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**Comparative study of valorization of pomegranate and
wine wastes-
Added value products and biological activities**

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Background

Pomegranate fruit (*Punica granatum* L.)

- rich in polyphenolic compounds and tannins
- 3.000.000 tn annual production
- **peel** constitutes about 50% of the total fruit weight



Grapes are the largest fruit crop in the world

- 60.000.000 tn annual production
- ~80% for wine making
- **Wine wastes** (20% w/w total volume processed)



Agro-industrial by-products management practices

- **Animal feed** (unbalanced diet)
- **Composting** (time consuming and land required)
- **Incineration** (pollution problems)
- **Landfill** (decomposition, greenhouse gas production)

Valorization practices

- **Extraction of valuable components for nutraceutical and industrial applications** (polyphenols, flavonoids etc.)
- **Biosorption and heavy metals removal**
- **Conversion to bio-fuels** (bio-ethanol from wine wastes)

Aim of the Study: Extraction and isolation of phenolic compounds and evaluate their biological activities

A. Phenolics extracted from:

- Fresh pomegranate peels (*Wonderful* cultivar)
- White marc (Variety *Malagouzia*)
- Red marc (Variety *Syrah*)
- White lees (Variety *Malagouzia*)-before alcoholic fermentation

B. Biological activities examined:

- Antioxidant
- Anti-platelet (*in vitro*)
- Antibacterial
- Anti-inflammatory (*in vitro*)

Results

A. Ultrasound- assisted extraction of phenolic compounds

(Total Phenolic Content (TPC) expressed at mg gallic acid/ g dry weight)

B.1. Antiradical Activity- DPPH method (517 nm)

% inhibition of DPPH= $[(A_{DPPH} - A_{EXtr})/A_{DPPH}] * 100$ (eq. 1)

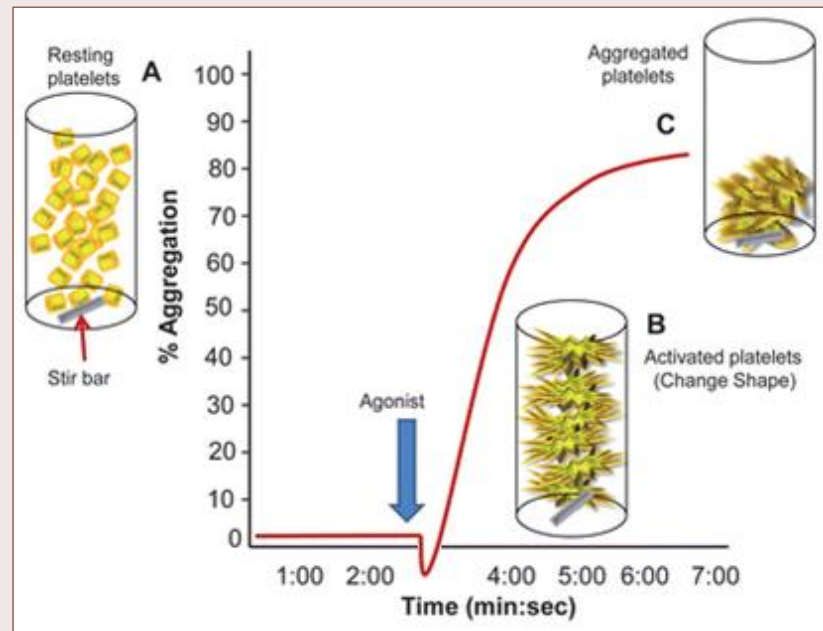
(referred to 200 μ g Total Phenolics)

Extracts	Total Phenolic Content (mg/g d.w.)	Antiradical Activity (%)
Pomegranate peel	89 \pm 2.91	85 \pm 1.54
White marc	18 \pm 1.73	91 \pm 1.73
Red marc	22 \pm 2.69	94 \pm 2.12
White lees	13 \pm 2.72	65 \pm 2.74

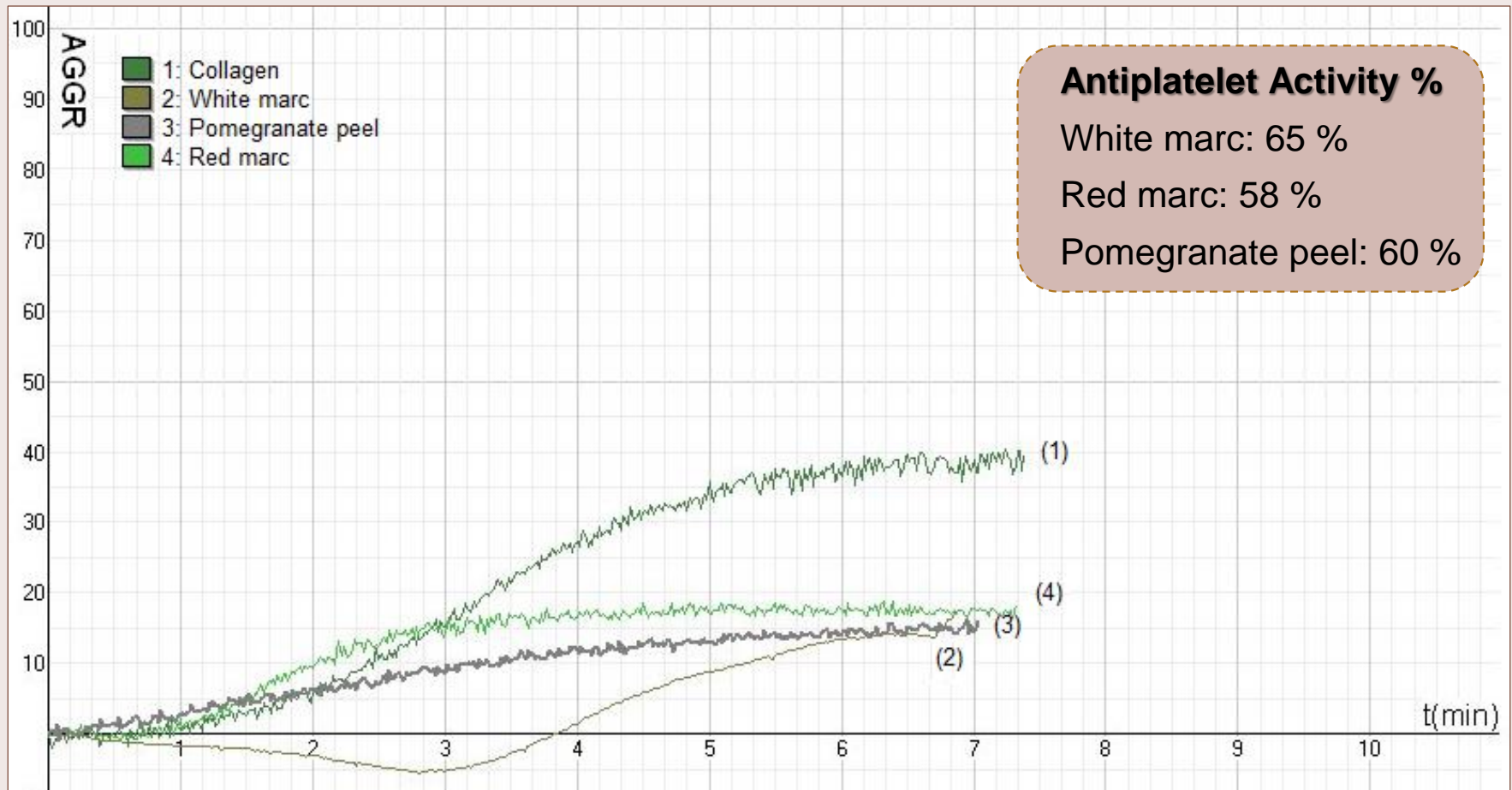
B.2. Antiplatelet Aggregation Experiments (*in vitro*)

- Aggregation agent: **collagen**
- **Platelets** were obtained from venous blood of healthy donors.
- Experiments were performed in a four channel aggregometer according to the photometric method of Born (1963).

$$\text{Antiplatelet Activity \%} = \frac{(\text{Max Aggregation collagen} - \text{Max Aggregation sample})}{\text{Max Aggregation collagen}} * 100\%$$



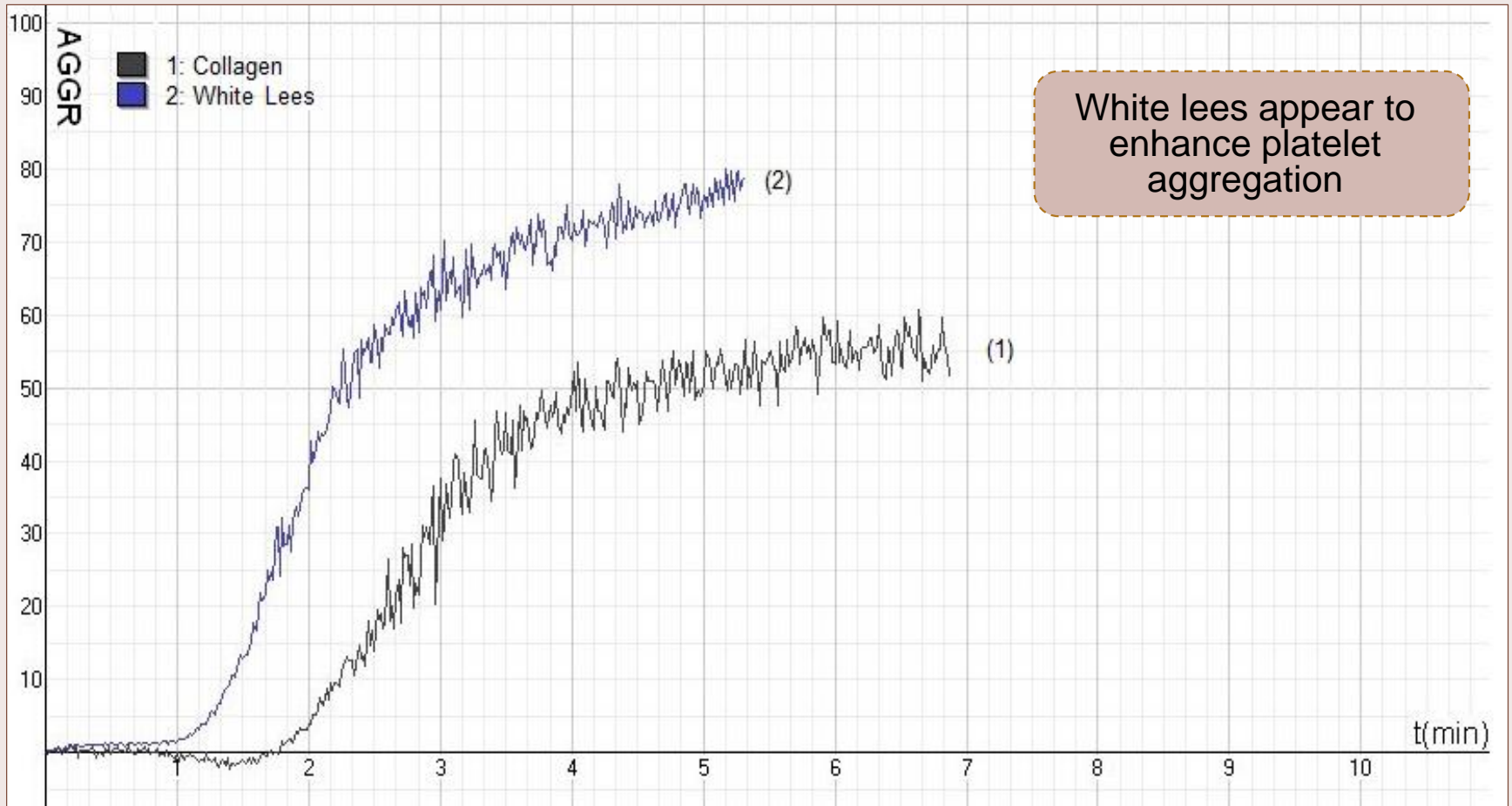
Antiplatelet Activity (*in vitro*)



Effect of white (2) and red (4) marc and pomegranate peel (3) extracts on human platelet aggregation induced by collagen (1).

(Initial concentration of phenolics for extracts: 38 $\mu\text{g/ml}$)

Antiplatelet Activity (*in vitro*)



Effect of white lees (2) extract on human platelet aggregation induced by collagen (1).

(Initial concentration of phenolics for extracts: 38 $\mu\text{g/ml}$)

B.3. Antibacterial Activity

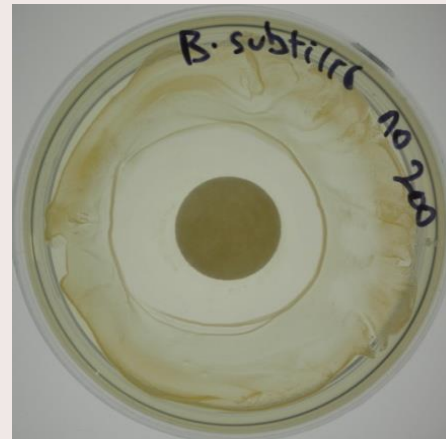
(Colaboration with Chemistry Department of A.U.Th., Laboratory of Biochemistry)

- 1. The antibacterial activity of the extracts against:
Escherichia coli (BL21 [DE3]),
Staphylococcus aureus (ATCC 6538),
Bacillus subtilis (ATCC 6633) and
Bacillus cereus (ATCC 11778)
- 2. Disc diffusion method (inhibition zone diameter, mm)

MeOH (blank)

White lees Extract 200 µg TP

*Bacillus
subtilis*



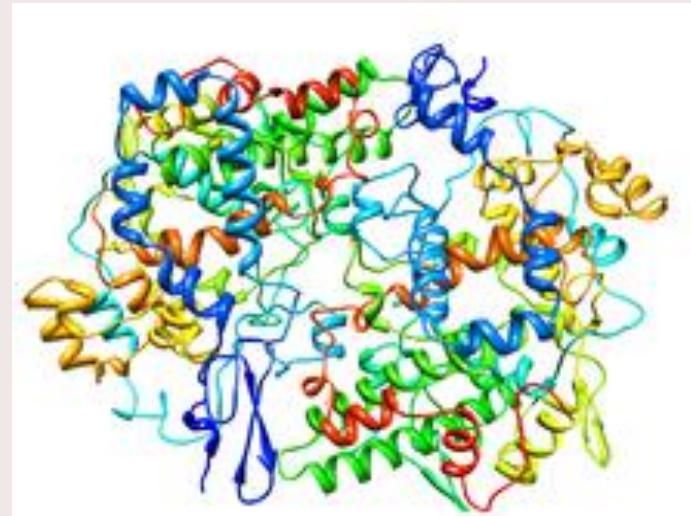
B.3. Antibacterial Activity

(Collaboration with Chemistry Department of A.U.Th., Laboratory of Biochemistry)

Bacteria	Increase of inhibition diameter zone (mm)			
	Pomegranate peels 200 µg TP	White Marc 200 µg TP	Red Marc 200 µg TP	White Lees 200 µg TP
Gram –Negative				
<i>E. coli</i>	10.01 ± 0.98	6.00 ± 0.71	8.67 ± 0,24	3.33 ± 1.00
Gram-Positive				
<i>S. aureus</i>	12.80 ± 1.09	6.84 ± 0.58	8.33 ± 0,61	4.09 ± 1.42
<i>B. cereus</i>	Not examined yet	5.00 ± 1.23	6.58 ± 2,20	7.00 ± 0.24
<i>B. subtilis</i>	Not examined yet	2.92 ± 0.32	3.92 ± 1,00	9.50 ± 0.24

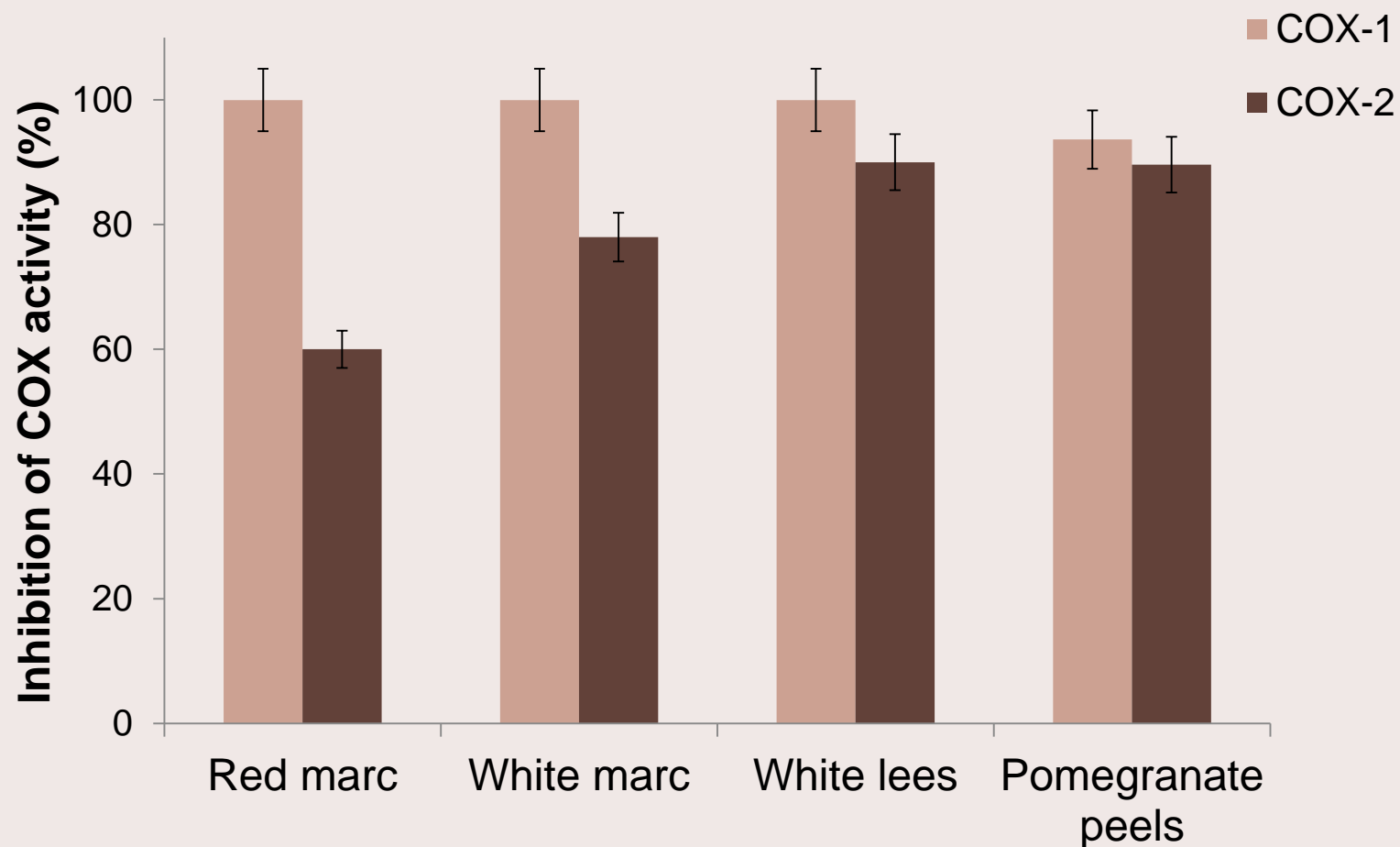
B.4. Anti-inflammatory activity measuring COX-1 and COX-2 activities (*in vitro*)

- **Cyclooxygenase enzyme (COX)** plays a catalytic role in the conversion of arachidonic acid to prostaglandins (PGs), which have an important role in inflammation .
- COX enzyme exists in two isoforms **COX-1** and **COX-2**.
- Most non steroidal drugs (NSAIDs) inhibit the activity of COX-1 and COX-2 and thereby the synthesis of prostaglandins.
- COX inhibitor screening assay kit (Cayman Chemical, USA)



Crystallographic structure of complex COX -1 with flurbiprofen

Effect of extracts on COX activity *in vitro*



Initial concentration of phenolics for extracts: 38 $\mu\text{g/ml}$

Conclusions

- ❖ Pomegranate peels and wine wastes extracts are two agrochemical by-products, rich in polyphenols and a good source of natural antioxidants.
- ❖ Antiplatelet activity and anti-inflammatory activity of the phenolic extracts obtained from both wastes together with the significant observed antibacterial activity should be a key point for the nutraceutical and pharmacological applications.
- ❖ Based on the results of the biological activities and the HPLC analysis of the extracts, which shows some common phenolics, the responsible phenolics for each action will be further elucidated.
- ❖ Valorization of wine wastes and pomegranate peels leads to added value products and should be applied in most cases than disposal.

*Thank you for your
attention!*