Biological activities and potential health benefits of plant bioactives in traditional foods of the Black Sea region

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Overall Research Aims:

(1) Elucidate the manner in which polyphenols in foods interact with the human body to promote healthy ageing

(2) Exploit this knowledge
   e.g. Supporting existing dietary policies
   e.g. in the development of novel health-promoting foods
   e.g. in supporting health claims
Dietary Polyphenols and Human Health

Foods Absorption/metabolism Pre-clinical (mechanisms) Clinical studies
Biological activities and potential health benefits of plant bioactives in traditional foods of the Black Sea region
The scope of the research in BaSeFood

Selection of plants and bioactives for study

Assessment of bioactivity of traditional foods
  • Direct antioxidant activities
  • Anti / pro-bacterial activities

Assessment of the biological activities of food plants and their bioactives
  • Expression of cell adhesion molecules on endothelial cells
  • Production of vasorelaxor and vasoconstrictor molecules by endothelial cells
  • Prevention of oxidative stress
  • Effects on cholesterol metabolism and trafficking
  • Effects on platelet activity
  • Effects on inflammatory cytokine production by blood-derived dendritic cells
The scope of the research in BaSeFood

Intervention studies in humans

• Effects of 6 weeks consumption of teas made from Sideritis scardica (Mountain tea) and Urtica dioica (nettle tea) from the Black Sea region on endothelial function and other markers of CVD risk

• Effects of 6 weeks consumption of ellagitannin-rich Montenegrin pomegranate juice on platelet reactivity and other markers of CVD risk

Directions for future research
Selection of traditional foods, plants and bioactives for study

- Prioritised list of traditional foods (>30 foods)
- Identification of food plant components of the traditional foods
- Selection of bioactive-rich food plants widely used in the traditional foods
- Selection of bioactives prevalent in the bioactive-rich food plants
- Identification and synthesis of human metabolites of selected bioactives
Selection of traditional foods, plants and bioactives for study

Prioritised list of traditional foods (>30 foods) → Identification of food plant components of the traditional foods

Selection of bioactive-rich food plants widely used in the traditional foods

Selection of bioactives prevalent in the bioactive-rich food plants → Identification and synthesis of human metabolites of selected bioactives
Selection of bioactive-rich food plants widely used in the traditional foods

1. Desk research to identify the major bioactives in each plant

2. Plants selected according to pre-defined criteria:
   a. Concentration of bioactives in plant-ingredients
   b. Proportion in traditional foods
   c. Prevalance of ingredients in prioritised list of traditional foods
   d. Prevalance of ingredients in Western European diets
   e. In-house bioactive expertise
Six highest ranking plant-ingredients were:

- Nettle (*Urtica dioica*)
- Kale
- Dill
- *Sideritis scardica* (Mountain tea)
- Persimmon
- Pomegranate
Identification of phenolic and glucosinolate bioactives in kale, dill, annual nettle (*Urtica urens*), *Sideritis scardica*, persimmon and pomegranate from the Black Sea region using LC-DAD-MS.

Saha S¹, Woodcock M¹, Konik-Risitic A², Glibetic M², Boyko N³, Jorjadze M⁴, Hayran O⁵ and Kroon PA¹

¹Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK; ²Institute of Medical Research, Belgrade, Serbia; ³Uzhhorod National University, Uzhhorod, Ukraine; ⁴Biological Farming Association (ELKANA), Tbilisi, Georgia; ⁵YEDITEPE University, Istanbul, Turkey.
Characterization of phenolic compounds using diode array

A diode array detector allows recording of UV-vis spectrum of each peak of the chromatogram and shows unambiguous attribution of each chromatographic peak to a certain class of polyphenols, since each class exhibits a characteristic UV-visible spectrum (see Figure 1).

A - Caffeic acid
B - Flavonols
C - Anthocyanidin
D - Punicalagin
E - Flavonoid-acetylglycoside
Determination of molecular ions using positive and negative polarity mass spectrometry

The identification was carried out using mass fragmentation and supported by the presence of adducts generated by the solvent/sodium and by molecular complexes.

Figure 2: Mass spectra of caffeoylquinic acid; A-positive polarity, B- negative polarity
HPLC-DAD-MS techniques were successfully used for the identification of both known and previously unknown phenolic acids, flavones, flavonols, flavan-3-ols, anthocyanins and glucosinolates in extracts of plants typical of the Black Sea region.

Nettle  Kale  Dill

*Sideritis scardica*  Persimmon  Pomegranate
Selection of important / representative bioactives and their human metabolites

**Quercetin** (a flavonol type flavonoid) (found in high quantities as glycosides in *nettle*, *dill* and *kale*)

A mix of human metabolites of quercetin:
- Quercetin-3’-Sulfate (Q-3’-S)
- Quercetin-3-Glucuronide (Q-3-GlcA)
- Isorhamnetin-3-GlcA (3’MeQ-3-GlcA)
Selection of important / representative bioactives and their human metabolites

**Sinigrin** (the major glucosinolate of **kale**)
**Allyl-isothiocyanate** (a breakdown product of sinigrin)
**Sulforaphane** (another ITC)

A mix of human metabolites of sulforaphane:
- Sulforaphane-cysteine
- Sulforaphane-cysteine-glycine
- Sulforaphane-N-Ac-Cysteine
- Sulforaphane-glutathione
Biological activities of traditional foods of the Black Sea region

- Prioritised list of traditional foods (>30 foods)
- Selected food plants typical of the Black Sea region

Bioactive-rich extracts prepared

- Antioxidant activities - direct antioxidant action
- Anti-/pro-microbial activities - antibiotic and probiotic activity
Screening of antioxidant capacity and phenolic content of selected Black Sea area traditional foods

Francesca Danesi\textsuperscript{a}, Federica Pasini\textsuperscript{a}, Mariia Mudryk\textsuperscript{b}, Bike Kocaoglu\textsuperscript{c}, Dmitry Karpenko\textsuperscript{d}, Leonid Kapreliants\textsuperscript{e}, Marjam Jorjadze\textsuperscript{f}, Alexandru Stroia\textsuperscript{g}, Iordanka Alexieva\textsuperscript{h}, Maria Caboni\textsuperscript{a}, L. Filippo D’Antuono\textsuperscript{a}, Alessandra Bordoni\textsuperscript{a}

\textsuperscript{a}University of Bologna, Italy; \textsuperscript{b}Uzhhorod National University, Ukraine; \textsuperscript{c}Yeditepe University, Turkey; \textsuperscript{d}Moscow State University of Food Production, Russian Federation; \textsuperscript{e}Odessa National Academy of Food Technologies, Ukraine; \textsuperscript{f}“Elkana”, Biological Farming Association, Georgia; \textsuperscript{g}Bucharest University of Economics, Romania; \textsuperscript{h}University of Food Technologies, Bulgaria.
Screening of antioxidant capacity and phenolic content of selected Black Sea area traditional foods

- 39 traditional foods of the Black Sea Area countries

- Foods prepared according to traditional recipes in:
  - Bulgaria, Georgia, Romania, Russia, Turkey, Ukraine

- Total Antioxidant Capacity (TAC) - evaluated by 2 assays:
  - ABTS and DPPH

- Total phenolics (TP), hydroxycinnamic acids (HI), and ortho-diphenols (ODI) content also quantified

- Correlation analyses between TAC and phenolics
Assessment of the probiotic and antibiotic activities of prioritised traditional foods and selected food plants

Dept of Microbiology, Immunology, Virology and Etiology of Infectious Diseases
LMMI, Faculty of Medicine
Uzhhorod National University, Ukraine

Viktoriya Bati
Nataliya Markush
Nadiya Boyko

Poster 36
State of the art

Existing literature data:
- Parsley (Antimicrobial)
- Nettle (Controversial)
- Carrot (Antimicrobial)
- Beet (Stimulatory)
- Dill (Antimicrobial)
- Apple (Inhibitory)
- Dogwood (Antimicrobial)
- Garlic (Antimicrobial)
- Cranberry (Antimicrobial, and no effect)

1. Lack of data describing effects of traditional foods prioritised in BaSeFood on selected bacteria
2. Current data largely concerned with inhibitory properties of isolated plant components on microorganisms
3. Little information available regarding effects on fungi
4. Available studies have not taken account of differences in strain origin (animal/human) or virulence
Comprehensive assessment of pro- and anti-microbial effects

- 36 traditional foods, &
- 80 plant ingredients

- 27 strains of 15 (16) bacterial species
- 14 (16) pathogen / opportunistic pathogens
- 10 (11) beneficial (commensal) bacteria

→ Assessed using 4 different methods
Why are these findings important?

A number of potentially useful effects have been demonstrated

- Pre-biotic effects (↑ beneficial bacteria)
- Anti-bacterial effects (↓ potentially harmful bacteria)

It is now recognised that the gut microbiota play key roles in:

- Regulation of the host immune responses
- Initiation of metabolic or immune misbalances in the host

Some of these foods have the potential to induce healthy gut flora profiles (enterotypes)
Biological activities of traditional foods of the Black Sea region

Selected food plants typical of the Black Sea region

Bioactive-rich extracts prepared

Biological activities in cultured cell models:
- endogenous antioxidant defences
- cardiovascular health
- immune function

Biological effects in animal models:
- Host immune function
- Gut flora composition
Cells and animal models used for testing of biologically active compounds of plant origin in traditional foods – results from the BaSeFood project

Viktor Petrov, Olga Levchuk, Viktoriia Bati, Nataliya Markush, Nadiya Boyko

Dept of Microbiology, Immunology, Virology and Etiology of Infectious Diseases, LMMI, Faculty of Medicine, Uzhhorod National University, Ukraine
Counteracting cellular oxidative stress

1. *Sideritis scardica*

2. Pomegranate

Several posters
**Sideritis scardica**

- In several countries, tea is the major source of antioxidant flavonoids, and high tea consumption has been ascribed with several favourable outcomes.

- Other plants used for the preparation of herbal teas are a source of phenolic antioxidant compounds; among them *Sideritis scardica* (SS) is used for the preparation of a popular drink (mountain tea) throughout Eastern and Central Europe.

- In this study the antioxidant effects of a SS extract have been compared to a *Camellia sinensis* (CS) extract in a biological system (HepG2 cells), to set the scientific basis for the exploitation of other herbal teas in counteraction of oxidative stress.
Methods

HepG2 cells seeded in 6-well plates

After 24 h, treat cells with:
- 50 µg/mL SS extract or
- 50 µg/mL CS extract or
- 20 µM TC, or
- Unsupplemented (US)-cells (fresh medium control)

After 24 h, treat cells with 300 µM of tert-butyl hydroperoxide (t-BOOH) to induce an oxidative insult
Other cells not treated with t-BOOH (basal condition).

After 3 hours, media were removed and analysed:
- Cytotoxicity
  a. Mitochondrial viability by MTT assay
  b. Lactate dehydrogenase (LDH) leakage in the media.

- Protection against oxidative stress was by TBARS (thiobarbituric acid reactive substance) assay and by evaluating the cellular TAC [4] and reduced glutathione (GSH) content [5].
Cell proliferative activity and lactate dehydrogenase (LDH) release in the medium under basal conditions

The possible cytotoxicity of the herbal extracts and TC at the supplemented concentration was verified under basal conditions.

Neither SS nor CS nor TC supplementation caused any negative effect, as evidenced by cell proliferative activity and LDH release into the medium.

Different letters represent significant differences ($p<0.05$).
Cell proliferative activity and lactate dehydrogenase (LDH) release in the medium in stressed condition.

Clear detrimental effects of tert-butylhydroperoxide (t-BOOH)
- decreased cell proliferation
- increased LDH activity in the medium.

Only α-tocopherol fully protected against t-BOOH.

CS was partially protective against increased LDH leakage due to the oxidative stress, but Sideritis had no effect.

Different letters represent significant differences ($p<0.05$).
Thiobarbituric reactive substances (TBARS) concentration in the medium under stressed conditions.

The onset of the oxidative stress after t-BOOH exposure was confirmed by the measurement of TBARS concentration in the medium.

In basal condition TBARS content was very low and similar in US and supplemented cells (n.s., not significant), and it increased after t-BOOH exposure in all cells but TC supplemented ones. This increase was higher in US cells than in SS and CS supplemented ones.
Cytosolic total antioxidant capacity (TAC) concentration in basal conditions (A) and stressed conditions (B).

In basal condition, SS, CS, and TC supplementation caused an increase of the antioxidant defences, as evidenced by cytosolic TAC (figure A); in US cells the oxidative stress did not significantly modify the TAC, which appeared on the contrary increased in all supplemented ones (figure B).
Reduced glutathione (GSH) concentration in basal conditions (C) and stressed conditions (D).

In basal condition cells supplemented with both herbal extracts showed an increased GSH level (figure C); the thiol concentration increased in all stressed cells, and to a greater extent in supplemented ones independent of the type of supplementation (figure D).
**Pomegranate (Punica granatum L.)**

- In recent years, the number of scientific papers concerning pomegranate (*Punica granatum* L.) and its health properties has increased greatly. Among the great variety of chemical components present in the pomegranate, ellagic acid, ellagitanins (including punicalagins), punic acid, anthocyanins, flavonols, flavan-3-ols, and flavones seem to be the ones responsible, at least in part, for most of the health benefits, which appear mainly related to its antioxidant potential [1].

- Pomegranate antioxidant activity could be due not only to a direct scavenging activity, but also to the modulation of the main antioxidant enzymes [2].

- To further elucidate the mechanisms of the reported antioxidant activity, HepG2 cells were supplemented with a Georgian pomegranate extract (POME), and then subject to an exogenous oxidative stress. The expression of the gene encoding for the main antioxidant enzymes (superoxide dismutases – SODs; catalase – CAT; and glutathione peroxidases – GPXs) was determined. To compare the biological activity of whole POME extract to its main phenolic component, some cells were supplemented with punicalagin (PUNI).
• Pomegranate fruits were collected in Georgia.
• First, POM extract cytotoxicity was assessed in order to select the appropriate concentration to be used in further experiments. HepG2 cells were supplemented with serial dilutions of POM extract (0.6-30 mg/mL of medium).
Comparison of POM extract with its main phenolic components

Punicalagin (PUNI) final concentration in the medium was 1 µM, corresponding to the concentration of the compounds in the medium after the addition of 0.6 mg/mL POM extract.

HepG2 cells were supplemented with POM extract or punicalagin (PUNI) for 24 h, then some cells were exposed to 300 µM tert-butyl hydroperoxide (t-BOOH) for 3 h.

SOD1, 2, and 3, CAT, and GPX1 and 4 gene expression was determined by quantitative real-time PCR. Cell viability [3], lactate dehydrogenase (LDH) leakage [4], and cellular lipid peroxidation [5] were also evaluated.
SOD1 (A), SOD2 (B) and SOD3 (C) gene expression in control and supplemented HepG2 cells in basal and stressed conditions.

CAT (A), GPX1 (B) and GPX4 (C) gene expression in control and supplemented HepG2 cells in basal and stressed conditions.
Conclusions - Sideritis scardica

• We have demonstrated that a bioactive-rich extract of the mountain tea plant (*S. scardica*) performs as well in improving cellular antioxidant status as a catechin-rich extract of *C. sinensis*, even though the phenolic content of the *S. scardica* extract was only about 18% of that of the *C. sinensis* extract and its TAC was 10-fold lower.

• Since extracts of *C. sinensis* have been demonstrated to induce a number of beneficial physiological effects in humans, many of which may be related with the capacity of the tea catechins to improve cellular antioxidant functions, we can conclude that consumption of *S. scardica* may have health benefits.
Conclusions - Pomegranate

• At the tested concentrations, POME extract and PUNI did not counteract the detrimental effects of t-BOOH. Gene expression data indicated that POME and PUNI had different effects on the transcription of the main antioxidant genes; in stressed conditions, the up-regulation of SOD1, SOD3, and GPX4 was evident in POME cells, while SOD2 only was modulated in PUNI cells.

• Experiments are in progress on the evaluation of the activities of SOD, CAT and GPX enzymes, and conclusions on the direct/indirect POME antioxidant activity cannot be drawn without considering the incoming results. The absence of protective effect by POME and PUNI could be related to the low concentrations used.
Effects on cholesterol metabolism and trafficking

1. Phytosterols (oilseeds)

2. Kale and dill
**Phytosterols**

- PS are well-known cholesterol-lowering agents (Abumweis et al, 2008), and several theories have been proposed to explain their action (Rozner & Garti, 2006), which is considered mainly related to the inhibition of cholesterol absorption.

- Notwithstanding the low plasma concentration, due to their similarities with cholesterol, PS can be taken up by cells, incorporated into cell membranes, and affect their composition and functionality (Danesi et al, 2011).

- Since PS chemical structure is very similar to cholesterol, they could mimic its action within the cell, and their increased concentration could reduce nuclear SREBP concentration, and therefore cholesterol synthesis.

**Sunflower**  
**Pumpkin**  
**Tahini**
Impact on cell function - mechanisms

Human Umbilical Vein Endothelial Cells

**Endothelium:**
- Lines inner side of blood vessels
- Large secretory tissue (720g in human)
- Produces various mediators (NO, ET-1, prostacyclin, prostaglandin) that are important for haemostasis & fibrinolysis, and regulation of vascular tone.
- Expresses adhesion molecules involved in recruitment and binding of monocytes
- Endothelial dysfunction reflects an imbalance in the production of mediators

**We have investigated:**
- Adhesion molecules (ICAM-1, VCAM-1, E-selectin)
- Inflammatory cytokines (IL6, MCP1)
  → inflammatory status
- Vasomodulators (ET-1, cGMP, iNOS, eNOS)
  → vasomodulator balance
- Related signalling pathways (e.g. PI3K/Akt)
The three major metabolites inhibited VCAM surface expression in HUVECs at physiological concentrations.
Are polyphenols found in plants from the Back Sea region able to attenuate TNFα induced increases in adhesion molecules in endothelial cells?

Winterbone M¹, Woodcock ME¹, Cichello F¹, Konic-Ristic A², Glibetic M², Boyko N³, Jorjadze M⁴, Hayran O⁵ and Kroon PA¹.

¹Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK
²Institute of Medical Research, Belgrade, Serbia
³Uzhhorod National University, Uzhhorod, Ukraine
⁴Biological Farming Association (ELKANA), Tbilisi, Georgia
⁵YEDITEPE University, Istanbul, Turkey.
Compounds and mechanisms responsible for the \textit{in vitro} increases in nitric oxide in endothelial cells treated with a phenolic-rich pomegranate extract

Winterbone M\textsuperscript{1}, Woodcock ME\textsuperscript{1}, Cichello F\textsuperscript{1}, Konic-Ristic A\textsuperscript{2}, Glibetic M\textsuperscript{2}, Boyko N\textsuperscript{3}, Jorjadze M\textsuperscript{4}, Hayran O\textsuperscript{5} and Kroon PA\textsuperscript{1}.

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\textsuperscript{5}YEDITEPE University, Istanbul, Turkey.

\textbf{M Woodcock poster talk and Poster 43}
The effects of food plants and their bioactives on platelet function
Study objectives:

1. Inhibition of human platelet aggregation, platelet activation, platelet/monocyte and platelet/granulocyte (neutrophil) conjugate formation – *ex vivo* study

2. Inhibition of human platelet aggregation, platelet activation, platelet/monocyte and platelet/granulocyte (neutrophil) conjugate formation – *in vitro* study

Institute for Medical Research
Studies:

1. Inhibition of human platelet aggregation, platelet activation, platelet/monocyte and platelet/granulocyte (neutrophil) conjugate formation – *ex vivo (acute intervention)* study

2. Inhibition of human platelet aggregation, platelet activation, platelet/monocyte and platelet/granulocyte (neutrophil) conjugate formation – *In vitro* study

3. Inhibition of human platelet aggregation, platelet activation, *platelet/monocyte and platelet/granulocyte (neutrophil)* conjugate formation – *ex vivo (long term intervention)* study

P-32, P-34 & short talk
Design of experiments:

1. Extracts and metabolites tested:
   - Nettle
   - Dill
   - Mountain tea
   - Pomegranate
   - Kale
   - Persimmon

Metabolites:
   - quercetin-3-glucuronide
   - 3’-methyl-quercetin-3-glucuronide
   - quercetin-3’-sulfate
   - sulforaphane cysteine glyceine

**Negative control:** HTP/DMSO (0.1%/0.2%)
**Positive control:** Aspirin
2. Subjects:
   - patients with metabolic syndrome
   - healthy volunteers

3. Agonist:
   - arachidonic acid 300 µM

4. Markers:
   - P-selectin
   - GpIIb/IIIa
   - CD61+ / CD14+
   - CD61+ / CD11b+
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**Summary table**

**Metabolics (100/20)**

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**Metabolics (200/40)**

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**Healthy (200/40)**
Acute effects of selected herbs on ex vivo effects of agonists

1. **Patients:**
   88 patients with metabolic syndrome included (22 per group)

2. **Intervention:**
   water extract of 2g of dried herbs with 50 g of white bread
   control intervention: 50 g of white bread

3. **Parameters analysed:**
   - platelet activation:
     P-selectin) expression (% in CD61+ cells)
     GPIIb-IIIa expression (% in CD61+ cells)
   - platelet aggregation:
   - CD11b+CD61+ cells (platelet/ granulocyte aggregates)
     (% in CD11b+ cells)
   - CD14+CD61+ cells (platelet/ monocytes aggregates)
     (% in CD14+ cells)
Selection of subjects for chronic study:

Normalisation of parameters obtained
1. Ranks in correlation to quintiles in distribution
   - for all parameters and all subjects
   - algorithm applied:
     FINAL score: NoR5*5 + NoR4*4 + NoR3*3 + NoR2*2 + NoR1*1/total NoR
2. Omission of 20% of patients in two tails of distribution
The Effects of Plant Bioactives on Platelet Function

Wendy Hollands, Mark Woodcock, Sandra Konic-Ristic, Maria Glibetic, Nadiya Boyko, Mariam Jorjadze, Osman Hayran, Paul A Kroon
Study objectives

1. To quantify the anti-clotting activity of plant extracts and component compounds.

2. To compare differences in the anti-clotting activity between plant extracts and their conjugated plasma metabolites.

3. To investigate the effects of different concentrations of conjugated flavonoids and ITCs on anti-clotting activity.
Study design

Subject recruitment (n=15)

Whole blood collection for assessment of platelet function

Effects of crude extracts (n=10)
Persimmon, Pomegranate, Kale, Dill, Nettle & Sideritis (100 µg/ml)

Effects of plasma metabolites (n=5)
Q-conjugates (Q-3-GlcA/3’Me-Q-3-GlcA/Q-3’-sulf). Mix of 3 metabolites (5, 20 and 100 µM)
SFN and SFN-cys-gly (2 and 20 µM)

Day 1
- Treatment
- ADP

Day 2
- Treatment
- Epinephrine

Day 1
- Treatment
- ADP

Day 2
- Treatment
- Epinephrine

Assessment of platelet function (PFA-100)
Whole blood collected into 3.2% buffered sodium citrate tubes and rested for 30 mins.

Sub-samples of citrated whole blood aliquoted into tubes (in duplicate) containing treatments.

Aspirin or abcixima b

DMS O

Crude extracts and plasma metabolites

Aspirin or abcixima b

DMS O

Incubation time = 30 mins
Methodology Cont:

Post incubation 800 uL of mix is pipetted into self contained cartridges containing either Collagen/epinephrine (CEPI) or Collagen/ADP (CADP).

Simulates in-vitro platelet adhesion and aggregation that occurs following vascular injury.
Anticoagulated whole blood is aspirated from sample reservoir through a capillary tube and aperture which exposes platelets to high shear flow conditions.

Adherence of platelets to one another to form aggregates which build a platelet thrombus at the membrane aperture thereby arresting blood flow. The time it takes for the aperture to occlude is referred to as closure time.

An Inhibition of platelets (and therefore positive anti-platelet effect) is expressed as prolongation of closure time.

Platelet function analyser (PFA-100)
Results

1. Untreated whole blood samples

<table>
<thead>
<tr>
<th></th>
<th>Reported ref range</th>
<th>This study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADP - CT</td>
<td>56 - 102</td>
<td>87</td>
</tr>
<tr>
<td>CEPI - CT</td>
<td>80 - 184</td>
<td>104</td>
</tr>
</tbody>
</table>

*n=15

2. Negative Control - No significant differences in CT between start and end of each 4-h experimental period.

3. Positive control – Maximal platelet inhibition achieved in most subjects after treatment with positive control chemicals.

Therefore method worked and any absence of change real effect
4. Effects of crude extracts on platelet function

Key points

- CEPI-CT ↑ after treatments with persimmon, pomegranate and Kale but anti-platelet effect not significant.

Conversely, Sidertitis, nettle and Dill ↓ CEPI-CT

- CADP-CT were ↓ after treatment with 5 out of 6 extracts which was significant for Kale and Dill.

- ? Pro-inflammatory effect of Kale and Dill

n=10 subjects
*p= 0.03
5. Effects of conjugates on platelet function

Key points

- CEPI-CT ↑ after treatments with Q-mix at all three conc. and SFN at 2 µM conc.

  But ↓ after treatments with high conc. SFN and both conc. SFN cys gly

- CADP-CT prolonged after treatments with 100 µM Q-mix and 20 µM SFN compared with control.

- None of the effects were significant.

n=5 subjects
Comparisons with Literature

Plant foods initially chosen partially because of literature evidence that these foods or components of these foods show anti-platelet effects.

1. Pomegranate (rich source of anthocyanins)

<table>
<thead>
<tr>
<th>Food/Beverage</th>
<th>Method</th>
<th>Time/inc</th>
<th>Agonist</th>
<th>% change</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate juice (6-7ml/kg BW)</td>
<td>PFA-100</td>
<td>2 h</td>
<td>CEPI</td>
<td>↑8% (n/s)</td>
<td>Polagruto</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 h</td>
<td></td>
<td>↑19% (p&lt;0.05)</td>
<td>(2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 h</td>
<td>CADP</td>
<td>↑5% (n/s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 h</td>
<td></td>
<td>↑6% (n/s)</td>
<td></td>
</tr>
</tbody>
</table>

Polagruto - Pomegranate juice significantly prolongs CEPI –CT 6h post ingestion.

? Which bioactive compound caused effect

IFR - Marginal increase in both CEPI and CADP-CT but not to levels of significance
2. Persimmon (Rich source of monomeric flavanols and procyanidins)

<table>
<thead>
<tr>
<th>Food / Beverage</th>
<th>Method</th>
<th>Time</th>
<th>Agonist</th>
<th>% change</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa beverage (897mg flavanols)</td>
<td>PFA-100</td>
<td>2h</td>
<td>CEPI</td>
<td>↑8% (n/s)</td>
<td>Rein (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CADP</td>
<td>↑16% (n/s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CEPI</td>
<td>↑31% (p=&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CADP</td>
<td>↑13% (n/s)</td>
<td></td>
</tr>
<tr>
<td>Grape seed extract</td>
<td>PFA-100</td>
<td>1h</td>
<td>CADP</td>
<td>↑8% (n/s)</td>
<td>Shenoy (2007)</td>
</tr>
<tr>
<td>130mg/flavanols/d/8wks</td>
<td></td>
<td>2h</td>
<td></td>
<td>↑24% (p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6h</td>
<td></td>
<td>↑14% (p&lt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>

Shenoy/Rein – Prolonged CT 2 & 6h post ingestion of epicatechin rich grape seed extract and cocoa.

IFR - Marginal increase in CEPI - CT only
2. Nettle and Dill (Rich sources of Quercetin)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Method</th>
<th>Conc.</th>
<th>Inc. period</th>
<th>Agonist</th>
<th>% change</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>Flow cytometry (PRP)</td>
<td>10 µM, 20 µM</td>
<td>30 mins</td>
<td>Collagen (2mg/L)</td>
<td>↓18% (n/s)</td>
<td>Pignatelli (2000)</td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td>10 µM, 20 µM</td>
<td></td>
<td>Collagen (4mg/L)</td>
<td>↓50% (p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓11% (n/s)</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓43% (p&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>Aggregometry</td>
<td>0.25 - 2500 µM</td>
<td>10 mins</td>
<td>Collagen ADP</td>
<td>↓95% (p&lt;0.05)</td>
<td>Janssen (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓97% (p&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(all at 2500 µM)</td>
<td></td>
</tr>
</tbody>
</table>

Pignatelli – Isolated compounds significantly inhibit platelet function using flow cytometry as model for assessment.

Janssen – Significant inhibition in platelet function but at conc. not achievable in diet.

IFR - Shortened CADP/CEPI-CT after treatments with nettle and dill extracts but prolonged after treatments with quercetin conjugates at all conc.
Conclusions

• None of the six bioactive-rich extracts containing various flavonoids, glucosinolates and other bioactive compounds significantly increased closure time in the PFA-100 model for assessing functional changes in platelet activity.

• None of the human metabolites of flavonoids such as quercetin and isothiocyanates such as sulforaphane were effective in increasing closure time using the PFA-100.

Poster 30
EFFECTS OF 6-WEEK OF POMEGRANATE JUICE CONSUMPTION ON PLATELET FUNCTION IN SUBJECTS WITH METABOLIC SYNDROME: A RANDOMISED CONTROLLED TRIAL

Sandra Konic-Ristic, Maria Glibetic, T Srdic-Rajic, N Kardum, Wendy Hollands, Paul A Kroon

Oral Poster, Friday

Institute for Medical Research
Effects of 6 weeks consumption of bioactive-rich nettle and *Sideritis* teas on endothelial function and other markers of cardiovascular disease risk in an at-risk group: A Randomised Controlled Trial

Mykola Rishko, Taras Chendey, Olexandr Kutsyn, Olena Plyska and Tetyana Vasylovka

Uzhhorod National University, Uzhhorod, Ukraine
Primary objective:

To evaluate the effects of *Sideritis* and *Urtica* in the form of water decoction (tea) on endothelial-dependent flow-mediated vasodilatation in patients with coronary artery disease (CAD) or high cardiovascular risk.

Three groups in parallel (n-27 per group)
1. Nettle tea
2. Sideritis tea
3. Control group (no intervention)

Secondary outcomes
- Blood pressure
- Plasma lipoproteins and triglycerides
To evaluate the effects of *Sideritis* and *Urtica* in the form of water decoction (tea) on endothelial-dependent flow-mediated vasodilatation in patients with coronary artery disease (CAD) or high cardiovascular risk.

Three groups in parallel (n-27 per group)
1. Nettle tea
2. Sideritis tea
3. Control group (no intervention)

Secondary outcomes
- Blood pressure
- Plasma lipoproteins and triglycerides
Human study: activities

• LEC approval obtained (Mar 2011)
Primary endpoint: FMD in study groups

Nettle  |  Sideritis  |  Control

Day 1  |  Day 42

FMD, %

p=0.04
Secondary endpoints: blood pressure and plasma lipids
Conclusions

• Neither sideritis nor nettle consumption for 6 weeks did affect FMD in patients at high CV risk.

• There was a trend towards decrease in FMD in nettle group, also FMD after 6 weeks was not statistically different from control group.

• Neither sideritis nor nettle consumption for 6 weeks did affect blood pressure levels or lipid profile in patients at high CV risk.
Benefits of the research

New high quality data for some regional plant foods

More detailed research for several plants
  - extracts, isolated bioactives and human metabolites
  - cardiovascular and gut effects

High quality RCT data

A series of papers concerned with health benefits of Black Sea region plants / foods
Directions for future research

Traditional food plants consumed in the BSAC have been shown to possess potential to exert favourable effects on human health

What next?

• Focus on those with the greatest potential
e.g. Pomegranate, kale
• Careful consideration of gut and human metabolism
• Integrated approaches to explore potential cause and effect relationships
• Human studies then mechanistic studies!
• Industry involvement – focus...
A big thank you!
Beneficial effects of dietary bioactive peptides and polyphenols on cardiovascular health in humans

BACCHUS

Coordinating organisation: Institute of Food Research, Norwich
Coordinator: Paul Kroon
Co-coordinator: Paul Finglas
Project Manager: Dawn Wright

Programme “FP7 Cooperation Work Programme: Food, Agriculture and Fisheries, and Biotechnologies”
Call identifier: “FP7-KBBE-2012-6”
Proposal No. 312090 BACCHUS
Overall aim:

To develop tools and resources that will facilitate the generation of robust and exploitable scientific evidence that can be used to support claims of a cause and effect relationship between consumption of bioactive peptides and polyphenols and beneficial effects related to cardiovascular health in humans.