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Chemical fingerprinting and evaluation of free-radical scavenging activity of multivitamin supplements

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The sales of multivitamins have been growing fast ever since and the natural-concept and/or plant-based products are easily found in the market nowadays. The natural plant-based multivitamins lead consumers to anticipate additional health benefits from phytochemicals that accompany the vitamins in whole plants. Phytochemicals are expected to be beneficial in the human body, especially as antioxidants. Growing evidence suggests that phytochemicals can act as free radical-scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors, and synergists[1]. To develop a model to verify the identity of plant-based multivitamins, 15 commercially available vitamin supplements were investigated for the evaluation of *in vitro* free-antioxidant activities based on chemical fingerprinting. To determine the contribution of nutrients or phytochemicals to the total antioxidant activity of the multivitamins, net effects[2] of each part was computed after variable selection using F-tests. The repeatability was evaluated with 3 lots of test products.

Antioxidant activities were estimated and compared by using a range of techniques, such as Oxygen reducing antioxidant capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant capacity (FRAP), and 2,2-Diphenyl-1-picrylhydrazul (DPPH). Fourteen phytochemicals were identified and quantified using high performance liquid chromatography coupled with photo diode array detection and mass spectrometry (q-Tof) to identify major contributors to their antioxidant effect; the most abundant phytochemicals in descending order were the hesperidin (9 samples), β-carotene (5 samples), and ellagic acid, zeaxanthin, lycopene (2 samples).

In an attempt to quantify oxidative stress (OS) in a cell model, we examined OS induced by incubating for 1 hour with various free radical generators in HepG2 cells by using the dichlorofluorescein (DCF) assay. In addition, total phenolic contents were also estimated using the Folin-Ciocalteau method.

Most, but not all, of the correlations could be explained based on similarities in the mechanisms of antioxidant effect and many correlations/non-correlations were also observed in this study. For example, strong positive correlations ($P<0.01$) were found between ORAC and Vitamin C, keracyanin, hisperidin, lutein and lycopene. Similarly, a strong positive correlation was observed between TAS and Vitamin C, quercetin, and lutein ($P<0.01$). Significant positive correlations between intracellular FRAP and Vitamin C, quercetin, and lutein ($P<0.01$). There was a strong correlation ($P<0.01$) between DPPH and Vitamin C, E, quercetin, and lutein. Interestingly, strong correlations ($P<0.01$) were found between total polyphenols and Vitamin C, keracyanin, and lutein.

Compounds	ORAC	TAS	FRAP	DPPH	Total
Vitamin C	$P<0.0001$	$P<0.0001$	$P = 0.0021$	$P = 0.0094$	$P = 0.0073$
Vitamin E	$P = 0.0256$	–	$P = 0.0177$	$P = 0.0086$	–
Selenium	$P = 0.0186$	–	–	–	–
Keracyanin	$P = 0.0031$	–	–	–	$P<0.0001$
Ellagic acid	$P = 0.0149$	$P = 0.0438$	–	–	–
Hesperidin	$P<0.0001$	–	–	–	–
Lutein	$P = 0.0051$	$P<0.0001$	–	$P = 0.0003$	$P = 0.0029$
Lycopene	$P<0.0001$	–	$P<0.0001$	–	–
Quercetin	–	$P<0.0001$	$P<0.0001$	$P<0.0001$	–

Values show a contribution of multiple variables to five biological of ORAC, TAS, FRAP, DPPH, and Total polyphenols.

The research shows intervarietal differences among multivitamin supplements in terms of antioxidant properties and phytochemicals that could prevent oxidative stress.

1. Hayes JE, Allen P, Brunton N *et al.* (2011) *Food Chemistry* **126**, 387–393.
2. Cook RD. (1998) Regression Graphics. Wiley, New York.