

Chemical Composition and Cytochrome C reducing properties of Lithuanian propolis

Sonata Trumbeckaite, Mindaugas Liaudanskas

**Lithuanian University of Health Sciences,
Medical Academy, Kaunas, Lithuania**

- Propolis possesses broad spectrum of **biological properties** related to its chemical composition.
- An intracellular antioxidant activity of propolis flavonoids may occur *via* several mechanisms within various subcellular compartments, **and mitochondria** may play an important role.

- **Mitochondria** are organelles that produce **ATP** by **oxidative phosphorylation** and also control **cell survival and death**, regulates mitochondrial ROS production etc.
- To accomplish their energetic function they have to be structurally and functionally intact.

In many pathological situations (**ischaemia, ischemia/reperfusion**) injury occurs, which is manifested as

- inhibition of oxidative phosphorylation
- loss of mitochondrial membrane integrity,
- loss of cytochrome *c* from mitochondria

- One of the **earliest events** in ischaemic heart damage is the **release of cytochrome c** from mitochondria which may lead to apoptosis by the mechanism involving binding of cytosolic cytochrome c to adaptor protein Apaf-1.
- The **redox state of cytochrome c** may regulate the process of caspase activation because according to several studies reduced cytochrome c is less potent in caspase activation than its oxidized form
- Further studies are required to determine which other propolis compounds provide most powerful cytochrome c reducing activities as they may have a potential application in inhibiting apoptosis

Polyphenols are generally strong antioxidants and possible reductants

- Our previous study show that some reductants (anthocyanins) block apoptosis by reducing cytosolic cytochrome c, which prevents caspase activation (Skemiene et al, 2013, Liobikas et al., 2016).

- Various plant flavonoids known as strong natural antioxidants
- In principle, they could reduce cytosolic cytochrome c and block caspase activation
- This has not been investigated yet

- **The aim** of this research was to determine **qualitative and quantitative composition** of Lithuanian propolis using UPLC-ESI-MS/MS analysis.
- and to **investigate the ability** of some propolis flavonoids **to reduce cytochrome c**, a component of the mitochondrial electron transport chain.

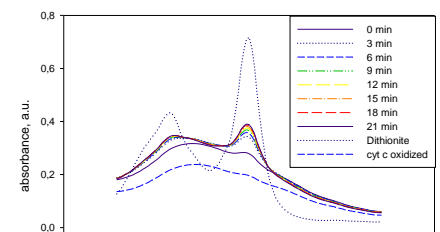
Methods

Preparation of propolis ethanolic extracts : crude propolis was grounded into powder and macerated in 70 % ethanol (1:10 w/v) by shaking. Extraction time - 72 h in room temperature.



Analysis of extracts by Acquity H-class UPLC system equipped with triple quadrupole tandem mass spectrometer with an electrospray ionization source (ESI) to obtain MS/MS data.

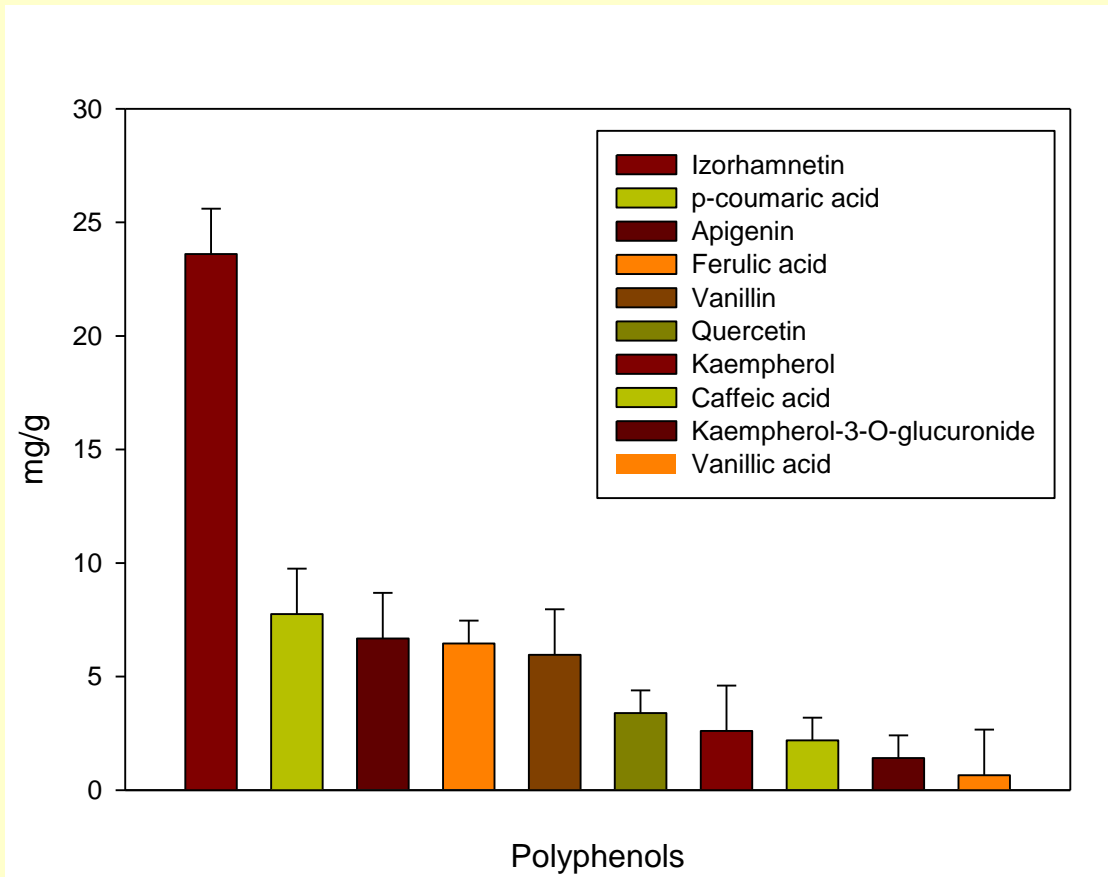
The **cytochrome c reduction** level by flavonoids was recorded **spectrophotometrically** (500 – 600 nm)



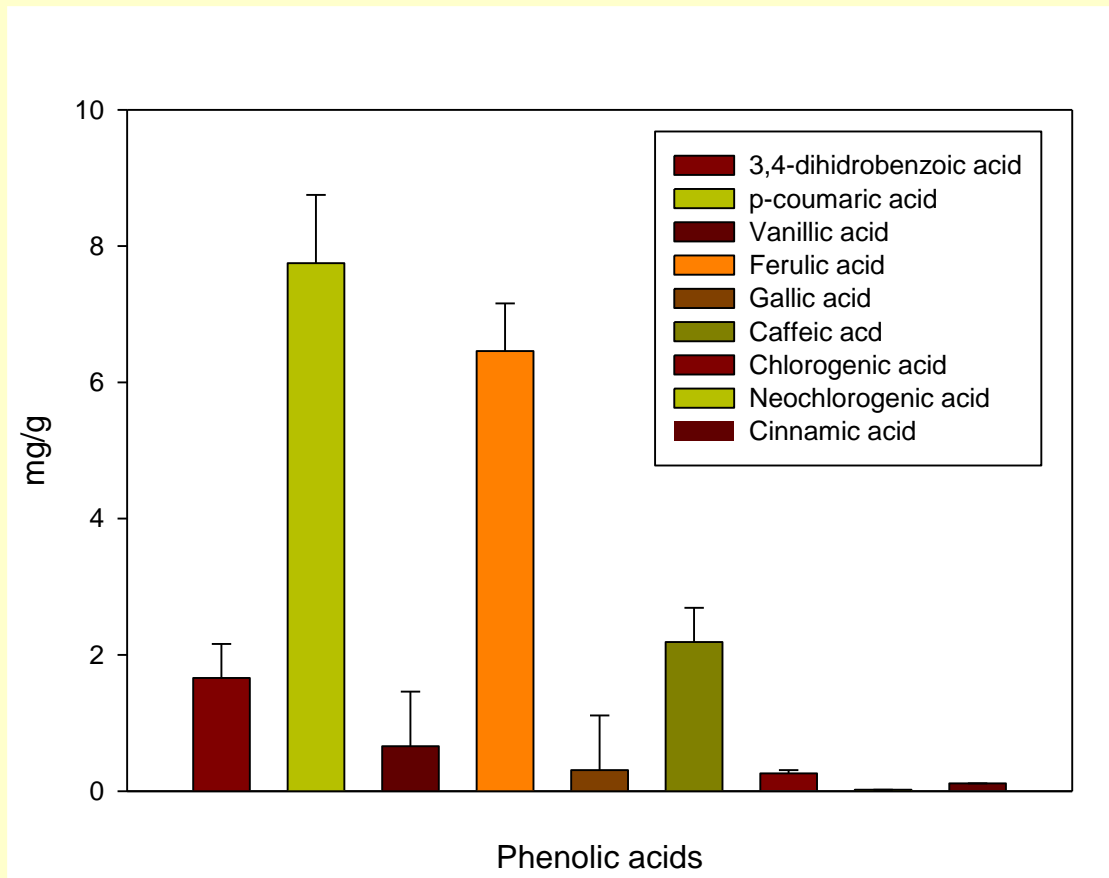
Results (1)

Chemical composition of Lithuanian propolis

The main polyphenols of Lithuanian propolis



The main phenolic acids of Lithuanian propolis

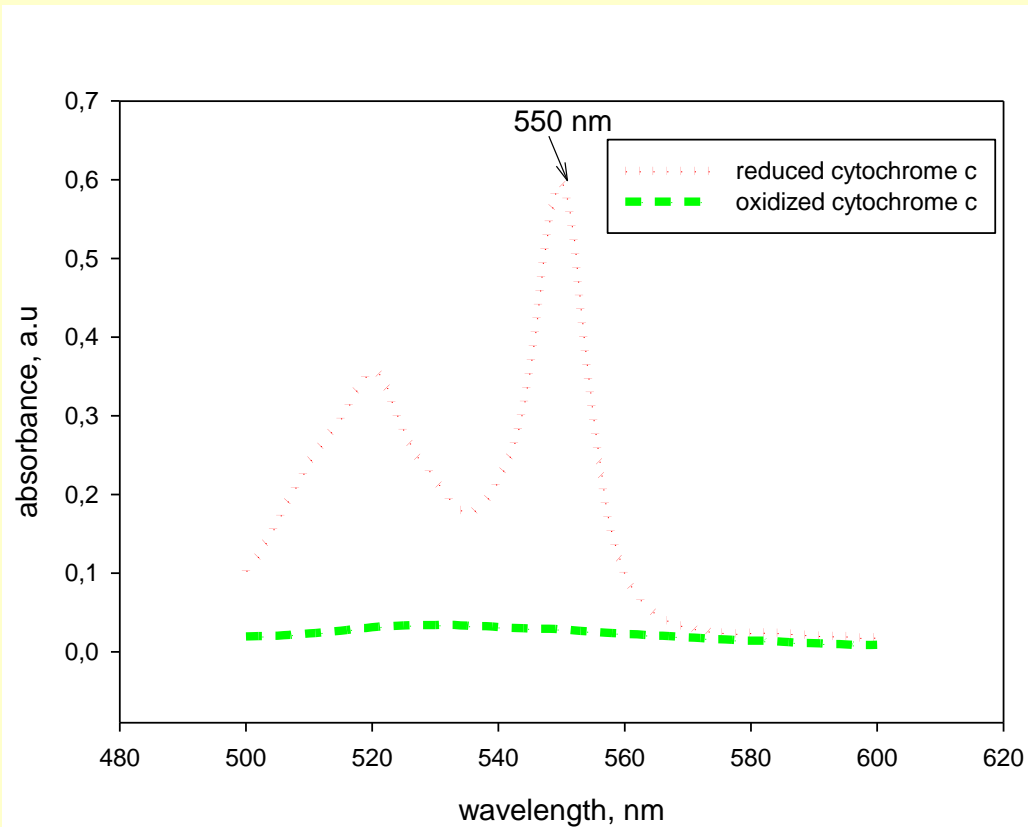


Results (2)

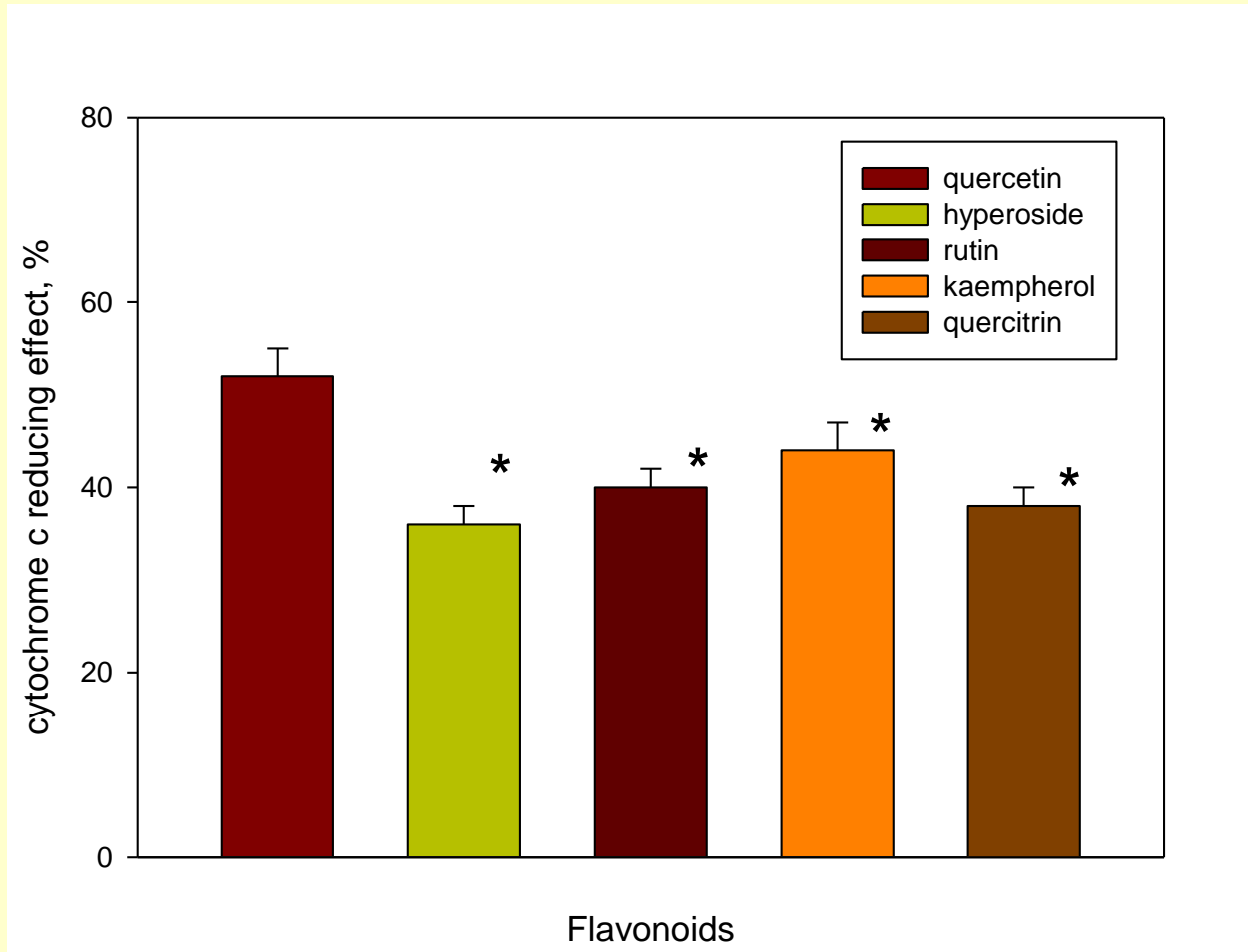
**Cytochrome c reducing properties
of some propolis flavonoids**

Measurement of cytochrome c reduction level

- The **cytochrome c reduction** level was recorded **spectrophotometrically** (500 – 600 nm)



Effect of flavonoids on cytochrome c redox state



Conclusions

- The most predominant Lithuanian propolis compounds of all identified and quantified analytes were izorhamnetin followed by apigenin, p-coumaric acid, ferulic acid, vanillin, quercetin and kaempferol
- Our results showed the efficient cytochrome c reducing capacities of propolis flavonoids: quercetin is able to reduce cytochrome c rapidly whereas the cytochrome c reducing activities of hyperoside, rutin and quercitrin are lower