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Antioxidant Potential of Polyphenols: In Need for Critical Assessment of *In Vitro* Results

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Editorial

Antioxidants have become OTC (Over the Counter) prophylactic dietary supplements hit of the 21st century. Polyphenols, including flavonoids and phenolic acids, are widely distributed antioxidants and are present in medicinal drugs, fruits and vegetables (food) e.g. propolis, wine, chocolate… [1]. Individuals tend to reach for polyphenols as a source of longevity which can be related to their ability to reduce oxidative stress. Oxidative stress has been postulated as a cause of many diseases e.g. rheumatoid arthritis, diabetes, skin diseases, cataract, Alzheimer and Parkinson disease… Question that we often face is how effective commercially available product as antioxidants is. Predominantly this answer is gained through *in vitro* testing that includes spectrophotometry and chromatography.

Spectrophotometry methods of choice determine quantity of polyphenols or their antioxidant potential. The Folin-Ciocalteu method is used for quantification of total polyphenols and is based on the reduction of MoO^{4+} to MoO^{3+} that can be monitored at 765 nm as change in color from yellow to blue. Polyphenols in propolis, wine, herbal drugs and foods have been determined by this method [2]. Total content of flavonoids can be determined by Christ-Müller's method after acid hydrolysis as liberated aglycones in a complex with Al^{3+} in a methanol-ethyl acetate-acetic acid medium at 425 nm. This method is effective in determining of content of flavonoids in medicinal plants, e.g. Rhamnus and Frangula species [3]. Total phenolic acids can be determined by measuring the absorbance of a complex formed between phenolic acids and sodium molybdate / nitrite at 505 nm. This is official method for determination of rosmarinic acid in *Melissae folium*, and has been successfully employed in determination of phenolic acids in Ilex leaves [4]. Total polyphenols, non-tannin polyphenols and tannins can simultaneously be determined by a spectrophotometric method using phosphorous-tungsten acid and hide powder e.g. *Teucrium* sp. [5].

Potential of polyphenols as antioxidants can be assessed using free stable colored radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). While DPPH comes in radical form, ABTS has to be activated with potassium persulphate. In ABTS test radical reduction is accompanied by change from blue-green and DPPH from violet to yellow. Loss of color is monitored at 730 nm and 518 nm, respectively. These methods have quite often been used for antioxidant potential assessment of foods and medicinal plants [2,6,7]. These are just some of the most used spectrophotometric methods, although list of methods for determination of polyphenols and their activity is rather extensive.

Results of spectrophotometric tests for determination of total polyphenols are usually expressed as equivalents of one phenolic acid or flavonoid e.g. gallic acid or quercetine. Expressing results of antioxidant is less uniform. It goes from expressing just percentage of discoloration compared to control, IC_{50} to more complex kinetic models. Although percentage of discoloration is easy to express it is highly dependent on experimental conditions and results of one research cannot be easily extrapolated and compared. Ascorbic acid and tocoferol are often used as controls and results should be expressed as equivalents of either of these vitamins or another polyphenol. While this will be satisfactory for most heterogeneous samples, problems emerge with similar samples with high antioxidant potential, e.g. propolis where expressing kinetic parameters was of use [8].

Most widely known and used antioxidants rich in polyphenols are propolis and wine. Beneficial effect of wine is well known under a catchphrase French paradox, while propolis has been in use mostly because of it antimicrobial potential. Both propolis and wine are complex mixture from which polyphenols can easily be separated and analyzed by liquid chromatography. As flavonoids and phenolic acids are UV active reverse phase High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD), as well as High Performance Thin Layer Chromatography (HPTLC) with densitometry are methods of choice. HPLC and HPTLC have been used in separation, determination and quantification of polyphenols in wine, propolis and medicinal plants [1,9]. As assessors of scientific manuscripts most common mistake we observe is tendency to determine and quantify individual polyphenols by chromatography and, at the end, come to a conclusion about total polyphenols as a sum of individual compounds. HPLC and HPTLC will not give an answer about total polyphenols content, spectrophotometric methods should be used for determination of total content.

Polyphenols have potential for many beneficial effects on human health. It has been shown that propolis consumption decreases malonaldehyde production (degradation product of peroxidation of polyunsaturated fatty acids) by 23.2% and increases superoxide dismutase activity (antioxidant enzyme) by 20.9%. This observation was sex dependent [10]. *In vitro* tests of platelet aggregation showed that flavonoids reduce platelet aggregation in concentrations that can be achieved *in vivo*. Moreover, flavonoids can interfere with diagnosis of von Willebrand factor related blood clothing disorders [11].

Many of pharmacological actions of polyphenols can be related to their antioxidant activity; same was has also been observed in platelet function tests. However, it should be noted that doses used and concentrations achieved in blood, as flavonoids can also have proaggregatory effect depending of concentration [11]. Thus the same

as for vitamins, exposure to polyphenols should not go above the recommended daily values. It should also be noted that data from *in vitro* studies cannot easily be extrapolated to *in vivo* observations, as *in vitro* data tends to overestimate the pharmacological effect.

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