00001	25
GRAM +	*Listeria innocua* WDCM 00017	24
GRAM +	*Clostridium perfrigens* WDCM 00007	n.i.
GRAM +	*Staphylococcus aureus* WDCM 00034	25
GRAM -	*Pseudomonas aeruginosa* WDCM 00025	n.i.
GRAM -	*Klebsiella pneumoniae* WDCM 00097	20
GRAM -	*Enterobacter aerogenes* WDCM 00175	16
GRAM -	*Escherichia coli* WDCM 00013	17



n.i.= no inhibition

A.D. 1308 unipg

Conclusions

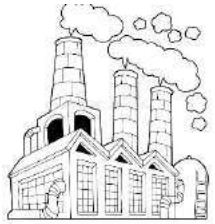
Patient

The bioadhesive film represents an effective treatment and it is safe, comfortable, free from bacterial resistance problems, easy applicable to injured skin, pain-free, easy removable by washing.

Environment

Onion skins are wastes.
Their use is an eco-friendly solution able to recycling and valorizing waste material and not substract biomass from the food sector, in accordance with the principles of food ethics and circular economy.

Industry



dry skin extract production is performed by green methods without production of special wastes and are low cost

Dynamic Maceration

Best Extraction method

Freeze dried petals
(2.46 g)

EtOH 70%

filtration under
vacuum

Petals were frozen immediately after the separation from stigma and freeze-dried in order to maintain the fresh flower original features, and then used for the extraction.

EtOH 70% **SE-A_1**

Freeze-drying

Drying
(rotary evaporator)

Extraction (Method B) → Maceration in ultrasonic bath

Freeze dried petals
(2.46 g)

EtOH 70% (200 ml), 10 min and
kept overnight in static conditions
at R.T.

filtration under vacuum

Drying
(rotary evaporator)

Freeze-drying

SE-B

Yield%: 56.70± 3.62

water solubility: 262.3 mg/ml

Antioxidant activity

Sample	Total phenols		FRAP		ABTS		DPPH	
	mg GAE/g	SD	mg TE/g	SD	mg TE/g	SD	mg TE/g	SD
SE-A_1	**5.14**	0.02	**0.33**	0.00	**9.76**	0.74	**2.62**	0.12
SE-B	**3.89**	0.02	**0.20**	0.00	**6.12**	0.13	**1.77**	0.03

UHPLC

Chemical analysis (UHPLC analysis)

Gallic Acid	Chlorogenic Acid	Caffeic Acid	EGCG	Syringic Acid	Orientin	Rutin	Isoquercetin	3-OH-Benzoic Acid
58.86 ± 8.95	54.67 ± 10.22	0.55 ± 0.11	5.29 ± 0.87	0.24 ± 0.04	0.15 ± 0.02	<LOQ	3.37 ± 0.53	<LOQ

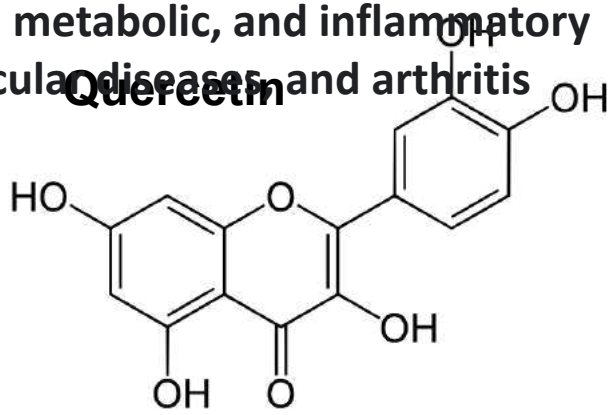
p-Coumaric Acid	Ferulic Acid	Myricetin	Luteolin	Quercetin	Apigenin	Kaempferol	Naringenin	Isoxhanthoumol
1.86 ± 0.31	0.32 ± 0.06	3.88 ± 0.69	0.35 ± 0.06	23.09 ± 3.46	0.02 ± 0.01	8.77 ± 1.46	5.09 ± 0.92	2.43 ± 0.36

Topic #2 - Crocus sativus L.

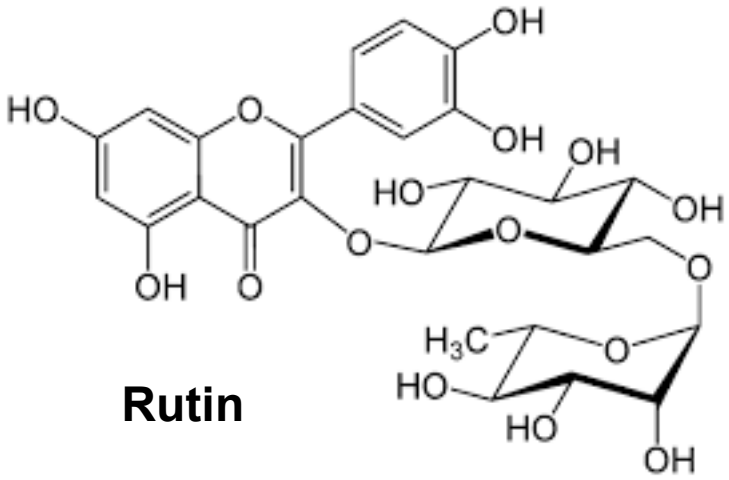
sent in several food products that
cological activities, such
tiprotozoal, and antimicrobial
gic, metabolic, and inflammatory
ascular diseases, and arthritis

Saffron petals….
a source of active ingredients

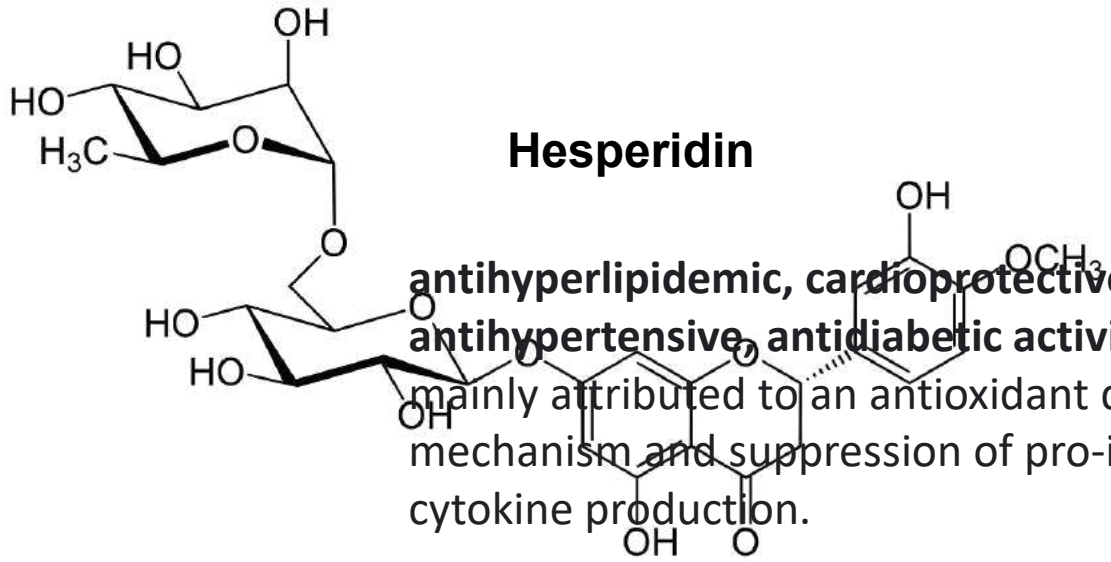
Quercetin



Rutin



Hesperidin

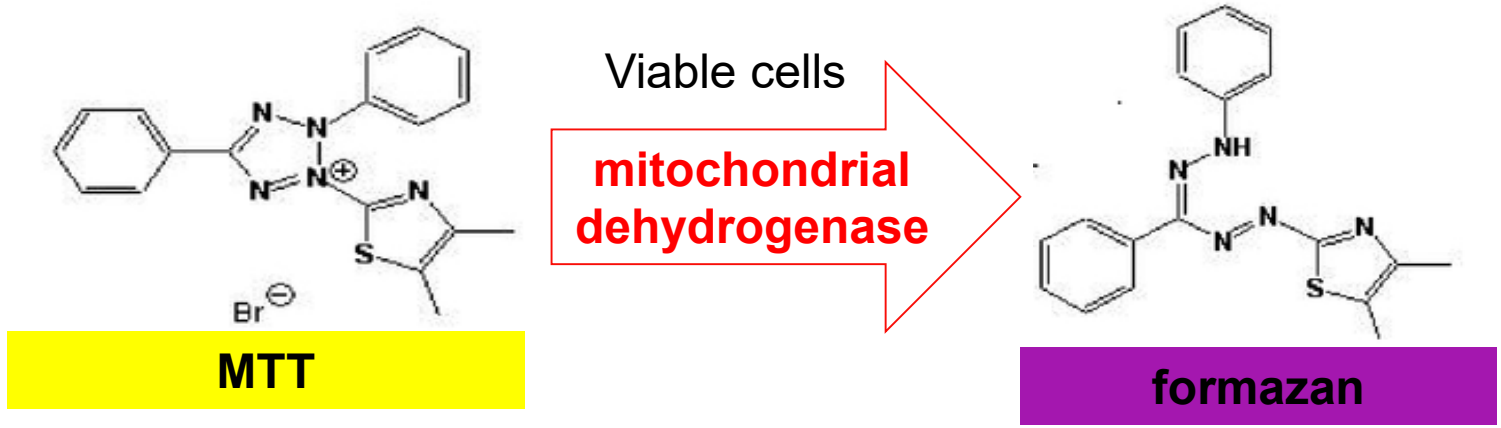


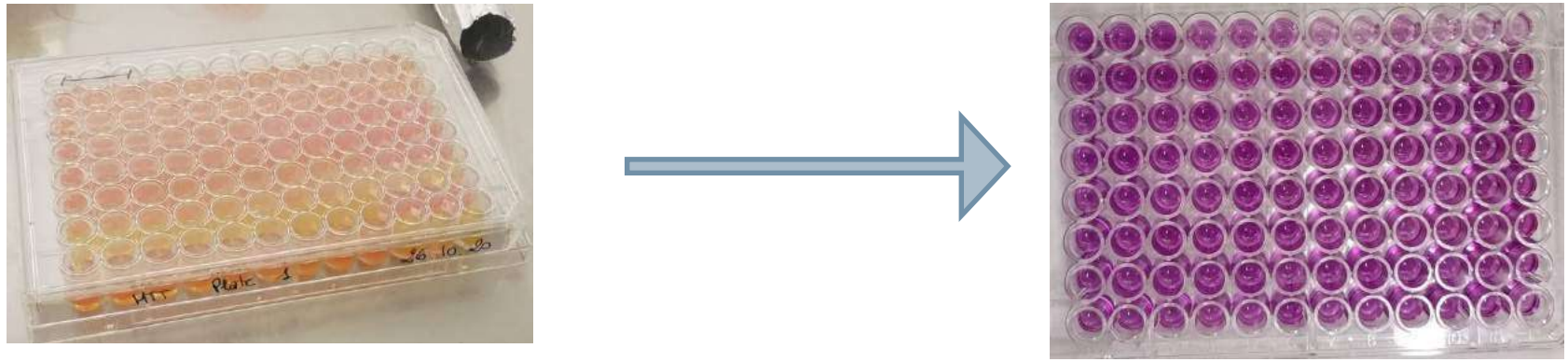
antihyperlipidemic, cardioprotective, antihypertensive, antidiabetic activities, mainly attributed to an antioxidant defer mechanism and suppression of pro-inflar cytokine production.

antibacterial, antiprotozoal, antitumor, antiinflammatory, antiallergic, antiviral, cytoprotective, vasoactive, hypolipidaemic, antiplatelet, antispasmodic, and antihypertensive

Extract characterization → Effect on cells

Cytotoxicity → HaCaT (immortalized human keratinocytes) model cell system representative of epidermidis





SE-A_1 solubilized in *DMEM (1 mg/ml)

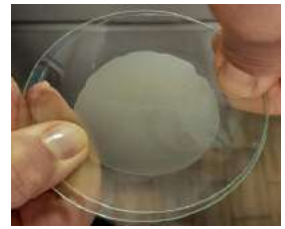
Dulbecco's Modified Eagle Medium (DMEM)

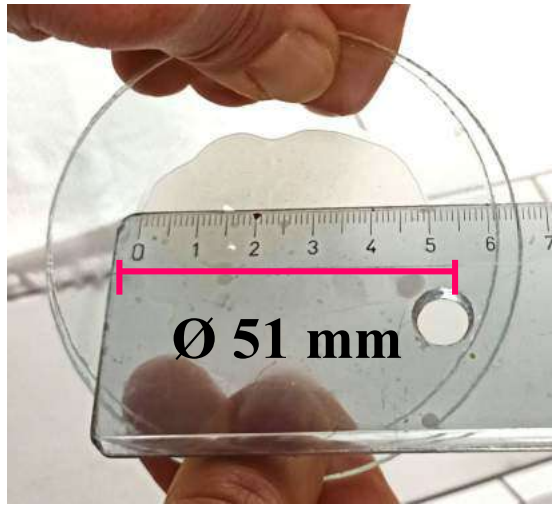
Spreadability assay

Determination of Spreadability

One gram of emulsion was pressed between two horizontal plates 20 cm square, upper one of which weighed 125 g, and its diameter (∅) was measured after one min.

Under the experimental conditions, the following classification was adopted : semi-stiff creams ∅ ≤ 50 mm and semi-fluid creams 50 < ∅ ≤ 70 mm (7).





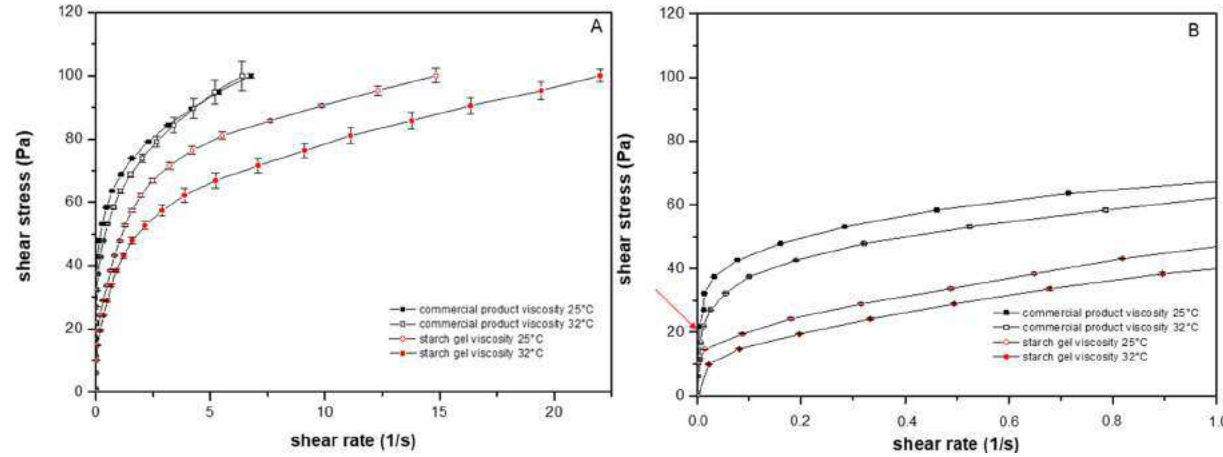
Semifluid creams

easy application on the skin

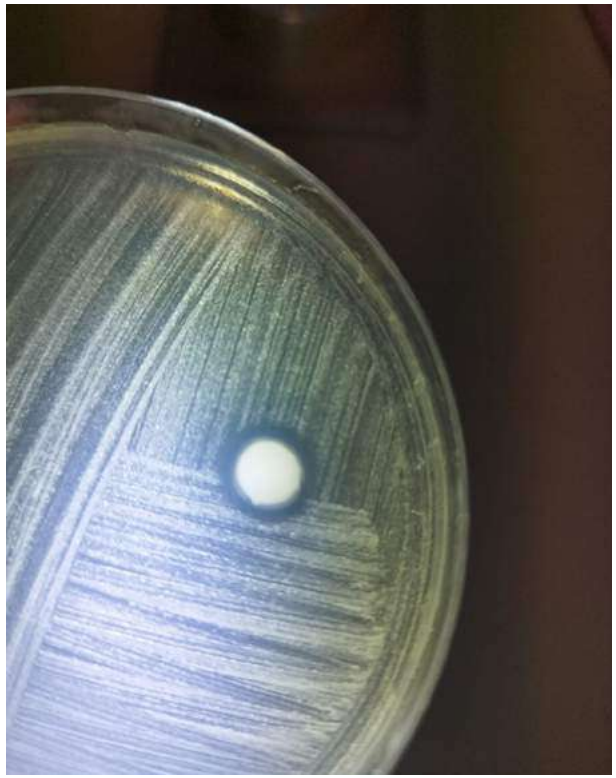
Formulation	Sample after eight 4°C/45°C cycles of 24 h	Sample after eight -20°C/25°C cycles of 24 h	Sample after centrifugation	Observations
Commercial gel				Temperature cycling tests: 1. discoloration; 2. appearance of black particles (red circles) for the sample treated -20°C/25°C. Centrifugation: sedimentation of solid particles/aggregates dispersed in the gel (red arrow).
Starch gel loaded with SE-A1-CS				Temperature cycling tests: no changes observed. Centrifugation: no changes observed.

Comparison with a commercial product

- Stability
- Rheological properties



Antibacterial activity



S. epidermidis

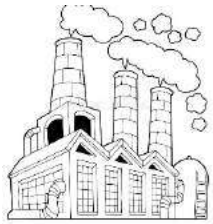
Agar diffusion technique

activity attributable to phenolic compounds, especially gallic and chlorogenic acids that are the most abundant in the extract.

CONCLUSIONS and ADVANTAGES

patient as the bioadhesive film represents an effective treat and it is safe, comfortable, free from bacterial resistance problems, easy applicable to injured skin, pain-free, easy removable by washing.

environment as onion skins are wastes.
Their use is an eco-friendly solution able to recycling and valorizing waste material and not substract biomass from the food sector, in accordance with the principles of food ethics and circular economy.



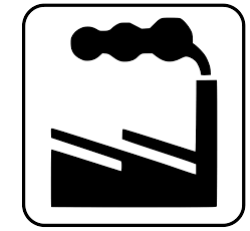
industry as dry skin extract production is performed by green methods without production of special waste

Conclusions

- ✓ the starting materials used derivers from cultivars for textile, food and cosmetic use as well as from food wastes as chitosans (obtained from shrimp exoskeleton and crab shell).
- ✓ The developed formulations are innovative, useful for topical treatments avoiding systemic treatments and consequent cross reactions;
- ✓ promising alternative to conventional treatments;
- ✓ useful for self-administration;
- ✓ the extraction and production procedures are eco-friendly, scalable and low cost.

environment

patient



industry

Topic #2

MORINGA OLEIFERA

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Preparation and characterization of polymeric microparticles loaded with *Moringa oleifera* leaf extract for exuding wound treatment

Cinzia Pagano[a], Luana Perioli[a,*], Claudio Baiocchi[b], Alessandra Bartoccini[a], Tommaso Beccari[a], Francesca Blasi[a], Paola Calarco[a], Maria Rachele Ceccarini[a], Lina Cossignani[a], Alessandro di Michele[c], Roberta Ortenzi[d], Stefania Scuota[d], Maurizio Ricci[a]

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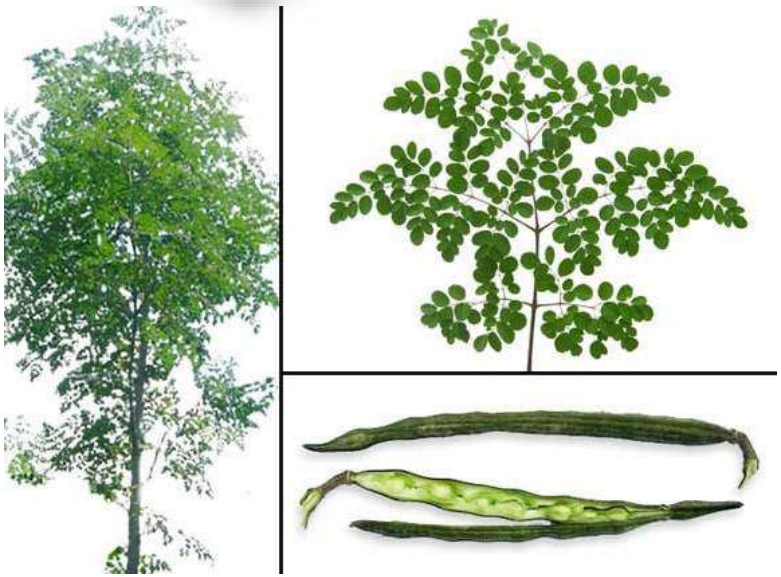
[b] Department of Molecular Biotechnology and Health Sciences, Sect. Analytical Chemistry, Via Pietro Giuria 5, 10125 Torino, Italy
[c] Department of Physics and Geology, University of Perugia, via Pascoli, 06123 Perugia, Italy
[d] Istituto Zooprofilattico dell'Umbria e delle Marche, via G. Salvemini, 1, Perugia, Italy

Introduction



Rich of many bioactive compounds useful in health field

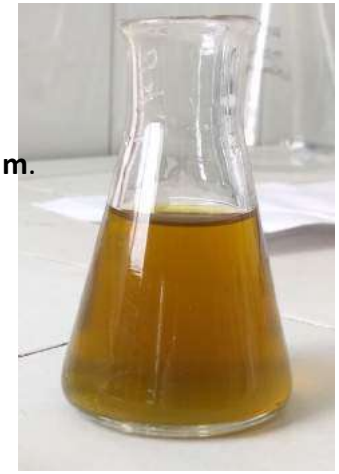
- **minerals**: calcium, potassium, zinc, magnesium, iron and copper
- **vitamins**: β-carotene, folic acid, pyridoxine, nicotinic acid, vitamin C, D and E,
- **phytochemicals**: tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, reducing sugar and their glucosinolates, isothiocyanates and glycoside compounds.

Extration process

Moringa leaf powder
(660 mg)

EtOH/water 50:50 v/v (40 ml), 45°C, 90 min, 400 rpm.

Centrifugation (4000 rpm 10 min). Filtration under vacuum.



Solvent evaporation

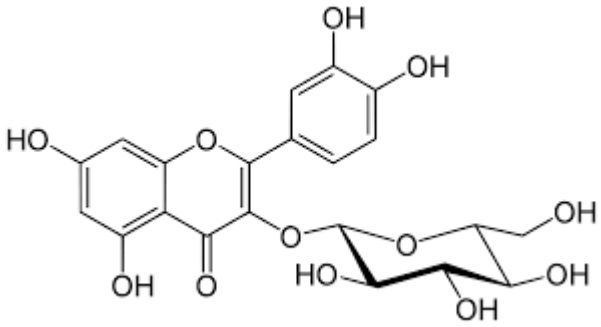
Dissolution of the residue in distilled water and freeze-drying.

Freeze-dried extract (MOE)

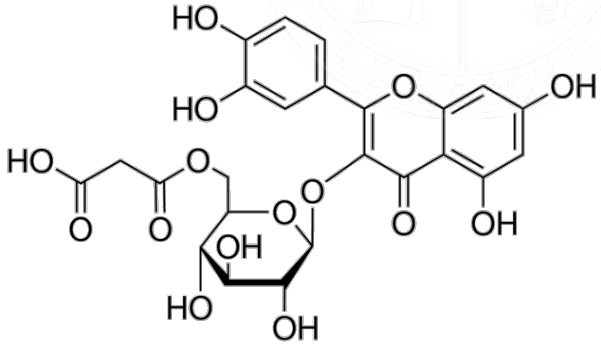
Yield MOE/ leaf powder = 29%
Water solubility 25°C =46.72 mg/mL

Characterization of extracts

Freeze-dried extract (MOE)



quercetin-O-glucoside



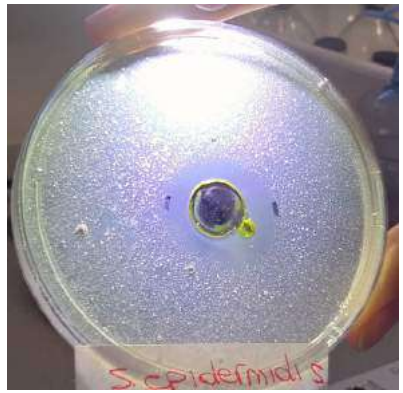
quercetin-O malonyl glucoside

Sample	MO leaves (mg)	MOE (mg)	TPC mg GAE/g	FRAP µmol Fe^{2+} /g	ABTS mg TE/g
mean	330.20	117.25 ± 0.63	14.87 ± 0.74	150.07 ± 14.45	23.50 ± 0.45

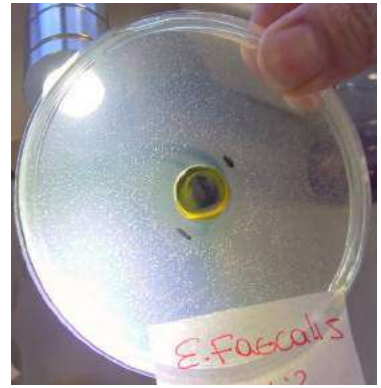
Antimicrobial activity

Agar diffusion method:
Incubation 37°C for 24 hours (48 hours for *S. pyogenes*).

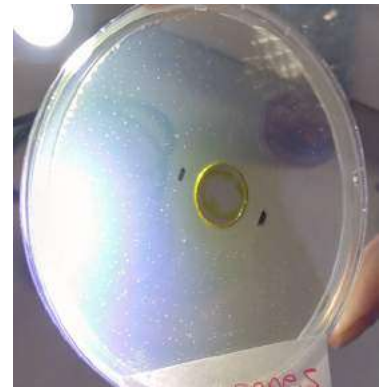
50 mg/ml



S. Epidermidis (26 mm)



E. faecalis (21 mm)



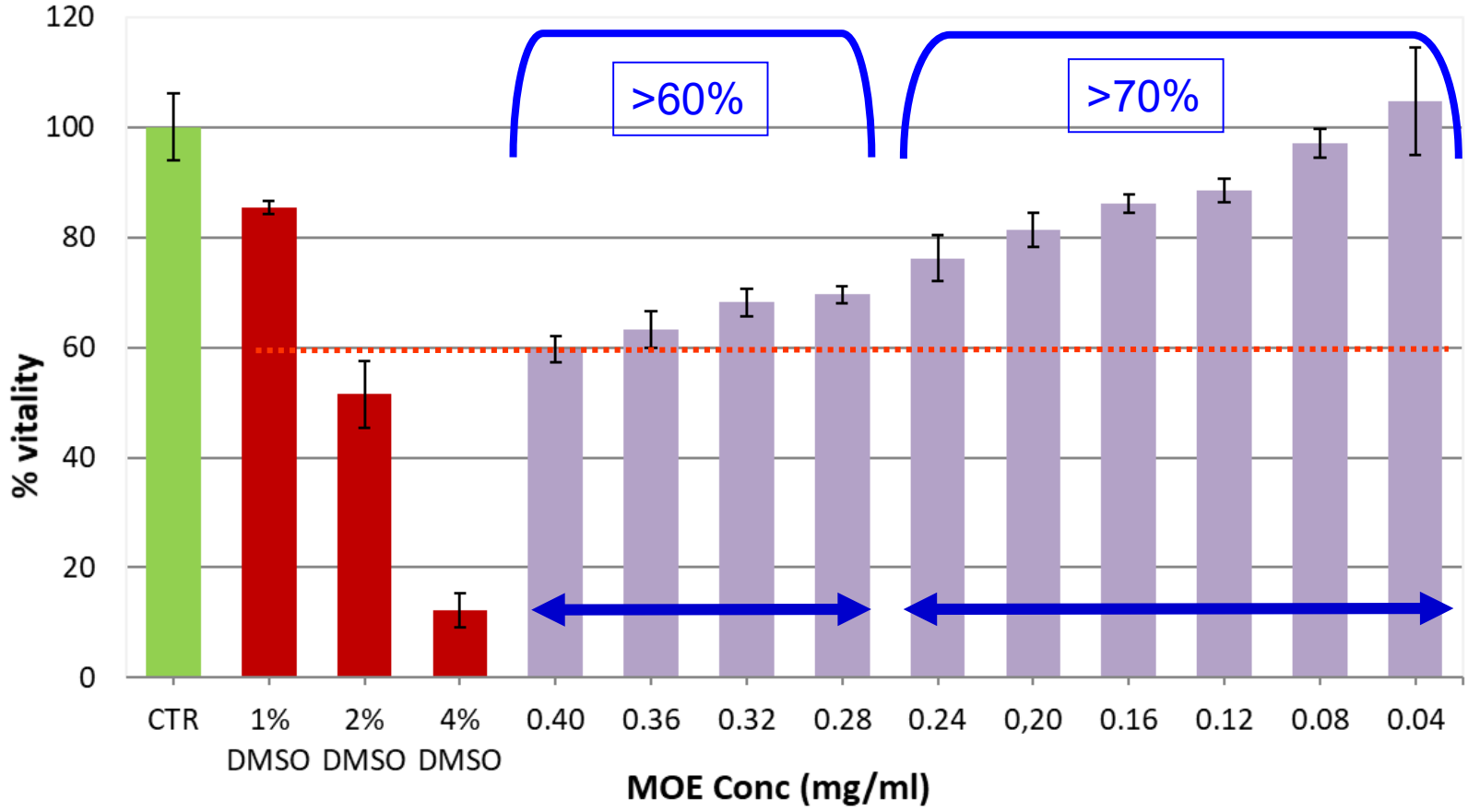
S. Pyogenes (24 mm)

S. Aureus (22 mm)

minimum inhibitory concentration (MIC) Minimum bactericidal concentration (MBC)

	Staphylococcus epidermidis	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Enterococcus faecalis***
MIC (mg/ml)	12.50	6.25	0.78	25.0
MBC (mg/ml)	-	12.50	3.13	-

Studies on cells: HaCaT (immortalized human keratinocytes)



viability > 60% denotes the safety of the tested samples

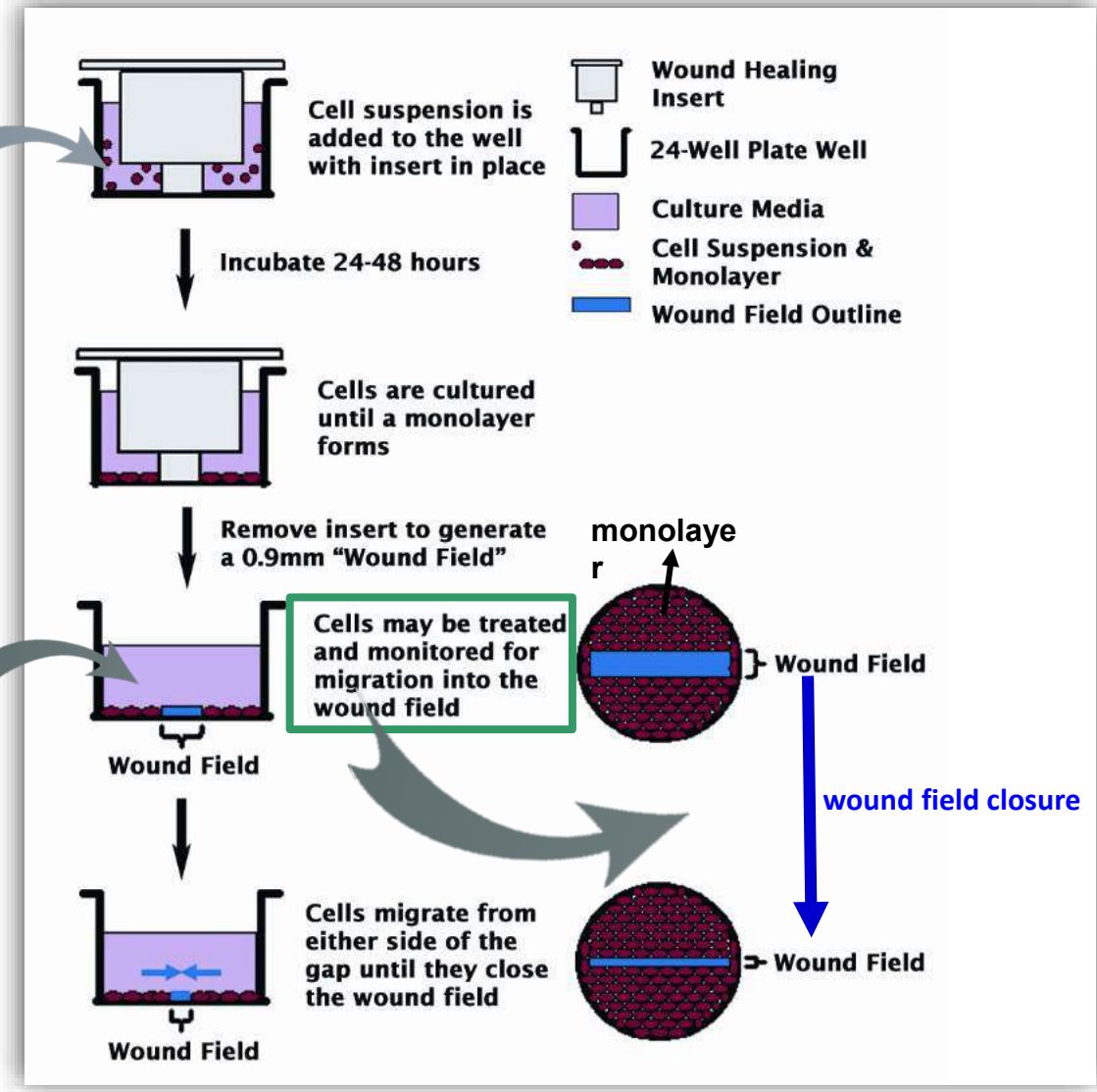
MOE is safe for keratinocytes at the tested concentrations

CytoSelect™ Wound Healing Assay Kit

HaCaT
(DMEM containing 10% FBS)

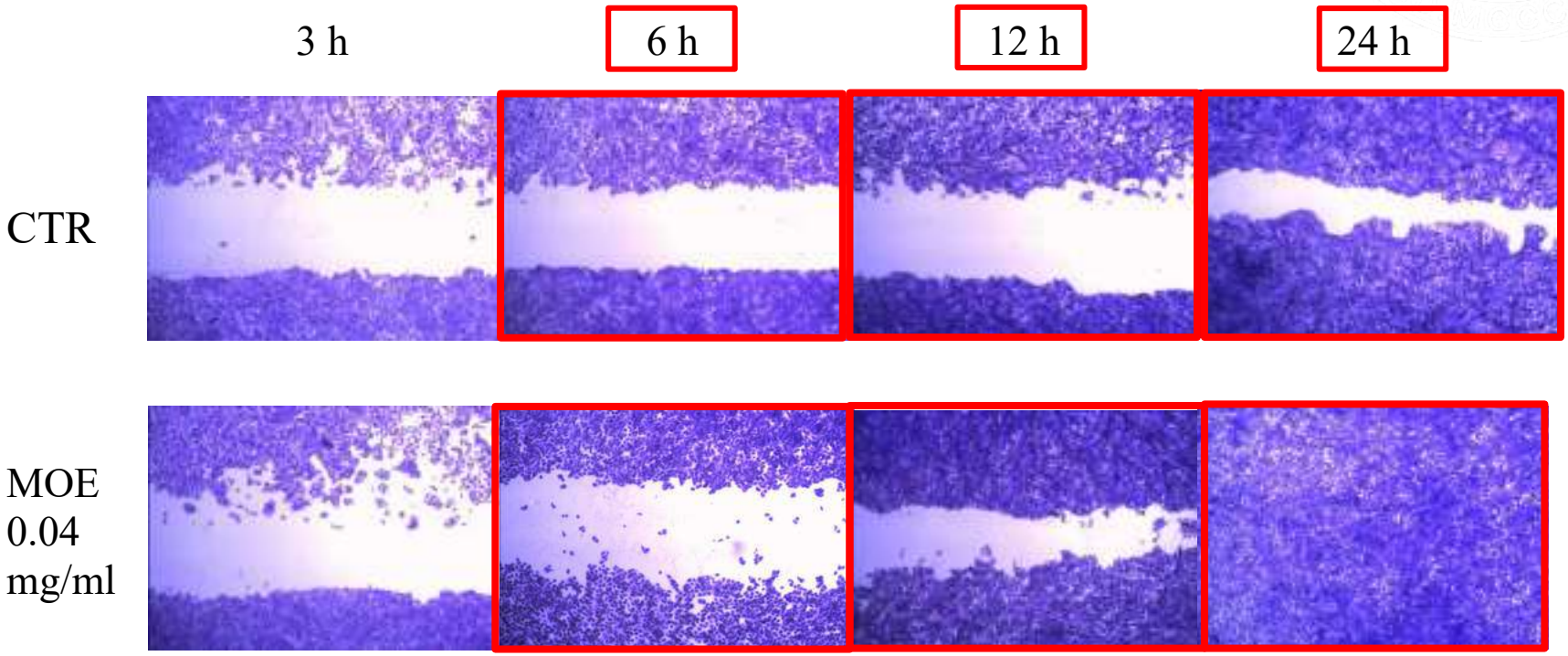
can MOE stimulate cell growth?

MOE solution
in DMEM



Wound field closure evaluation: 3, 6, 12 and 24 hours

Characterization of extracts



MO

- Antioxidant
- Anti-inflammatory
- Antimicrobial

formulation advantages

- Easy application
- Protection from mechanical solicitation and external agents
- High residence time
- Atraumatic/pain free removal (by washing)

microparticles preparation

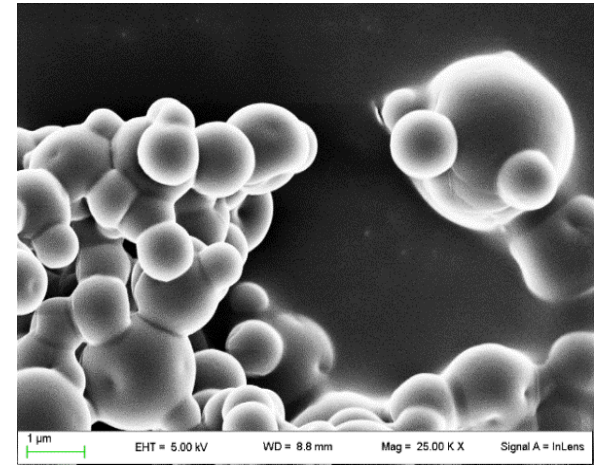
B290 mini spray-dryer (Büchi, Italy)

Microparticles (MP):

- **Chitosan** low molecular weight (75-85% deacetylated, in 1% (v:v) acetic acid solution)
- **MOF** (4:1 CS:MOF ratio wt:wt %)

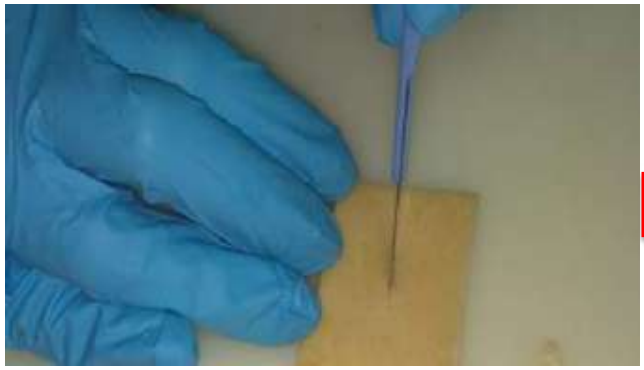
spray-drying conditions:

-aspiration rate 27 m^3/h,
-feed flow rate 3.5 ml/min,
-atomizing air flow 357 l/h
-Inlet temperature 110°C



F.C. Stenger Moura, L. Perioli, C. Pagano, R. Vivani, V. Ambrogi, T.M. Bresolin, M. Ricci, A. Schoubben. *Chitosan composite microparticles: A promising gastroadhesive system for taxifolin.* 2019, Carbohyd Polym, Vol. 218, p. 343-354.
L. Cruz, E. Fattal, L. Tasso, G. C. Freitas, A. B. Carregaro, S. S. Guterres, A. R. Pohlmann, , N. Tsapis. *Formulation and in vivo evaluation of sodium alendronate spray-dried microparticles.* 2011, J Control Release, Vol. 152, p. 370–375.

ex-vivo swelling capacity



simulated wound (3 cm) produced on pig skin sample obtained by a scalpel

simulated wound filled with SWF (100 µl)

form a complete hydrated film

deposition of 10 mg of MP on the wound.

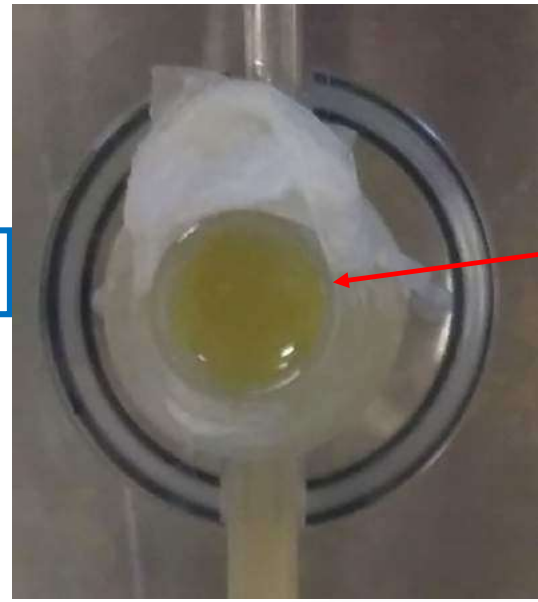
cover and protect the wound

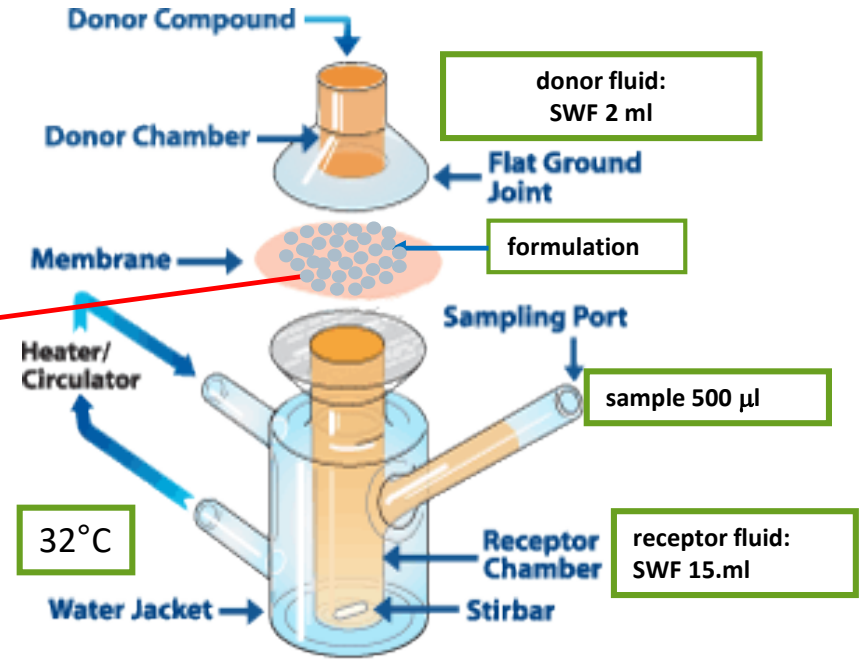
rapid film formation (10 min)

In vitro release studies

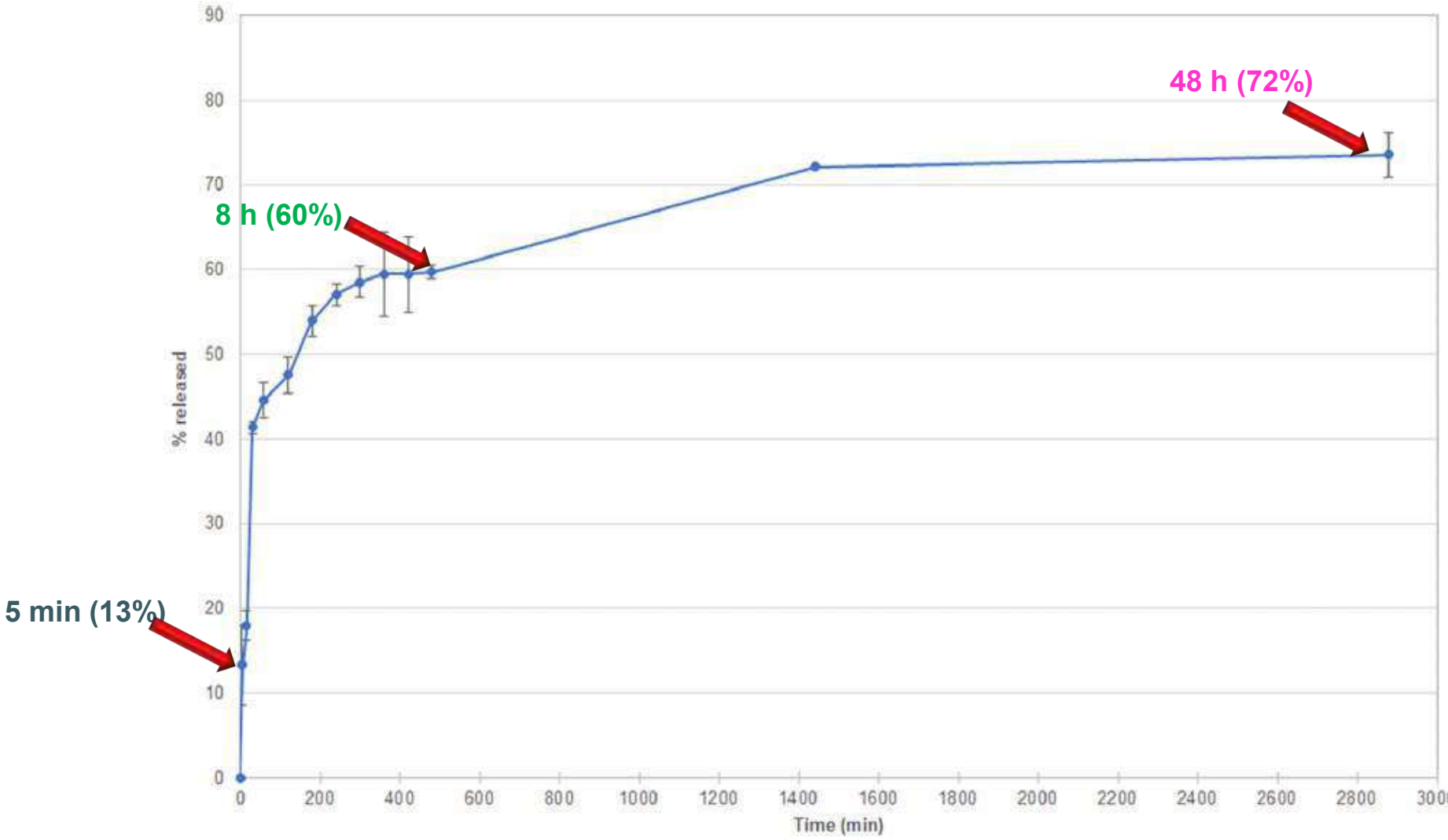
Franz cell

Microparticles (40 mg) hydrated with 2 ml SWF





In vitro release



Concluding remarks

Patient

- The **PETAL SAFFRON EXTRACT**, showed suitable antioxidant activity as well as ability to stimulate keratinocytes growth
- The extract formulated as starch-based hydrogel (showing suitable rheological properties as well as spreadability) demonstrated antimicrobial activity toward *S. epidermidis* and self-preserving capacity

The extract, thus formulated, can be considered very useful to treat skin diseases such as superficial wounds

Concluding remarks

Patient

- The bioadhesive film represents an effective treatment and it is safe, comfortable, free from bacterial resistance problems, easy applicable to injured skin, pain-free, easy removable by washing

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environment

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- ✓ promising alternative to conventional treatments;
- ✓ useful for self-administration;
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patient

industry

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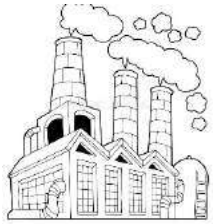
Onion skins are wastes.
Their use is an eco-friendly solution able to recycling and valorizing waste material and not substract biomass from the food sector, in accordance with the principles of food ethics and circular economy.

Industry



dry skin extract production is performed by green methods without production of special wastes and are low cost

WHO PRIORITY PATHOGENS LIST FOR R&D OF NEW ANTIBIOTICS

Priority 1: CRITICAL#

- ***Acinetobacter baumannii***, carbapenem-resistant
- ***Pseudomonas aeruginosa***, carbapenem-resistant
- ***Enterobacteriaceae****, carbapenem-resistant, 3rd generation cephalosporin-resistant

Priority 2: HIGH

- ***Enterococcus faecium***, vancomycin-resistant
- ***Staphylococcus aureus***, methicillin-resistant, vancomycin intermediate and resistant
- ***Helicobacter pylori***, clarithromycin-resistant
- ***Campylobacter***, fluoroquinolone-resistant
- ***Salmonella*** *spp.*, fluoroquinolone-resistant
- ***Neisseria gonorrhoeae***, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- ***Streptococcus pneumoniae***, penicillin-non-susceptible
- ***Haemophilus influenzae***, ampicillin-resistant
- ***Shigella*** *spp.*, fluoroquinolone-resistant

* Enterobacteriaceae include: ***Klebsiella pneumonia***, ***Escherichia coli***, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp, *Morganella* spp.

Currently, at least **700,000 people** die each year due to drug-resistant diseases, including 230,000 people who die from multidrug-resistant tuberculosis.

Around **2.4 million** people could die in high-income countries between **2015 - 2050** without a sustained effort to contain antimicrobial resistance.

Topical therapy to treat drug resistant infections

Report to the secretary general of the united nations (April 2019). No time to wait: securing the future from drug-resistant infections.

Pycnogenol (PYC)

extract from the bark of the French maritime pine, *Pinus pinaster* ssp. *Atlantica*

outer bark deeeply fessured

from the ancient Greek
$\pi\upsilon\kappa\nu o\sigma$ (condensed) + $\gamma\varepsilon\nu o\sigma$ (class, family)

extract powder

pharmaceutics

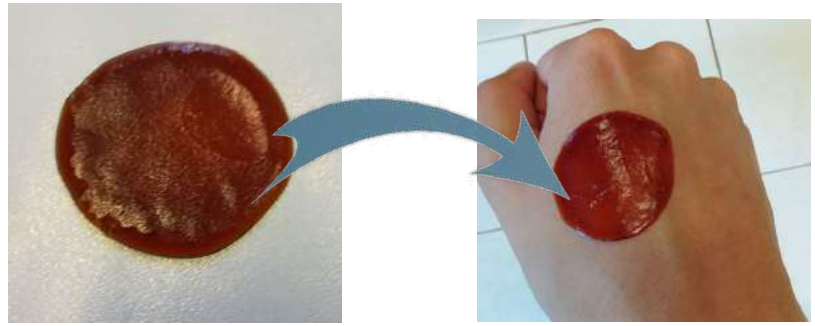
MDPI

Article

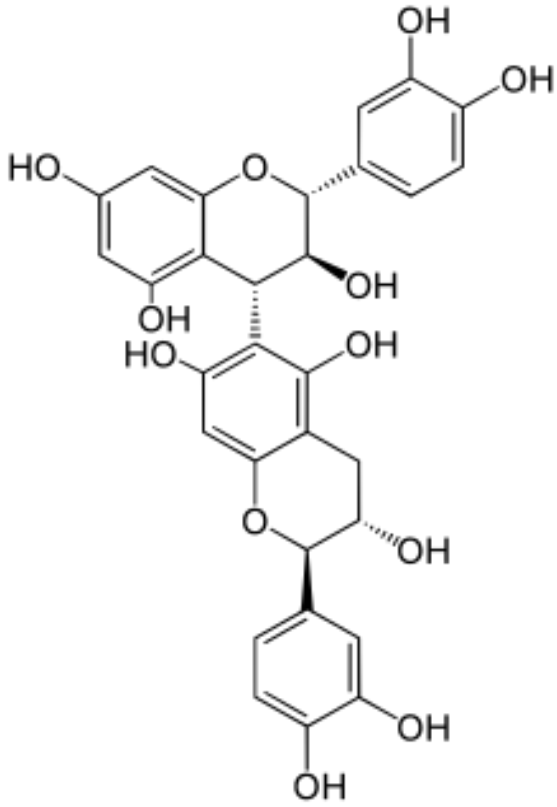
Development and Characterization of Xanthan Gum and Alginate Based Bioadhesive Film for Pycnogenol Topical Use in Wound Treatment

Cinzia Pagano [1,*], Debora Puglia [2], Francesca Luzi [2], Alessandro Di Michele [3], Stefania Scuota [4], Sara Primavilla [4], Maria Rachele Ceccarini [1], Tommaso Beccari [1], César Antonio Viseras Iborra [5], Daniele Ramella [6], Maurizio Ricci [1] and Luana Perioli [1,*]

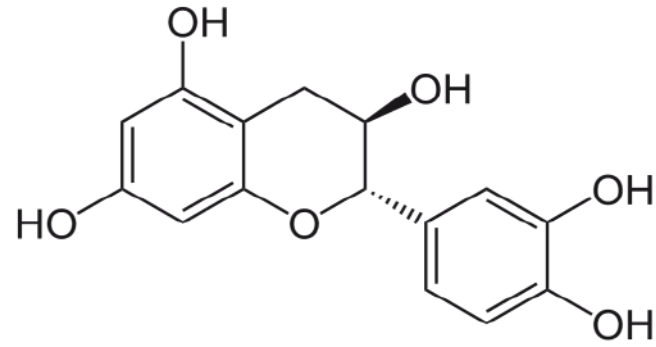
Pharmaceutics **2021**, *13*, 324. https://doi.org/10.3390/pharmaceutics13030324 www.mdpi.com/journal/pharmaceutics



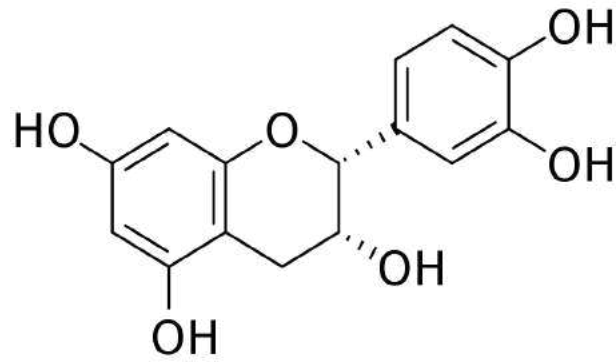
Active molecules



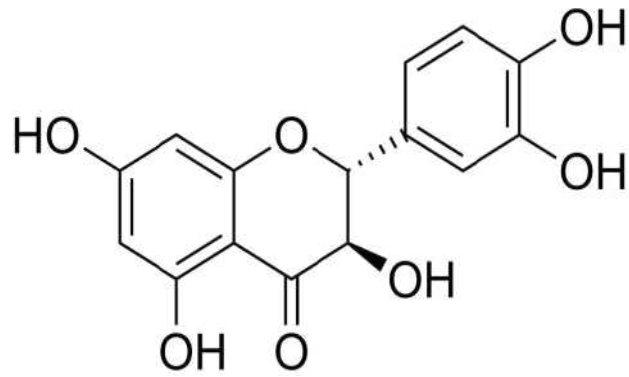
procyanidin



catechin



epicatechin



taxifolin

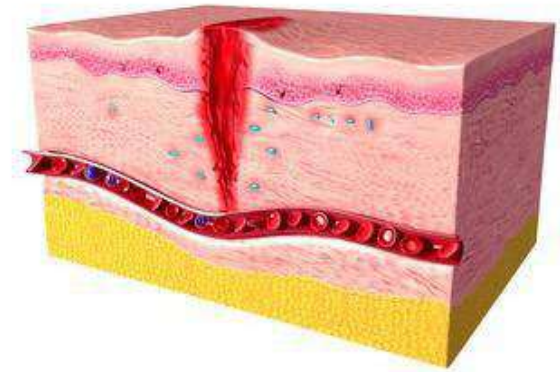
PYC activities and applications

✓ Traditional use is in the treatment of scurvy and wound healing

Anti-inflammatory

Antioxidant

Antimicrobial



Protection from UV-radiations

Stimulation of endothelial function

Useful for chronic venous disease, melasma

Polymers selection

Xanthan gum (XG)

natural heteropolysaccharide, produced by bacterium *Xanthomonas campestris*

Alginic acid sodium salt (AL)

co-polysaccharide composed of D-mannuronic acid and L-guluronic acid residues.

properties:
- biocompatible
- biodegradable
- non-toxic
- not irritating to the skin
- it has a wide range of applications

Pharmaceutical, food, cosmetic

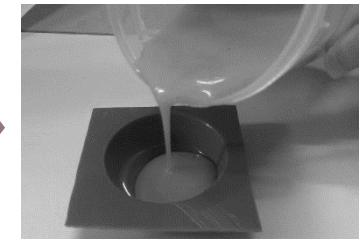
Film preparation

A) Hydrogel

B) Degassing

planetary mixer:
mixing: 3 minutes, 2000 rpm
defoaming: 5 minutes, 2000 rpm

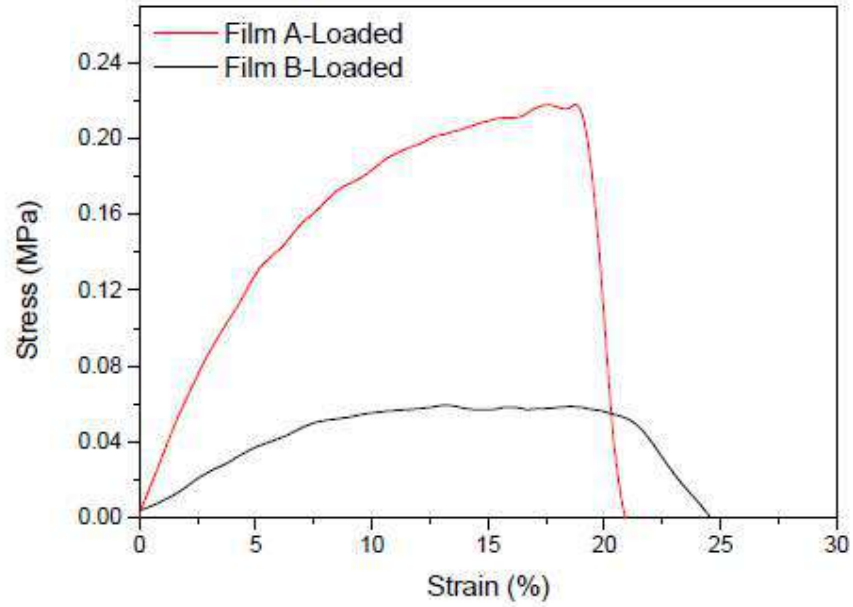
C) Casting



D) Drying

Film	AL (% wt/wt)	XG (% wt/wt)	PYC (% wt/wt)	glycerol (% wt/wt)	water (% wt/wt)
A-Loaded	1.50	3.02	20.10	40.20	35.17
B-Loaded	0.87	3.40	20.30	40.61	34.81

Mechanical characterization

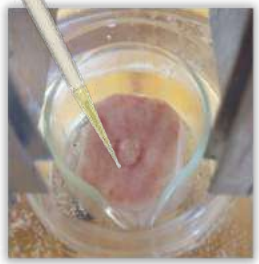


	σ_{max} (MPa)	$\varepsilon_{at\ \sigma max}$ (%)	E(MPa)
Film A-Loaded	0.215 ± 0.007*	17 ± 2**	3.070 ± 0.044***
Film B-Loaded	0.055 ± 0.005	18 ± 1	0.620 ± 0.044

Bioadhesion

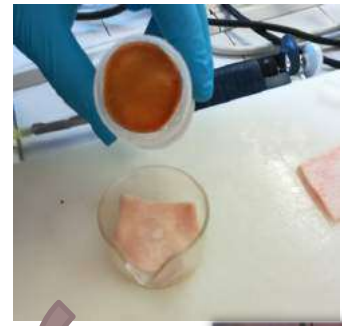
Simulated Wound Fluid (SWF) pH 6.5
NaCl (142 mM)
$CaCl_2$ (2.5 mM)

wetted 100 µl of SWF



Pig skin tissue (ear and shoulder region)

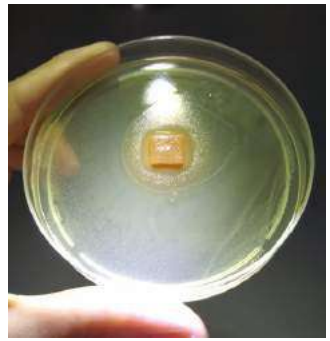
Film was attached at the bottom of a punch



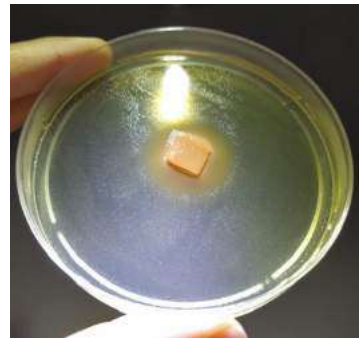
contact with skin (20 s) to induce adhesion

film	bioadhesion force (N) ± SD	bioadhesion time (sec) ± SD
A-loaded	0.25 ± 0.13	17.33 ± 4.50
B-loaded	0.07 ± 0.00	6.33 ± 0.57

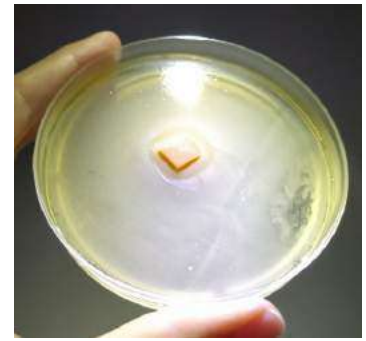
Antimicrobial activity



E. faecalis



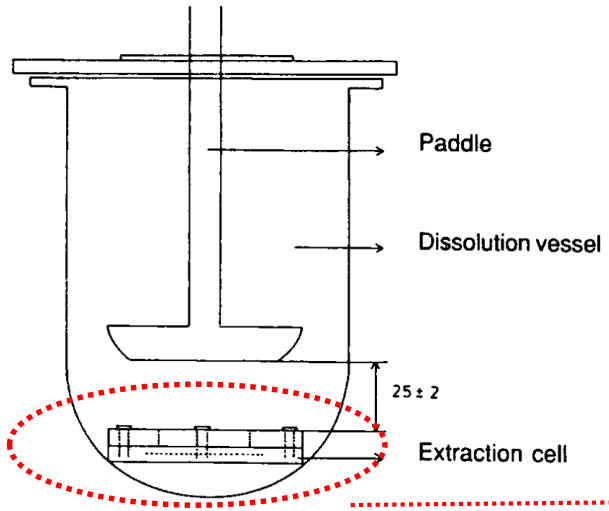
S. pyogenes

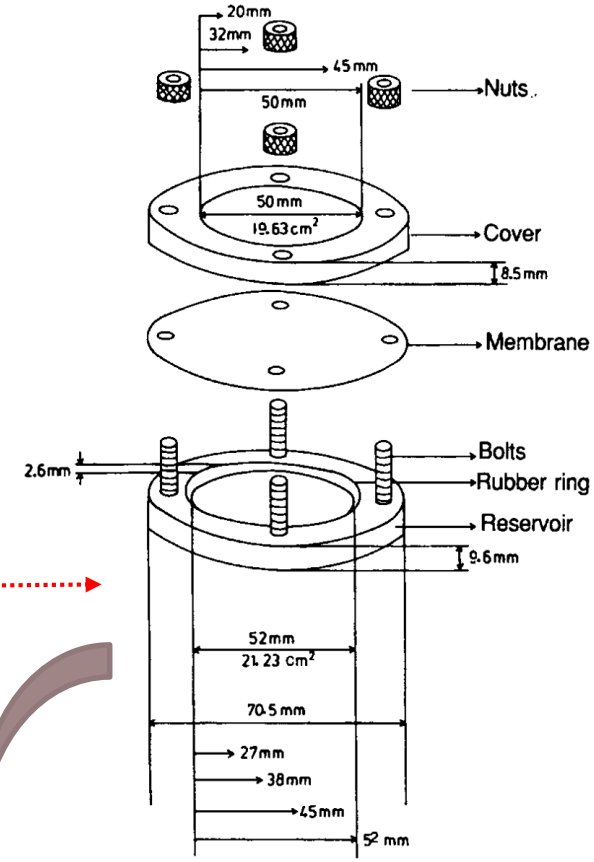


S. aureus

	film A-Loaded (mm)
K. pneumoniae	-
E. coli	-
P. mirabilis	-
S. aureus	19
S. epidermidis	-
E. faecalis	18
B. subtilis	-
S. pyogenes	24
P. aeruginosa	-
C. albicans	-

Release studies

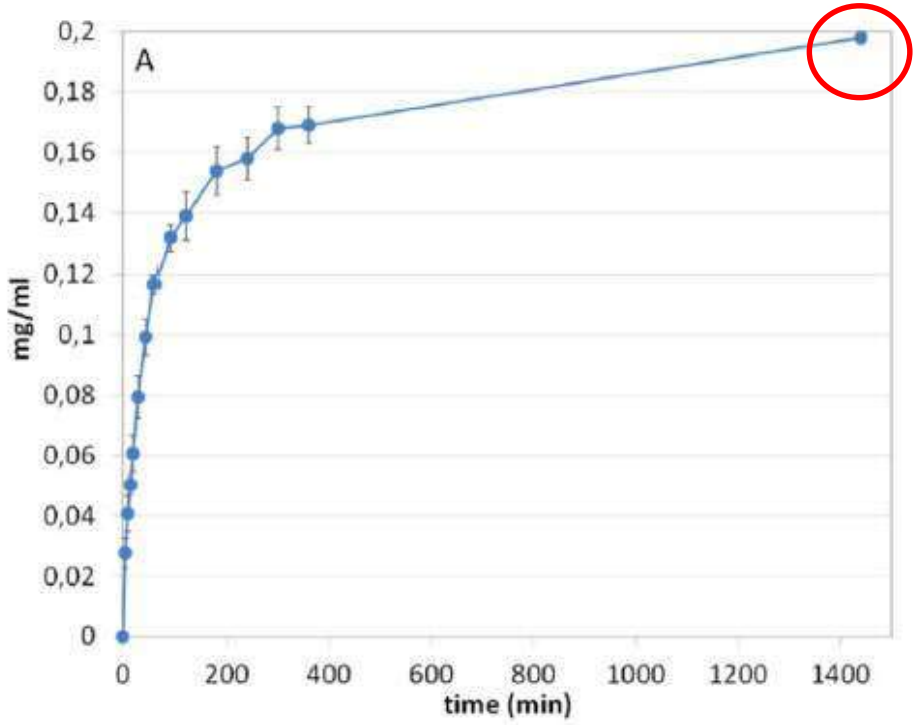


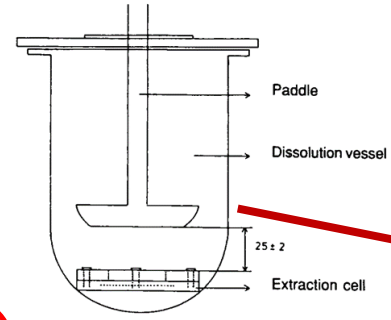


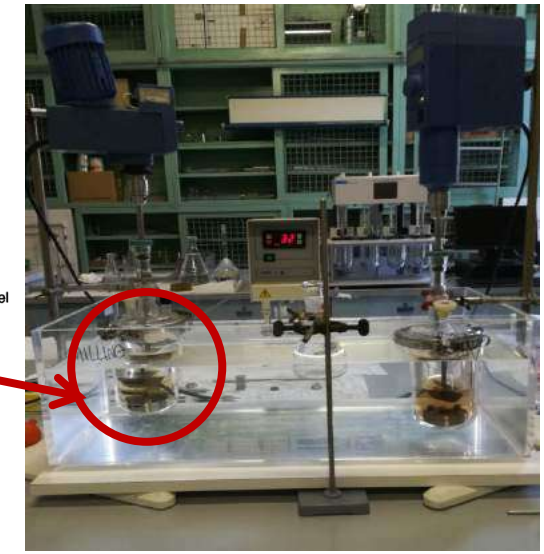
Transdermal films: extraction cell Ph. Eur. 10th Ed.

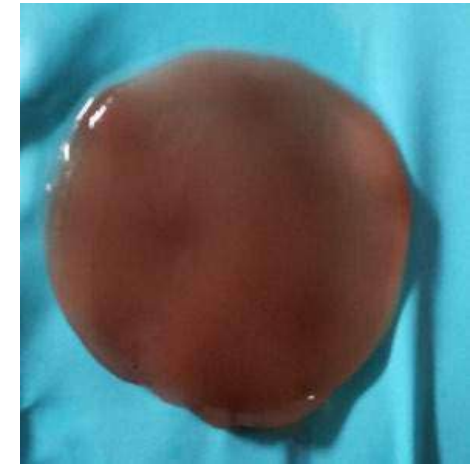
Experimental conditions:

- Dissolution media: SWF (Simulated Wound Fluid)
- Volume: 400 ml
- Temperature: 32°C
- Paddle speed: 40 rpm
- PYC quantification: UV spectrophotometry (λ_{max} = 281 nm)



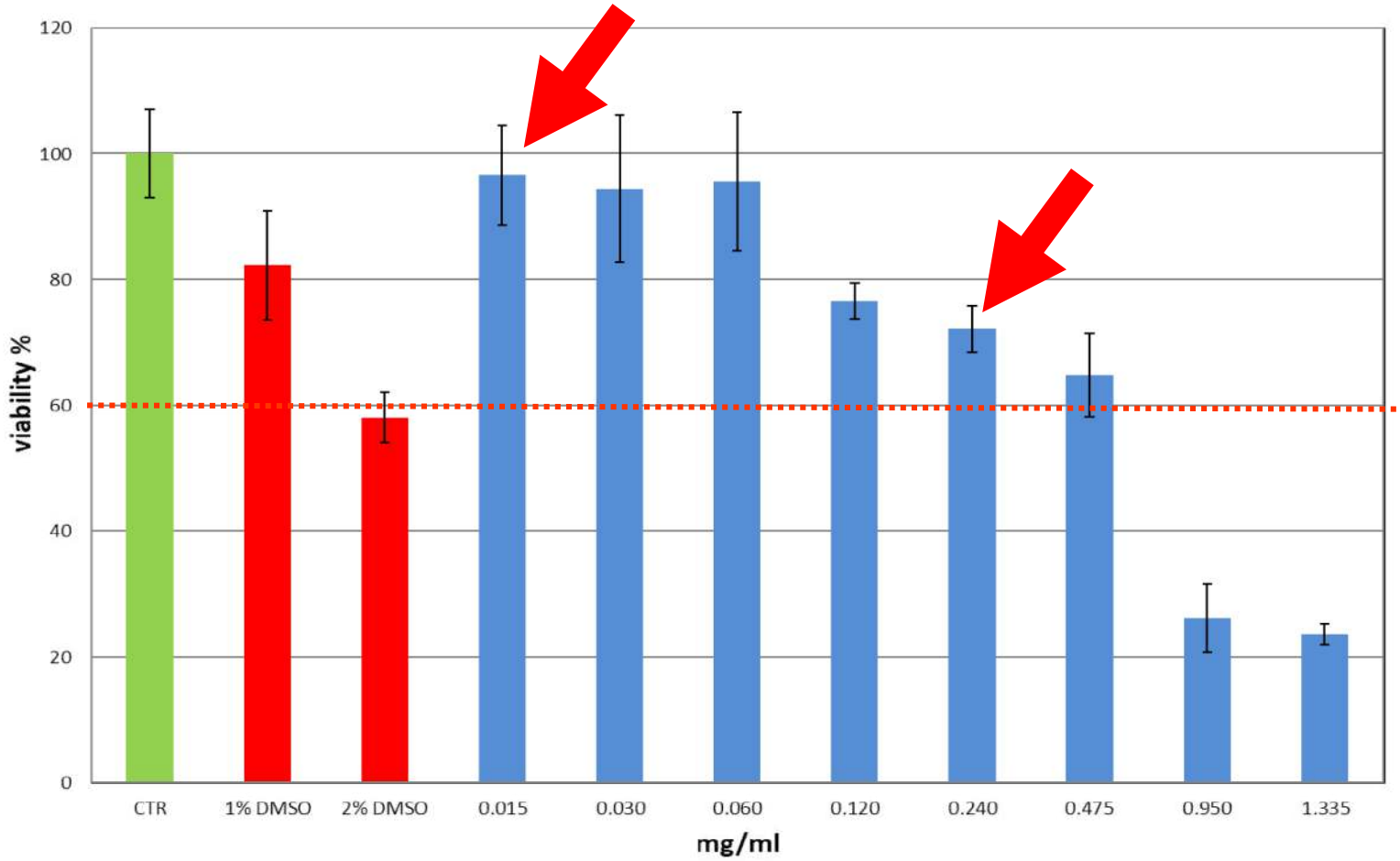






Hydrated film after 24 hours

Studies on cells: HaCaT (immortalized human keratinocytes)

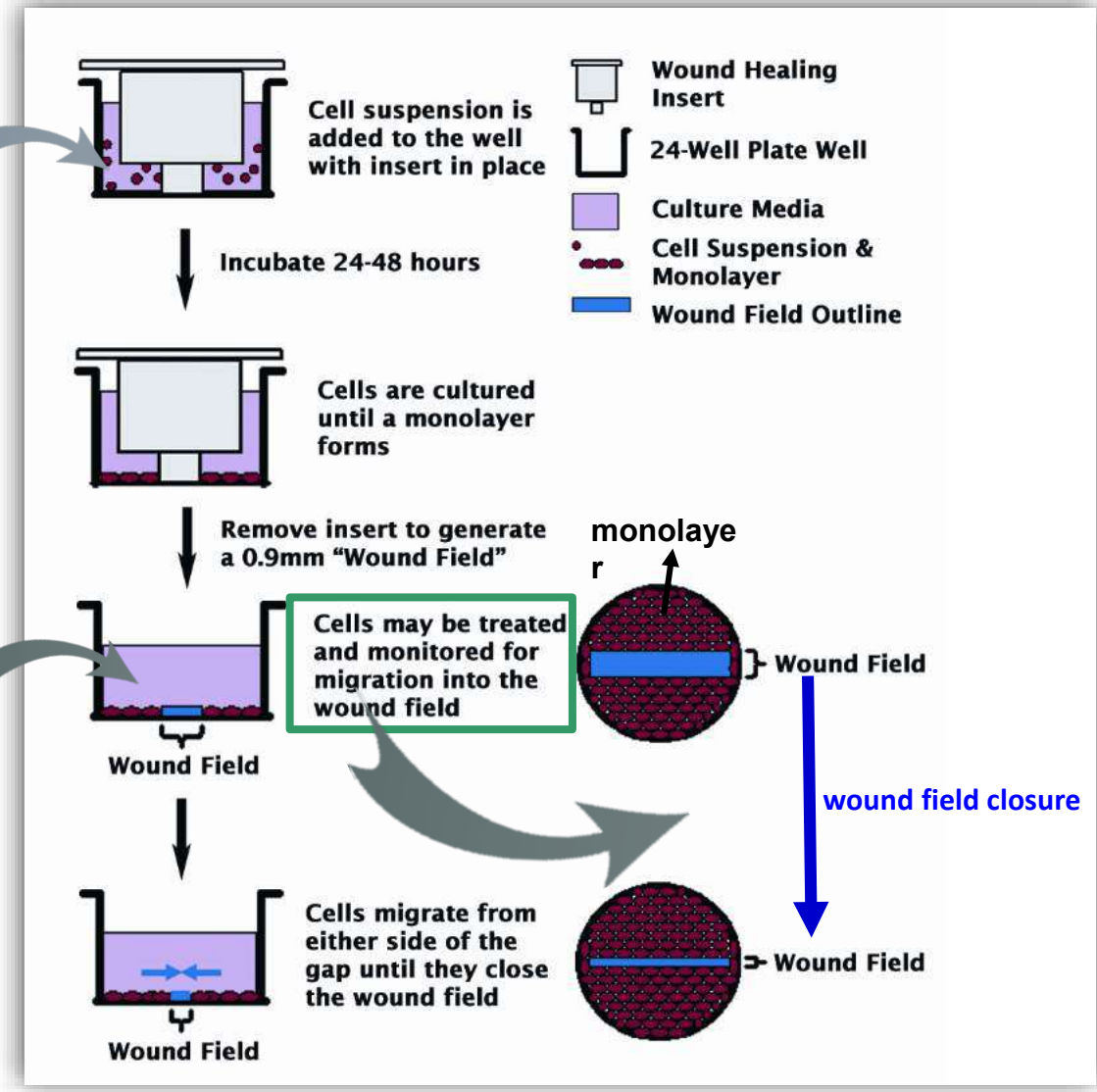


CytoSelect™ Wound Healing Assay Kit

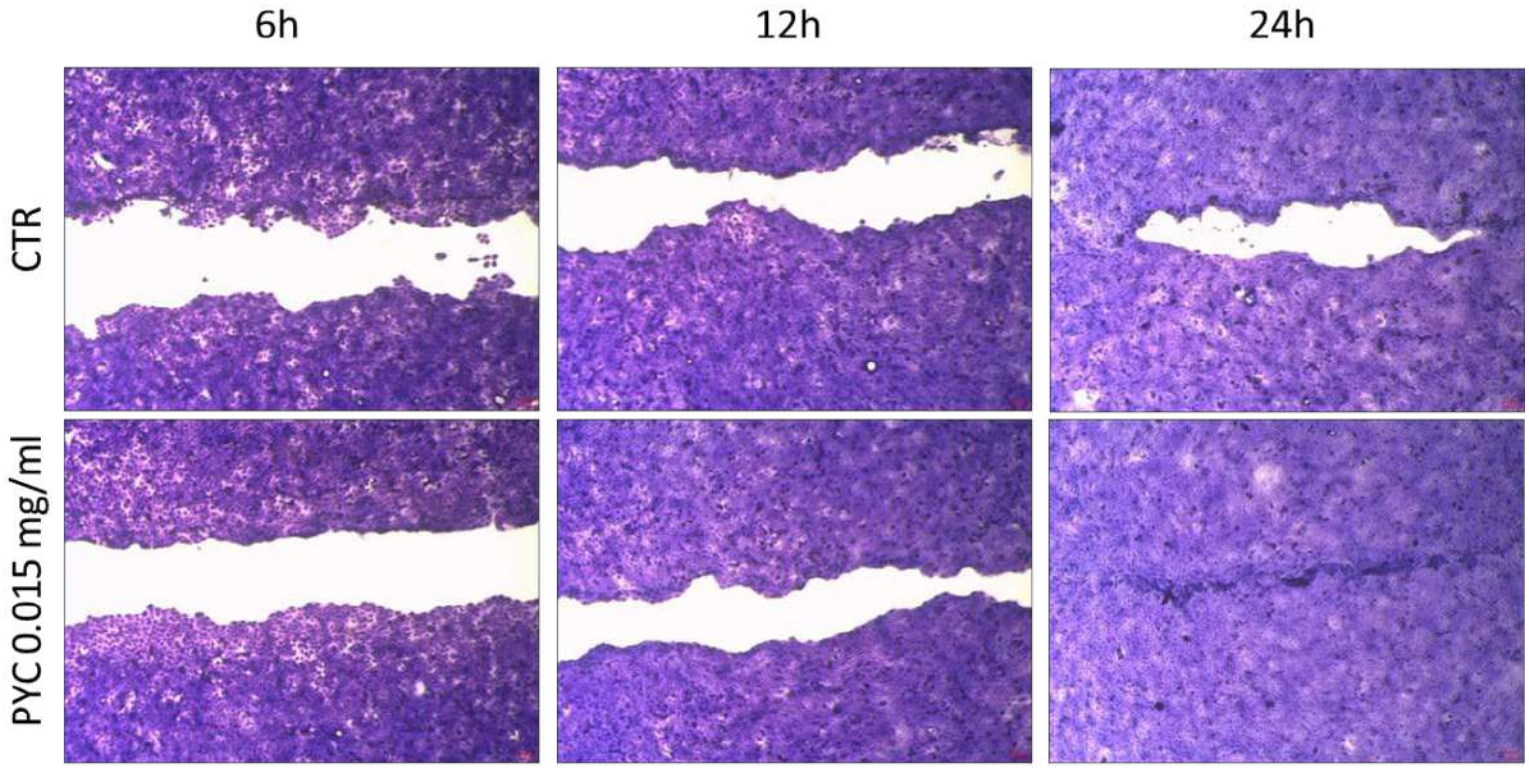
HaCaT
(DMEM containing 10% FBS)

can PYC stimulate cell growth?

PYC solution in DMEM



Wound field closure evaluation: 3, 6, 12 and 24 hours



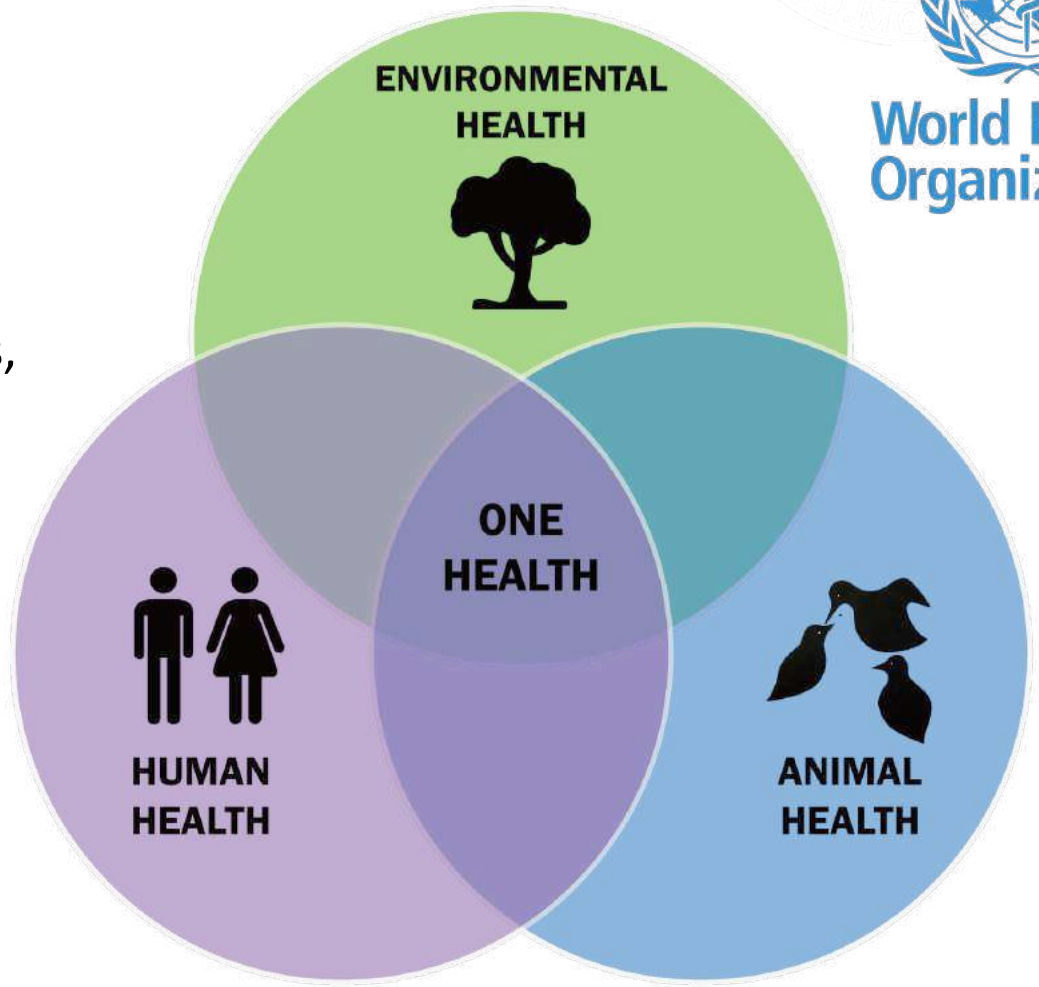
Stimulates keratinocytes growth

Wound healing

KEY-WORDS

- One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.
- It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.
- The approach can be applied at the community, subnational, national, regional and global levels,

World Health Organization



A.D. 1308 unipg

Questions

.....It was necessary to ask ourselves a few questions

- Do we have the skills?
- Do we have an interesting raw material to work on?

The answer was:

biosostenibilità e packaging

gel doccia ricaricabile

contenitori riciclabili

contenitore riutilizzabile

ricariche

balsamo solido

shampoo solido senza contenitore

Luana Perioli, Ph.D.

biosostenibilità e packaging

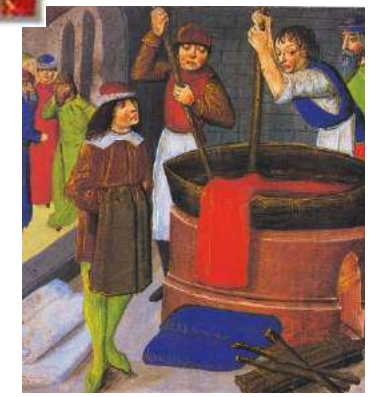
Cosmetica, il pacco è BIO
Ma NUDO è anche meglio

https://magazine.pambianconews.com

Luana Perioli, Ph.D.

Use of saffron in time…

1600-1500 B.C.



1400 sec. A.D.

Ebers Papyrus (1500 B.C.)

2021 A.D.

molecules

MDPI

Article

Bioadhesive Polymeric Films Based on Red Onion Skins Extract for Wound Treatment: An Innovative and Eco-Friendly Formulation

Cinzia Pagano [1,†], Maura Marinozzi [1,†], Claudio Baiocchi [2], Tommaso Beccari [1], Paola Calarco [1], Chielli [1], Ciriana Orabona [3], Elena Orecchini [3], Roberta Scuota [4], Maria Cristina Tiralti [1] and Luana Perioli [1,*]

ces, University of Perugia, via del Liceo 1-06123, Perugia, Italy; marinozzi@unipg.it (M.M.); tommaso.beccari@unipg.it (T.B.);

$$F = \sum_{i=1}^{n} E_i = \sum_{i=1}^{n} C_i q_i$$

Formula calcolo Impronta Ecologica

L'**impronta ecologica** misura l'area biologicamente produttiva di mare e di terra necessaria a rigenerare le risorse consumate da una popolazione umana e ad assorbire i rifiuti prodotti.

Si può esprimere l'impronta ecologica anche da un **punto di vista energetico**, considerando l'emissione di diossido di carbonio espressa quantitativamente in tonnellate, e di conseguenza la quantità di terra forestata necessaria per assorbire le suddette tonnellate di CO_2.

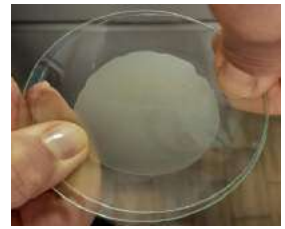
Luana Perioli, Ph.D.

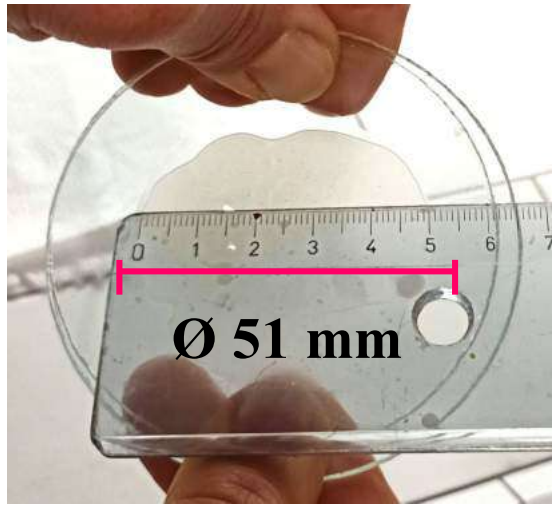
Spreadability assay

Determination of Spreadability

One gram of emulsion was pressed between two horizontal plates 20 cm square, upper one of which weighed 125 g, and its diameter (∅) was measured after one min.

Under the experimental conditions, the following classification was adopted : semi-stiff creams ∅ ≤ 50 mm and semi-fluid creams 50 < ∅ ≤ 70 mm (7).





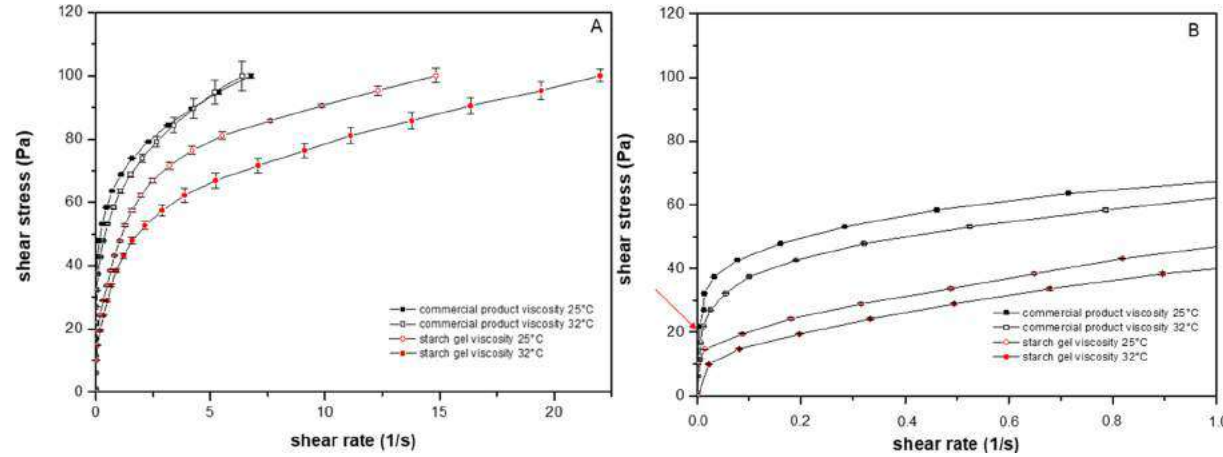
Semifluid creams

easy application on the skin

Formulation	Sample after eight 4°C/45°C cycles of 24 h	Sample after eight -20°C/25°C cycles of 24 h	Sample after centrifugation	Observations
Commercial gel				Temperature cycling tests: 1. discoloration; 2. appearance of black particles (red circles) for the sample treated -20°C/25°C. Centrifugation: sedimentation of solid particles/aggregates dispersed in the gel (red arrow).
Starch gel loaded with SE-A1-CS				Temperature cycling tests: no changes observed. Centrifugation: no changes observed.

Comparison with a commercial product

- Stability
- Rheological properties



pharmaceutics

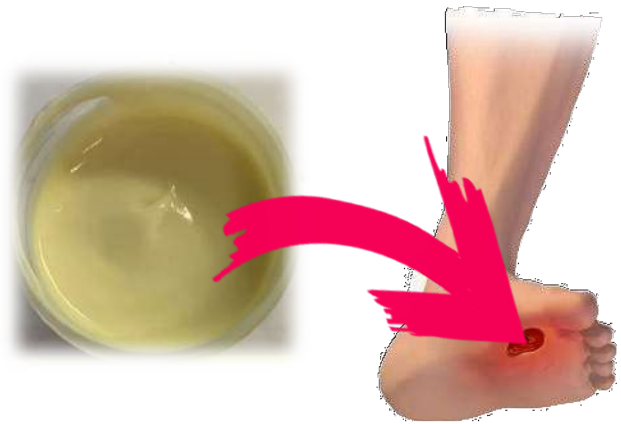
MDPI

Article

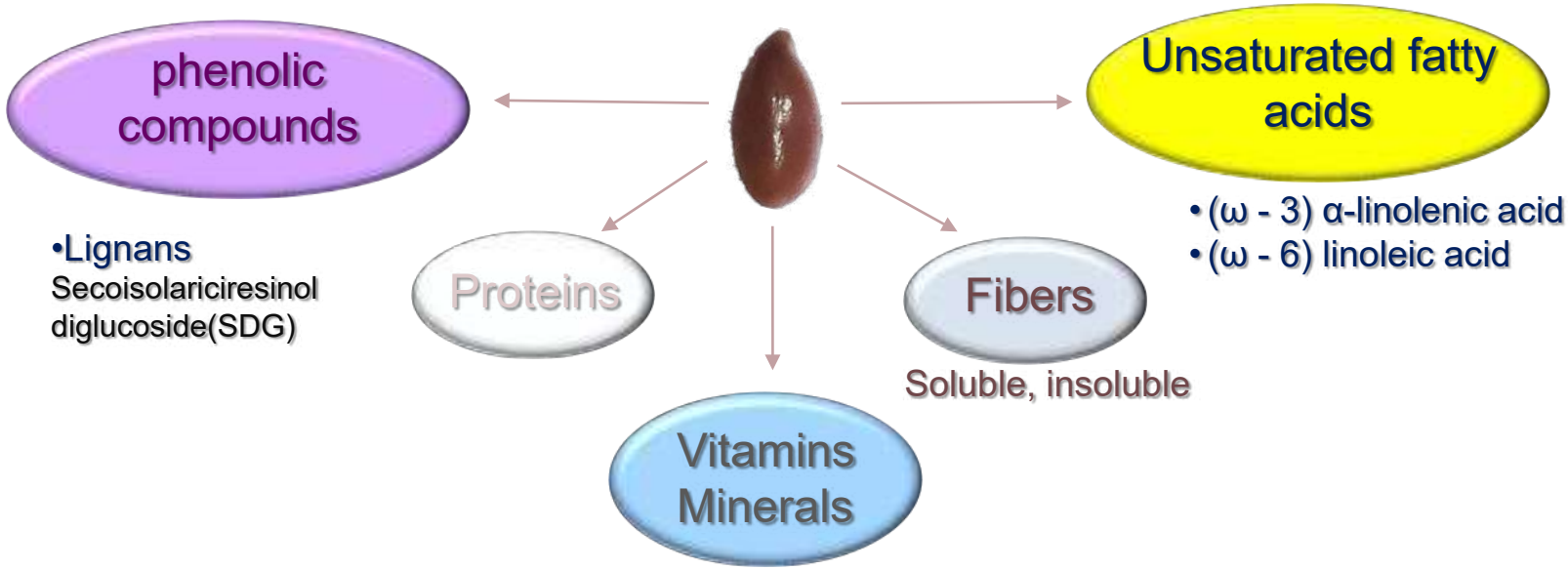
Emulgel Loaded with Flaxseed Extracts as New Therapeutic Approach in Wound Treatment

Cinzia Pagano [1], Claudio Baiocchi [2], Tommaso Beccari [1], Francesca Blasi [1], Lina Cossignani [1], Maria Rachele Ceccarini [1], Ciriana Orabona [3], Elena Orecchini [3], Enrico Di Raimo [4], Sara Primavilla [4], Laura Salvini [5], Alessandro Di Michele [6], Luana Perioli [1,*] and Maurizio Ricci [1]

Pharmaceutics **2021**, *13*, 1107. https://doi.org/10.3390/pharmaceutics13081107 https://www.mdpi.com/journal/pharmaceutics



Bioactive compounds



- anti-inflammatory
- antioxidant
- angiogenetic
- anti-atherosclerosis
- ↓ blood pressure
- ↓ total and LDL cholesterol

Y.Y. Shim et al. Flaxseed (Linum usitatissimum L.) bioactive compounds and peptide nomenclature: A review. Trends in Food Sci. & Technol. **2014**, 38, 5-20.
M. Parikh et al. Flaxseed: its bioactive components andtheir cardiovascular benefits. Am J Physiol Heart Circ Physiol. **2018**, 314: H146-H159.
B.D. Oomah. Flaxseed By-products. Food Wastes and By-products, R. Campos-Vega, B. D. Oomah and H.A. Vergara-Castañeda (Eds), John Wiley & Sons Ltd. **2019**, 267–289.

Dry extract (DE)

Flaxseeds flour
(2 g)

EtOH 60% (80 ml), 800 rpm, 45°C, 90 min

Centrifugation
(4000 rpm, 10 min)

Filtration under vacuum

Drying
(rotary evaporator)

Freeze-drying

DE
water solubility
2.5 mg/ml

Yield
12.5%

Liquid extract (LE)

Flaxseeds flour
(100 g)

EtOH 96%
(200 ml)

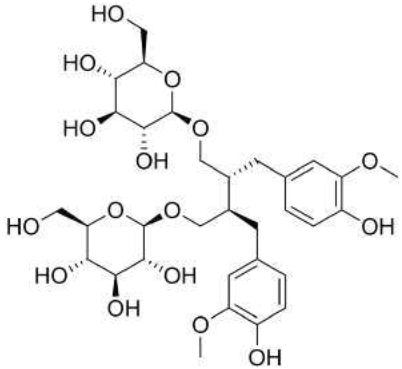
EtOH removal
(rotary evaporator)

Liquid extract

Yield
14.2%

Dry extract (DE)

Secoisolariciresinol diglucoside (SDG)

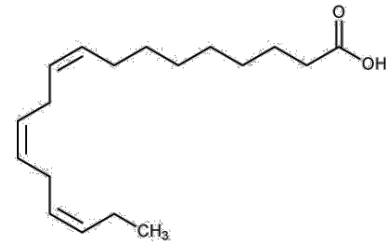


Antioxidant

Extract	TPC (Mean ± SD) mg GAE/g Dry Flaxseed Flour	FRAP (Mean ± SD) μmol Fe^{2+}/g Dry Flaxseed Flour	ABTS (Mean ± SD) mg TE/g Dry Flaxseed Flour
D.E.	1.94 ± 0.09	15.73 ± 3.10	5.25 ± 0.35
L.E.	1.62 ± 0.01	11.69 ± 0.21	0.62 ± 0.04

Liquid extract (LE)

α-Linolenic acid



Anti-inflammatory

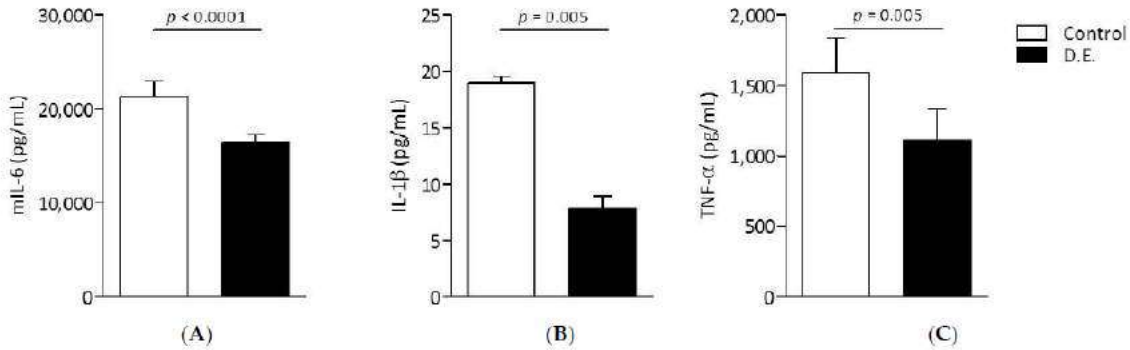


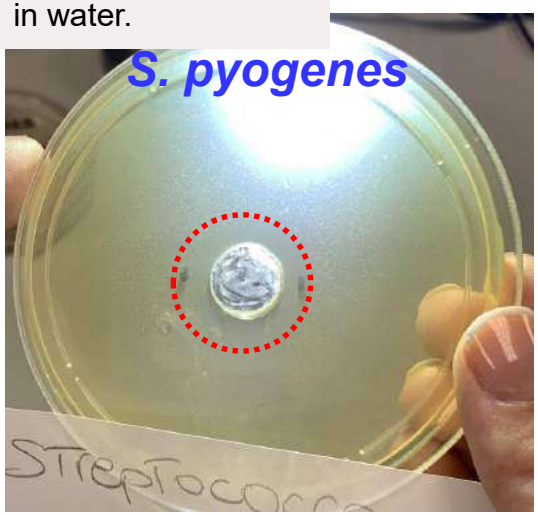
Figure 9. RAW 264.7 cells were in vitro co-treated with LPS (Control) and D.E. at 0.038 mg/mL for 24 h. Supernatants were collected and the concentrations of IL-6 (A), IL-1β (B) and TNFα (C) were determined by ELISA test. D.E. vs. Control (unpaired Student's *t*-test).

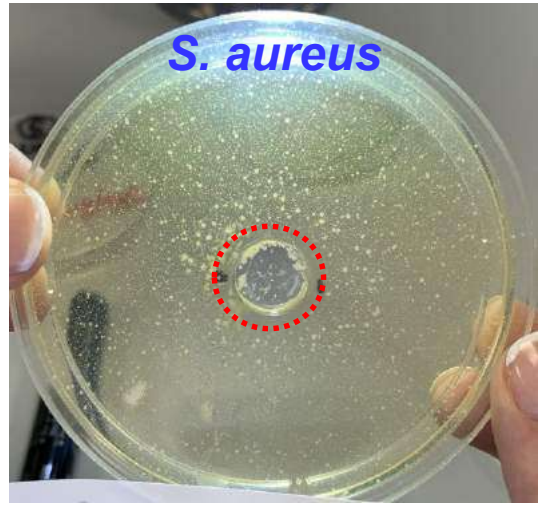
Antimicrobial activity

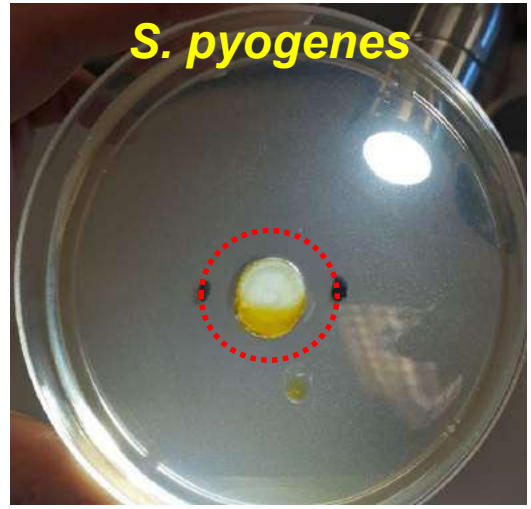
Agar diffusion method: the Petri dishes were prepared by adding to the previously dissolved agar 1 ml of a bacterial suspension containing 10^3 UFC of the chosen microorganism, cooled and then used to seed 100 µl of solubilized extract in water.

Strains	D.E. 100 mg/mL (mm)	D.E. 150 mg/mL (mm)	Marketed D.E. 100 mg/mL (mm)	Marketed D.E. 150 mg/mL (mm)	L.E. (mm)	Marketed Flaxseed Oil (mm)
Gram +						
S. epidermidis	-	-	-	16	-	-
E. faecalis	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
S. aureus	-	18	-	15	-	-
S. pyogenes	20	20	19	22	20	-
Gram –						
P. aeruginosa	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-
P. mirabilis	-	-	-	-	-	-
E. coli	-	-	-	-	-	-
Yeast						
C. albicans	-	-	-	-	-	-

-: no halo observed.







Dry extract (150 mg/ml)

liquid extract

Formulation

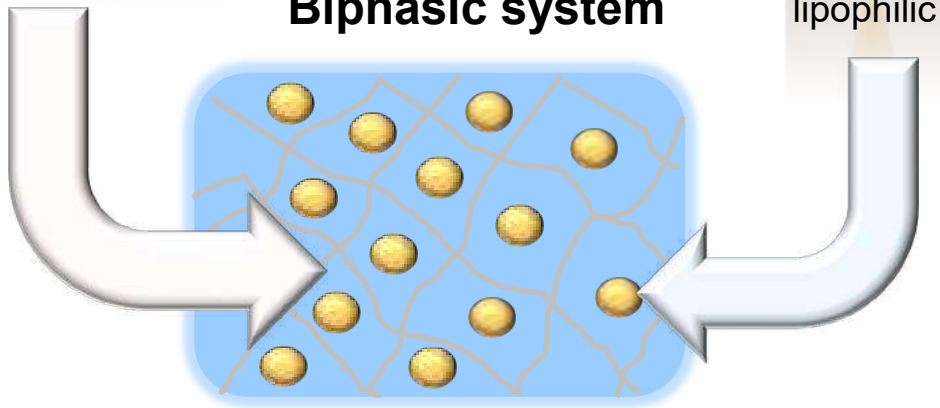
Dry extract (DE)

hydrophilic

Liquid extract (LE)

lipophilic

Biphasic system



EMULGEL

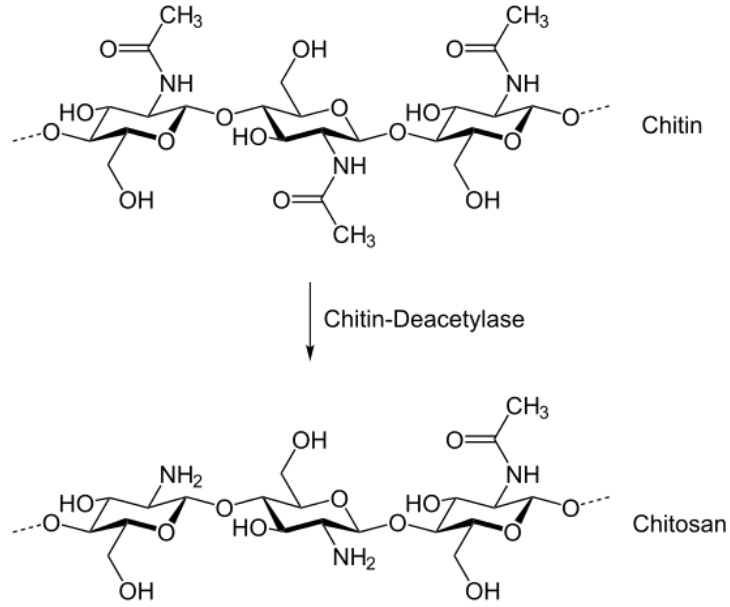
Oil phase (30% w/w)	Cetomacrogol 1000	2 g
	cetostearyl alchol	6 g
	Liquid Extract	22 g
Water phase (70% w/w)	**Dry Extract**	1 g
	Chitosan (1% w/v)	69 g

FG 90 chitosan*

deacetylation degree of 99.97%
Average MW100 kDa
Viscosity of 1% solution in 1% acetic acid 110 mPa· s

hydrogel

obtained from exoskeleton of crustacean, mollusks, insects and certain fungus



food waste materials

biocompatible, biodegradable, non-toxic

*Produced and characterized by Prof. Riccardo Muzzarelli, Department of Biochemistry, Biology and Genetics - Università Politecnica delle Marche-Ancona (Italy)

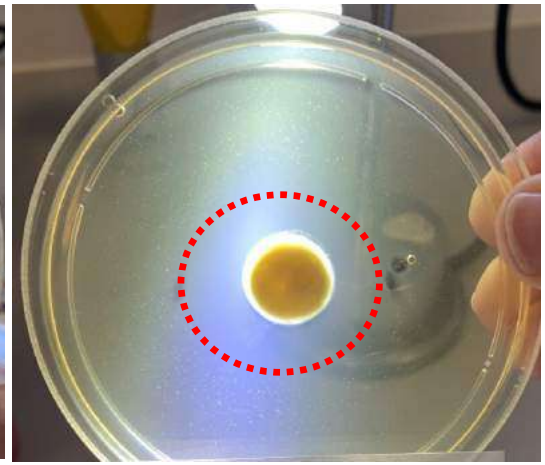
Emulgel – antimicrobial activity

Gram +

P. aeruginosa

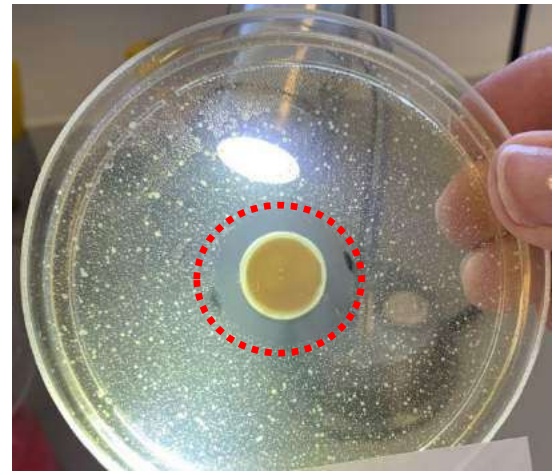
Inhibition halo: **31 mm**

S. pyogenes



Inhibition halo: **36 mm**

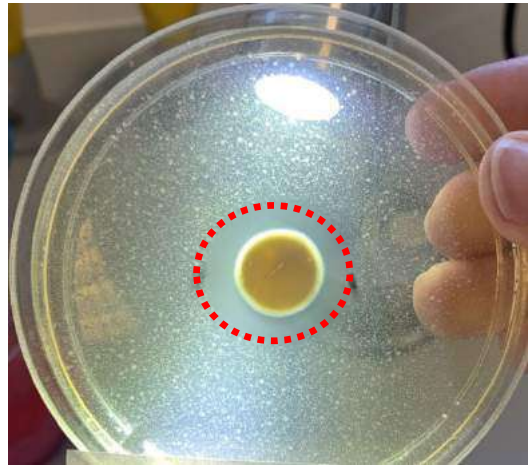
S. aureus



Inhibition halo: **24 mm**

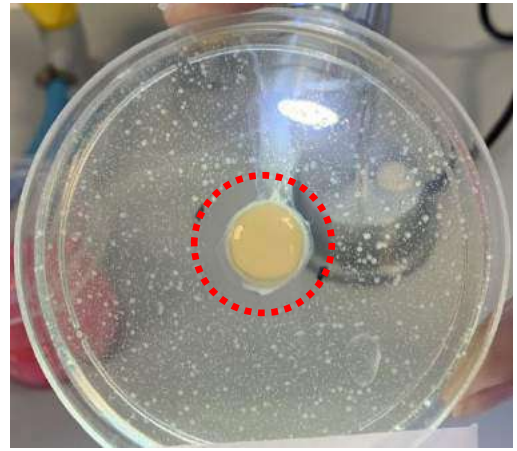
Gram -

K. pneumoniae



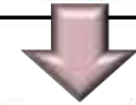
Inhibition halo: **27 mm**

E. coli



Inhibition halo: **26 mm**

Sample	MIC (mg/mL)	MBC (mg/mL)
Ciprofloxacin (control)	1 μg/mL	1 μg/mL
D.E.	0.59	1.17
L.E.	0.22	0.44
FG90	0.30	0.30
Emulgel	5.20	5.20



0.052 mg/mL of D.E., 1.14 mg of L.E. and 0.036 mg of FG90

Synergisms between extracts and chitosan

Literature data demonstrated that chitosan exhibits synergic effect with some antibiotics against many micro-organisms such as *S. aureus*

S.Tin. Activity of Chitosans in combination with antibiotics in Pseudomonas aeruginosa. Int. J. Biol. Sci. **2009** 5, 153-160.
A. Asli. Antibiofilm and antibacterial effects of specific chitosan molecules on Staphylococcus aureus isolates associated with bovine mastitis. PLoS ONE **2017** 12(5), e0176988.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2022
III SIMPÓSIO INTERNACIONAL
EM INVESTIGAÇÕES
QUÍMICO-FARMACÊUTICAS
I ENCONTRO IBERO-AMERICANO DE PLANTAS MEDICINAIS DR. MAHABIR GUPTA
I CONGRESSO LUSO-BRASILEIRO DE CIÊNCIAS E TECNOLOGIAS EM SAÚDE

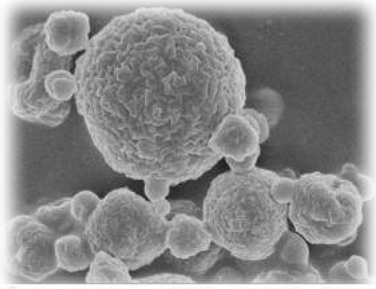
Wounds treatment → Circular health

NATURAL SOURCES

Moringa Oleifera

FORMULATION

Bioadhesive microparticles



Flaxseeds

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Emulgel