HINDERING ADVANCED GLYCATION END-PRODUCT (AGE) FORMATION BY NATURAL COMPOUNDS TO PREVENT OSTEOSARCOPENIA

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INTRODUCTION

The term "**osteosarcopenia**" indicates an age-associated and unresolved condition characterized by concomitant loss of bone (osteoporosis) and skeletal muscle tissue (sarcopenia) leading to increased risk of fractures, loss of independence, declined quality of life, morbidity and mortality, especially in old people. Common factors, such as low-grade chronic inflammation, elevated oxidative stress, and lifestyle factors (i.e., diet, smoking, alcohol) are responsible for bone and muscle loss [1]. **Advanced glycation end-products (AGEs)** are involved in musculoskeletal diseases and in the occurrence of osteosarcopenia [2,3]. During aging, AGE accumulation contributes to loss of mass and strength in human bone and muscle tissues by generating reactive oxygen species (ROS) and sustaining inflammation and tissue injury/breakdown by cross-linking extracellular matrix components and by interacting with the multiligand receptor for advanced glycation end-products (RAGE) [3]. Due to the increase in the life expectancy, osteosarcopenia has become a urgent medical and social problem. Since efficacious pharmacological treatments for sarcopenia and osteoporosis are still lacking, the use of natural compounds to counteract osteosarcopenia represents a potential strategy, considering the growing interest for phytotherapy extracts usable as food supplements [4].

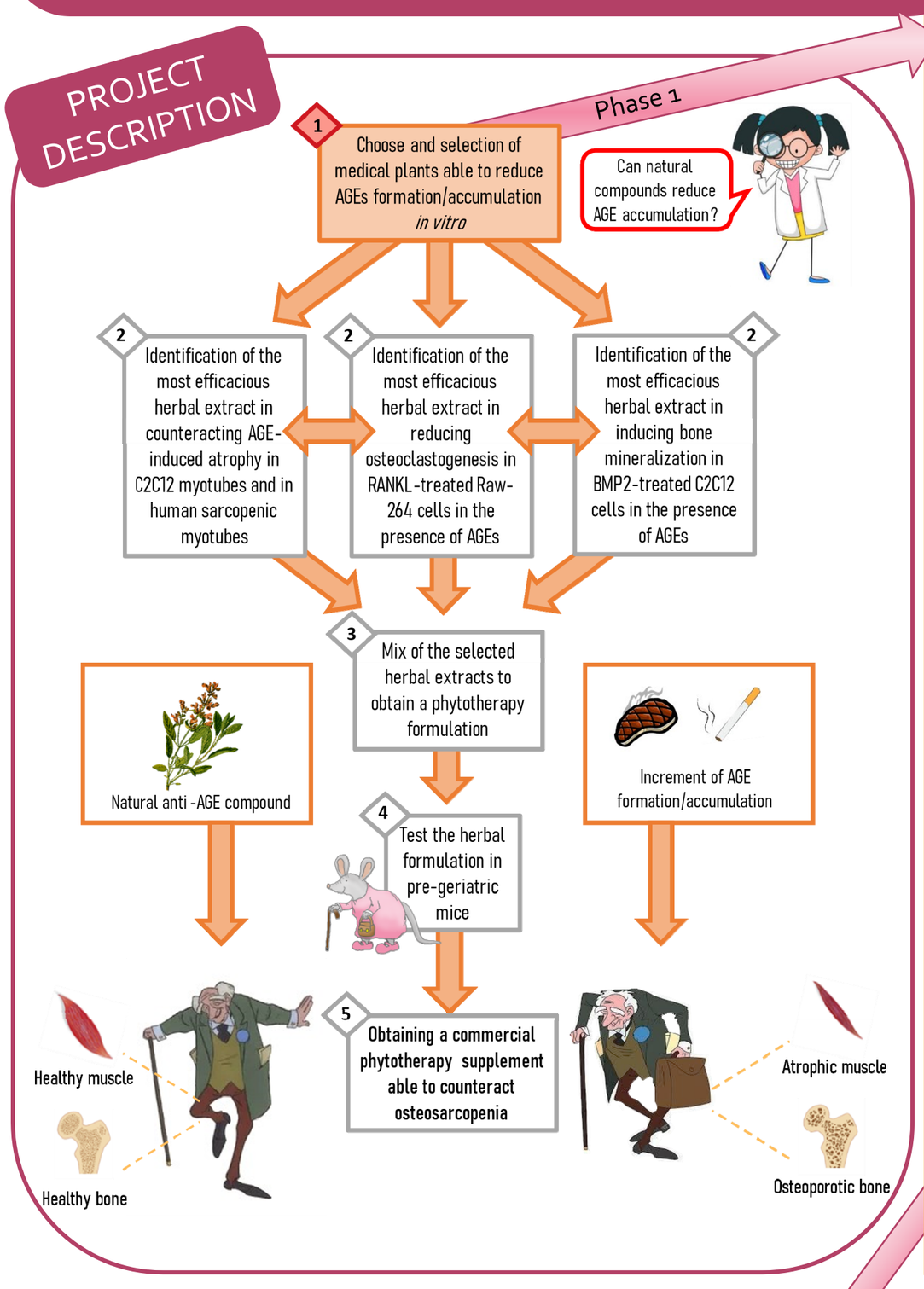
AIM and METHODS

The main project aims to identify an herbal formulation able to hinder AGE formation/activity to contrast age-related muscle atrophy and rebalance osteoblast/osteoclast activites. As a first step, a commercial kit (Albumin glycation assay kit) was used to analyze the ability of standardized dry extracts from officinal plants or mushrooms, synthesized active principles, and a food supplement in reducing AGE formation compared to aminoguanidine (0.8, 4 and 20 mM). Total fluorescence was measured with a microplate reader at 370 nm excitation and 440 nm emission filters.

FOCUS ON **AGEs**

Advanced glycation end-products (AGEs) are a heterogeneous group of non-enzymatic adducts especially formed by the glycation of proteins and lipids via Maillard reaction [3]. Exogenous AGEs derive from smoking and consumption of food typical of the Western diet; instead, endogenus AGE formation naturally occurs during aging, especially in hyperglycemia and oxidative stress conditions. AGEs are classified based on the ability to fluorescence and to cross-link with components of the extracellular matrix. AGEs are involved in the development of chronic degenerative diseases like diabetes, cardiovascular diseases, neurological disorders, and some types of cancer [2,3].

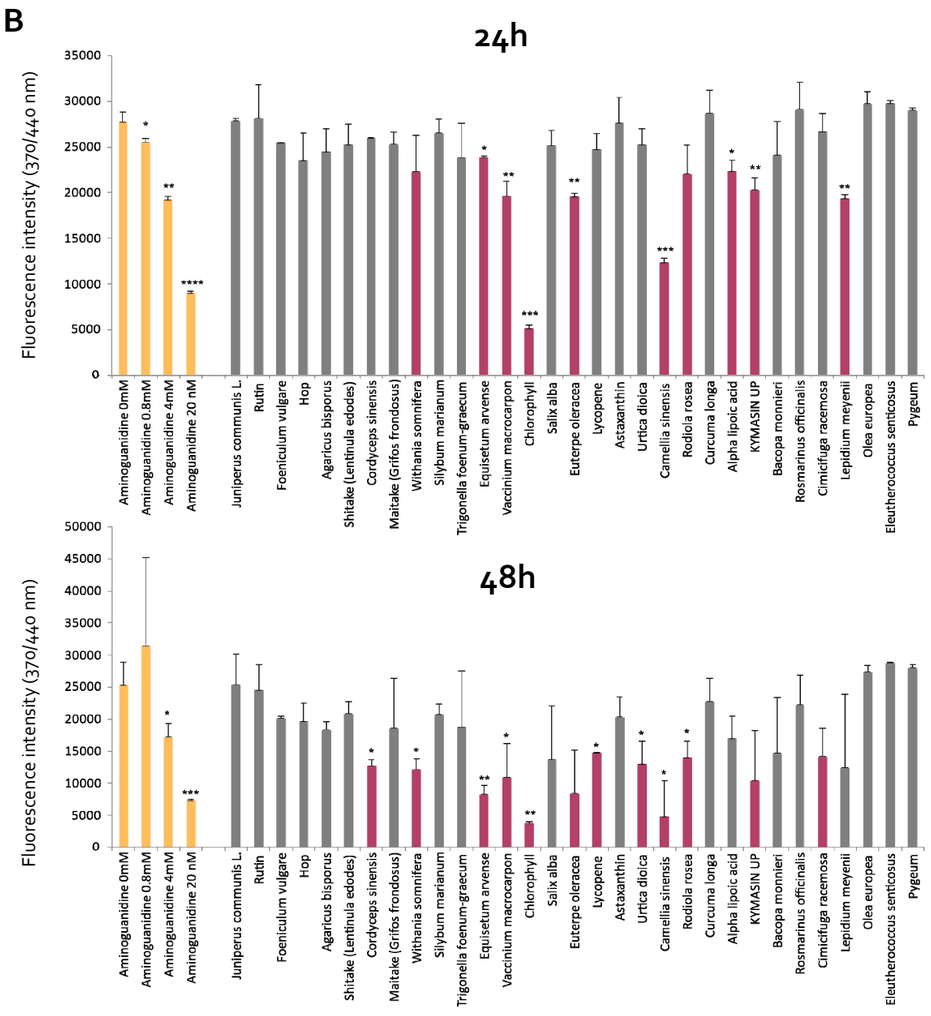
PROJECT DESCRIPTION



A

Tested compounds	
Offcinal plants	
B. monnieri	*O. europea*
Ca. sinensis	Pygeum
C. longa	*R. officinalis*
C. racemosa	*R. Rosea*
E. arvense	*S. alba*
E. oleracea	*S. marianum*
E. senticosus	*T. foenum-graecum*
F. Vulgare	*U. Dioica*
Hop	*V. macrocarpon*
Ju. communis L.	*W. Somnifera*
L. meyenii	
Mushrooms	
A. bisporus	Maitake (*G. frondosus*)
Cor. sinensis	Shitake (*L. edodes*)
Synthesized principles	
Alpha lipoic acid	Lycopene
Astaxanthin	Rutin
Chlorophyll	
Food supplement	
KYMASIN UP	

B



(A) Thirty standardized dry extracts and a food supplement (KYMASIN UP) were selected based on their activity as antioxidants and anti-inflammatory agents, their reported effects on muscle and bone, or their ethnobotanical use. **(B)** The extracts and the food supplement (100µg/ml) were tested for their ability in counteracting glyceraldehyde-derived fluorescent AGE formation after 24 and 48 hours. Statistical analysis was conducted using t-test *p<0.05, **p<0.01, and ***p <0.005, significantly different from control (aminoguanidine 0 mM).

AGE formation was significantly inhibited at the indicated time by

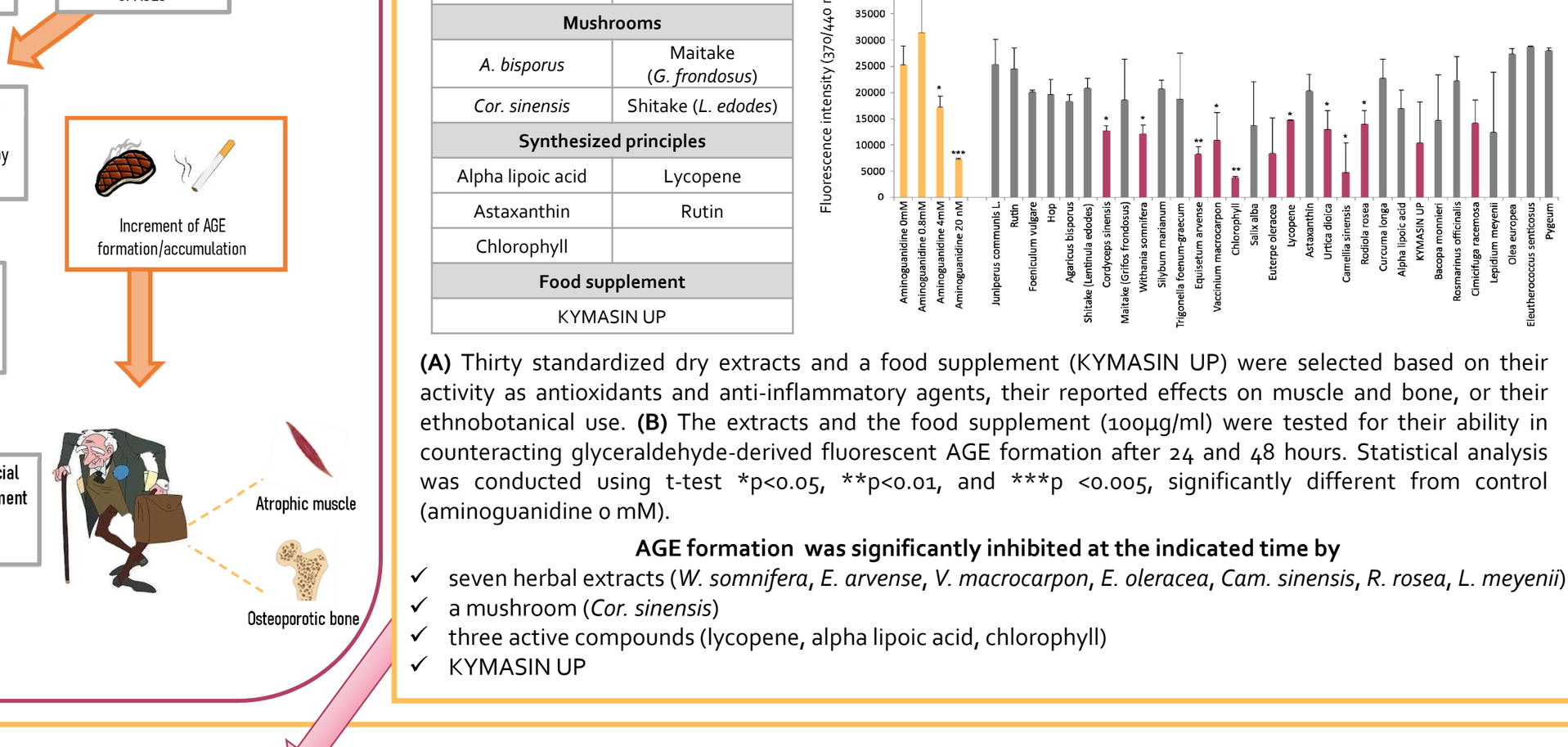
- ✓ seven herbal extracts (*W. somnifera*, *E. arvense*, *V. macrocarpon*, *E. oleracea*, *Cam. sinensis*, *R. rosea*, *L. meyenii*)
- ✓ a mushroom (*Cor. sinensis*)
- ✓ three active compounds (lycopene, alpha lipoic acid, chlorophyll)
- ✓ KYMASIN UP

A

Selected extracts	
Offcinal plants	
Ca. sinensis	*R. rosea*
E. arvense	*V. macrocarpon*
E. oleracea	*W. somnifera*
L. meyenii	
Mushrooms	
Cor. sinensis	
Synthesized	
Alpha lipoic acid	Lycopene
Chlorophyll	
Food supplement	
KYMASIN UP	

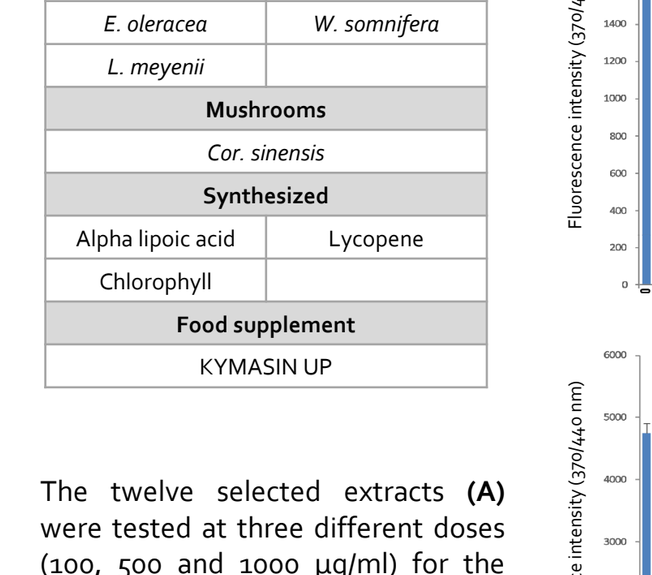
The twelve selected extracts **(A)** were tested at three different doses (100, 500 and 1000 µg/ml) for the ability to inhibit AGE formation **(B)**. AGE fluorescence intensity was measured every hour for 48h. Reported are the values at 6, 12, 24 and 48h. Statistical analysis was conducted using t-test *p<0.05, **p<0.01, and ***p <0.005, significantly different from control (aminoguanidine 0 mM).

B



The most efficacious compounds in reducing AGE fluorescence were

- ✓ *V. macrocarpon*
- ✓ *Cam. sinensis*
- ✓ Chlorophyll



The percentages of fluorescence intensity for the highest dose (1000 µg/ml) of each indicated extract was calculated with respect to the total fluorescence (untreated control) for 48h at the indicated time-points. Aminoguanidine 20mM (*blue line*) was used as a positive control. Reported are the means±SD.

- ✓ *V. macrocarpon*, *Cam. Sinensis* and Chlorophyll show a surprising ability in counteracting AGE formation in a dose-dependent manner starting from 3h.
- ✓ *Cam. Sinensis* (1000 µg/ml) completely abolished AGE-derived fluorescence starting from 6h.

CONCLUSIONS

Our results might lead to the development of a low-cost, non-toxic **phytotherapy product** that can **prevent AGE formation/accumulation** reducing the AGE-dependent **detrimental effects** on muscle and bone tissues occurring in **elderly**.

REFERENCES

1. Paintin J *et al.*,. *Br J Hosp Med (Lond)*. 2018; 2. Perrone A *et al.*, *Oxid Med Cell Longev*. 2020; 3. Suzuki A, *et al.*, *Methods*. 2020; 4. Qu Z *et al.*, *J Nutr Biochem*. 2021

Acknowledgements

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