The Biological Potentials of Olive Oil By-products: Valorization Through Recovery and Utilization of Bioactive Metabolites

ロジャース, ムワカルクワ

（様式３）Form 3

氏　　名　：Rogers Mwakalukwa (ロジャ-ス ムワカルクワ)
Name

論 文 名　：The Biological Potentials of Olive Oil By-products: Valorization Through
Title　　Recovery and Utilization of Bioactive Metabolites

(オリーブオイル副産物の生理活性：生理活性成分の回収・利用による付加価値創出)

区　　分　：Kou
Category

論 文 内 容 の 要 旨
Thesis Summary

A recent global increase in demand for olive oil leads to an increase in the amount of olive oil by-products (OOBPs) produced – olive mill waste (OMW) and leaves. The OOBPs raise environmental concerns due to high organic contents which are not easily biodegradable, especially in OMW. To address this problem, new ways to utilize these by-products are needed to save the environment and the general ecosystem. Fortunately, research has proved that they constitute useful bioactive compounds, more than 98% of the total olive biophenol content. This means that, OOBPs may be further utilized and can no longer be regarded as ‘wastes’ but as a ‘cheap source of valuable compounds’. One of the most promising ways to utilize them is through the recovery of those valuable compounds, which can be utilized in different fields – a process called valorization. To address the usefulness of OOBPs, the biological potential of the compounds recovered from OOBPs was explored in this work. In the process we targeted non-communicable diseases – allergy, diabetes, and Alzheimer’s (AD) because of the alarming rise in their prevalence, globally. To evaluate the anti-allergic and anti-diabetic activity, we used the OMW sample, and in both cases the bioassay-guided fractionation approach was employed and led to the isolation of ten anti-allergic compounds and eight anti-diabetic compounds, respectively. While for AD, the olive leaves sample was used, and contrastingly, a non-targeted metabolomics approach for the determination of anti-AD metabolites was employed using six cultivars of olive leaves.

In the anti-allergic study, the activity of the isolated compounds on the commonest form of an allergic reaction, ‘type 1 allergy’ was investigated. In type 1 allergy, basophil cells play important roles by releasing histamines or other cytokines after being mediated by IgE – a process called degranulation, which is accompanied by the rise of intracellular Ca^{2+} levels, $[Ca^{2+}]_i$. It was found that five compounds, including a novel one, actively inhibited degranulation by >80% (11-oxomaslinic acid, new HDOA, luteolin, hydroxytyrosol acetate, 1-acetoxypinoresinol). Additionally, we evaluated their ability to reduce; intracellular Ca^{2+} levels and the expression of calcium channel proteins in rat basophilic (RBL-2H3) cells, to assist the possible characterization of the mechanisms involved. Our findings revealed that they reduce $[Ca^{2+}]_i$ by decreasing the expression of calcium channel proteins, suggesting that they act mainly as ‘mast cell stabilizers’ to reduce RBL-2H3 cells’ degranulation – a marker of an allergic reaction.

While in the anti-diabetic study, we investigated the ability of isolated compounds to inhibit

α-glucosidase and *α*-amylase enzymes. These enzymes were targeted because, in controlling diabetes and its complications, it is of vital importance to control postprandial hyperglycemia, which is a result of hydrolysis of carbohydrates – which is primarily catalyzed by *α*-glucosidase and *α*-amylase enzymes. We found that four compounds showed strong inhibitory activity on both *α*-glucosidase and *α*-amylase enzymes with more potency or close to that of acarbose (Oleanolic acid, maslinic acid, 1-acetoxypinoresinol, and luteolin-7-*O*-*β*-D-glucoside). We also characterized their inhibitory kinetics to the enzyme *α*-glucosidase to understand the mechanisms involved, and the inhibitory kinetics analysis revealed that oleanolic acid, 1-acetoxypinoresinol, and luteolin-7-*O*-*β*-D-glucoside inhibit *α*-glucosidase in a mixed-type, non-competitive and uncompetitive mechanisms, respectively. Our findings were backed up by the calculated inhibition rate constants (K_i) values.

On the other hand, we applied a metabolomics approach as it is informative, predictive, and discriminative – thus it could inform, predict, and discriminate the anti-AD compounds in six cultivars of olive leaves. In the study, a novel UPLC/QTOF–MS-based non-targeted metabolomics with MS^n data acquired through all-ion fragmentation (AIF) mode, was carried out to analyze any cultivar-specific alteration in metabolites among six olive leaves cultivars. Briefly, LC/QTOF-MS in combination with the multivariate analysis was performed to achieve our aim. At first, the secondary metabolites in olive cultivars (phenolic and triterpenic compounds) were acquired from LC/MS analysis and exported as molecular features (MFs). Then, they were imported into a differential analysis software (MPP) for further processing including alignment, normalization, defining sample sets, and filtering by frequency (FbF) – in the end, after recursion, a total of 66 MFs were qualified as major metabolites in the cultivars. At last, the multivariate analysis (unsupervised principal component analysis (PCA) and supervised partial least square (PLS-DA) models), were applied for discrimination analysis whereby the marker metabolites from the cultivars were correlated with the anti-AD activity. Until now, to identify final metabolites that contribute the most to the anti-AD is ongoing.

In summary, our findings, even though they are based on *in-vitro* assays, highlight the potential of OOBPs and their constituents against allergy, diabetes and AD. This, in turn, attracts more attention to OOBPs as an important source of lead compounds that may be used as ingredients in food additives, food supplements, or for structural modification to develop novel drugs for the management/ treatment of allergies, AD and diabetes. Also, this work adds value to the importance of metabolomics application in the field of natural product chemistry especially in identifying the specific biomarkers in closely related plant samples (eg, cultivars or varieties) which may differ in terms of their bioactivity. That is to say, this work gives more scientific-driven evidence to support the utilization of the OOBPs in the cosmetic, pharmaceutical, and food industry.