

CONTRIBUTION OF POLYPHENOLS AND ASCORBIC ACID TO THE ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF CITRUS LUMIA RISSO JUICE

Marcella Denaro¹, Antonella Smeriglio¹, Clara De Francesco¹, Domenico Trombetta¹

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina - Italy

Introduction: Citrus fruits are rich source of bioactive compounds, which exert several health properties. The antioxidant activity and polyphenol content of several Citrus fruits have been evaluated. Recently, the polyphenol profile of albedo extract of an ancient Mediterranean species, *Citrus lumia Risso*, was investigated for the first time. It has proved to be an interesting source of bioactive compounds showing a very high polyphenol content, mainly flavanones, with antioxidant and cytoprotective properties. Moreover, the chemical composition and the antioxidant, anti-cholinesterase and neuroactive properties of *C. lumia* essential oil, was investigated showing that the fruit peel possess marked antioxidants activities that could be exploited for the prevention of oxidative stress-related diseases, especially neurodegenerative ones.

Materials and methods: The aim of this study was to investigate the polyphenol profile and the ascorbic acid content of *C. lumia Risso* juice by RP-LC-DAD-FLU-MS analyses as well as its antioxidant and anti-inflammatory activity by several in vitro assays. At this purpose, the determination of the antioxidant and free radical scavenging activity was carried out using several assays (DPPH, TEAC, FRAP and ORAC) to test the activity of the sample under different reaction environments. Moreover, a preliminary screening of its anti-inflammatory activity was carried out by bovine serum albumin heat-induced denaturation assay. Furthermore, an in-depth analysis of main polyphenols alone or in combination (polyphenol mix) as well as ascorbic acid contribution on the plant complex activity was investigated.

Results: Twenty-three polyphenols were identified and quantified by RP-LC-DAD-FLD-MS analysis. Flavonoids represent 94.22% of total polyphenols identified with flavan-3-ols as the most abundant class (79.23%) followed by flavonols (9.44%), flavanones (4.18%) and flavones (1.36%). Catechin was the main compound (8.974mg/100 mL) followed by hyperoside (0.678mg/100 mL), quercetin (0.368mg/mL) and esperidin (0.254mg/100 mL). The ascorbic acid content was 15.14mg/100 mL. The juice showed the strongest antioxidant and free radical scavenging activity, with the following order of potency expressed as half inhibitory concentration (IC_{50}): ORAC (IC_{50} 98.47nL) > TEAC (IC_{50} 209.81nL) > DPPH (IC_{50} 321.20 nL) > FRAP (IC_{50} 523.43nL). The main polyphenols (catechin, hyperoside, quercetin and esperidin) as well as ascorbic acid tested alone showed a weaker antioxidant and free radical scavenging activity with respect to *C. Lumia* juice. On the contrary, the polyphenol mix showed a superimposable activity in the TEAC assay, whereas in the other ones a five-fold lower activity with respect to the plant complex was observed. Regarding anti-inflammatory activity, diclofenac sodium (50 μ g/mL), used as positive control, showed an inhibitory activity of 60.51%. *C. lumia* juice, as tale, showed the best protein denaturation inhibition (77.35%). Polyphenols and ascorbic acid contribute to the plant complex activity with the following order of potency: ascorbic acid (15.20%) > hyperoside (11.40%) > quercetin (8.12%) > hesperidin (7.81%) > catechin (6.36%). According to previous results, the polyphenol mix showed the best protein denaturation inhibition (31.60%).

Discussion and conclusion: *C. lumia* juice showed the best antioxidant and anti-inflammatory activity. Polyphenols, polyphenol mix and ascorbic acid contribute only partially to the biological activities of the plant complex investigated highlighting a synergistic effect by the other juice matrix components. In conclusion, *C. lumia* juice could exert beneficial effects in oxidative stress-based as well as chronic inflammatory disorders.