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SHORT COMMUNICATION



## Isolation of polyphenol compounds from olive waste and inhibition of their derivatives for $\alpha$ -glucosidase and $\alpha$ -amylase

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### ABSTRACT

Olive waste was used as a sustainable resource because it contained a variety of valuable compounds. The polyphenols active fraction from enrichment by microporous resin and extraction with ethyl acetate were analysed by different chromatographic methods. A total of 14 polyphenolic compounds were isolated and identified by structure elucidation. Based on the above obtained compounds, tyrosol was selected as a characteristic polyphenol and participated in transesterification reaction to synthesise  $\beta$ -ketoester using  $\text{Yb}(\text{OTf})_3$ . Then the Biginelli reaction with benzaldehyde, urea and ketoester (1:1.2:1.2) was performed at 90 °C for 3.0 h under the acidic condition. In addition, the  $\beta$ -ketoester prepared using tyrosol with benzyl had a greater inhibitory effect on  $\alpha$ -glucosidase and  $\alpha$ -amylase, and the inhibition of enzyme activity for 3, 4-dihydropyrimidinone derivatives prepared using abovementioned  $\beta$ -ketoester was improved significantly. Meanwhile, fluorine-containing dihydropyrimidinone derivatives were considerable inhibitors for both enzymes.

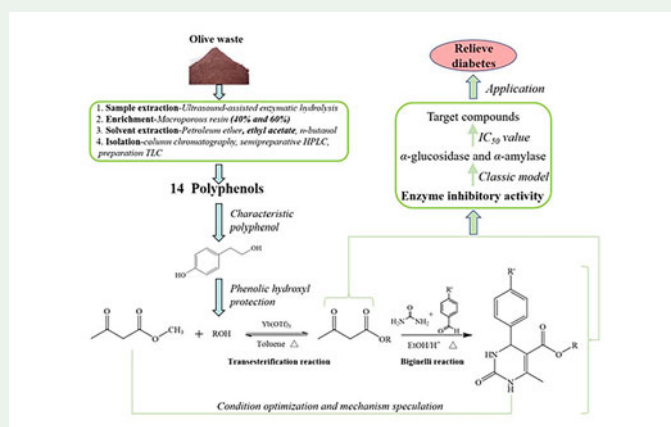
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## 1. Introduction

Olive oil has natural health functions due to its multiple active ingredients, and which is obtained from olive fruit by different extraction methods. It is worth nothing that 98% of the phenolic compounds from olive fruit were found in olive waste (Obied et al. 2005). Therefore, polyphenol compounds can be utilised efficiently and further derivatised due to the special hydroxyl structure by transesterification (Otera 1993). The  $\beta$ -ketoester from transesterification is a multi-coupling reagent with electrophilic and nucleophilic sites, and can be used to the preparation of dihydropyrimidinones, which is also an important pharmaceutical intermediate and has widely application prospects in biology and pharmacology (Chavan et al. 2003). Diabetes is a disease caused by endocrine disorders, especially type II diabetics, controlling postprandial hyperglycemia is one of the very significant treatments for relieving symptoms (Zhang et al. 2014). At present, the development of new drugs with inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase and safe, efficient and no side effects has been widely concerned (Custódio et al. 2015). In this study, some active compounds were isolated from the active fraction of olive waste, and tyrosol as a characteristic compound was selected for the preparation of target derivatives. Meanwhile, the inhibitory effect of these target products on  $\alpha$ -glucosidase and  $\alpha$ -amylase have been investigated.

## 2. Results and discussion

### 2.1. Isolation and identification of polyphenol compounds

Based on previous research results (Wang et al. 2017; Wang and Wang 2018), olive pomace extracted by ethyl acetate (OPE) was considered to be the active fraction and further investigated. The separation methods were considered using different chromatography technology (Figure S1). The structure was identified by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum data (Figure S2). SciFinder database and Spectral Database for Organic Compounds (SDBS) were used for the NMR spectra analysis. Due to differences in sample preparation methods, detection techniques and selected elution conditions, a total of 14 compounds (mainly phenolics or flavonoids group) were obtained (Figure S3), including ferulic acid (OPE-1), catechin (OPE-2), vanillin (OPE-3), protocatechuate (OPE-4), ethyl gallate (OPE-5), coumalic acid (OPE-6), tyrosol (OPE-7), hydroxytyrosol (OPE-8), eugenol (OPE-9), 4-hydroxybenzoic acid (OPE-10), quercetin (OPE-11), luteolin (OPE-12), apigenin (OPE-13), chlorogenic acid (OPE-14).

### 2.2. Transesterification reaction

Because the tyrosol structure contained phenolic hydroxyl and alcohol hydroxyl, the product of transesterification might be divided into two types. On the one hand, tyrosol was directly involved in transesterification (compound 1), on the other hand, the phenolic hydroxyl group of tyrosol was protected by the benzyl group, then the ketoester was obtained from above product through transesterification reaction (compound 2) (Figure S4). Compared to the yield (34.6%) of compound 1, the transesterification reaction of tyrosol protected for phenolic hydroxyl was more favorable for the

preparation of compound 2, and the  $\beta$ -ketoester yield reached to 75.7%. Ytterbium (III) trifluoromethanesulfonate ( $\text{Yb}(\text{OTf})_3$ ) was a mild Lewis acid catalyst and used in the transesterification (Hanamoto et al. 1996). The yield of product was higher with 0.3 equivalent of  $\text{Yb}(\text{OTf})_3$  compared with other amount of catalyst (Table S1). The special complex formed with methyl acetoacetate and  $\text{Yb}^{3+}$  was critical to the reaction (Dharma Rao and Kaushik 2011).

### 2.3. Preparation of dihydropyrimidinone derivatives

According to the results from above experiment, the ketoesters selected (Methyl acetoacetate and compound 2) were used in the preparation of dihydropyrimidinones derivatives through Biginelli reaction (Heravi et al. 2018; Kaur et al. 2017). Ethanol was chosen as ideal reaction solvent in the experiment (Zumpe et al. 2007). Considering the yield of the product and the cost of experiment, the ratio of reaction substrate was chosen as  $n$  (benzaldehyde): $n$  (urea): $n$  ( $\beta$ -ketoesters) = 1:1.2:1.2, and the result showed that the yield of target compound was more satisfactory at 90 °C (Table S2). Based on the reaction condition obtained, the Biginelli reactions of the  $\beta$ -ketoesters selected, urea and benzaldehyde with different substituent groups were performed. Subsequently, compounds 2a, 2b, 2c, 2d, 2a', 2b', 2c' and 2d' were obtained (Figure S5). The yield of product was affected by benzaldehyde containing different substituents, but their values were higher than 60% (Table S3). It was worth noting that the electrophilicity of carbonyl oxygen was increased and intermediate might be generated under acidic conditions. Furthermore, the nucleophilic addition reaction of N-acylimine ions with carbonyl isomerization was considered to be the critical step (Kaur et al. 2017).

### 2.4. Enzyme inhibitory activity of target products

The inhibitory effects of different derivatives obtained from above reactions on  $\alpha$ -glucosidase and  $\alpha$ -amylase activity were investigated (Hamid et al. 2015; Tan et al. 2017) (Table S4). The ketoester prepared from tyrosol, whose phenolic hydroxyl group was protected with benzyl group, had better inhibitory effect on the both enzyme activities, and the yield was also higher. Therefore, it was chosen as one of the reactants to participate in the preparation of dihydropyrimidinones derivatives. The inhibitory effect of the derivatives on these two enzyme activities had been significantly improved, and these compounds contained  $\beta$ -amino ketone structure. Fluorine-containing compounds have better inhibition on the  $\alpha$ -glucosidase and  $\alpha$ -amylase activities than other substituents. Because fluorine had strong electronegativity, relatively small size, lipophilicity and notable leaving group ability from anionic intermediates, it could achieve better inhibition of enzyme activity by replacing hydrogen atoms and induction effects (Robert 1992). Furthermore, dihydropyrimidinones prepared from the above selected  $\beta$ -ketoesters (compound 2) exhibited better inhibition to the both enzymes than other corresponding derivatives. The transesterification and Biginelli reactions with enzyme catalysis have been reported, but the reaction are often limited by the characteristic group of the compounds, the reaction condition and the reaction time. In future

research, in addition to further focusing on the activity evaluation of new compounds, it is very necessary to optimize the preparation process of the target compound by using green technology and novel catalyst.

## 4. Conclusions

In summary, a total of 14 compounds were isolated and identified from the olive waste active fraction, which was obtained by microporous resin enrichment and ethyl acetate extraction. Based on the obtained compounds, as a characteristic compound, tyrosol was chosen with a simple and effective transesterification reaction to synthesize  $\beta$ -ketoester using a catalyst of  $\text{Yb}(\text{OTf})_3$  and the reaction mechanism might be related to the formation of a complex system between  $\text{Yb}^{3+}$  and reactants. Then the Biginelli reaction with benzaldehyde, urea and ester (1:1.2:1.2) was performed at  $90^\circ\text{C}$  for 3.0 h under the acidic condition using  $\beta$ -ketoester derived from tyrosol. And the nucleophilic addition reaction of N-acylimine ions with carbonyl isomerization was crucial in this process. In addition, compared to the product of direct transesterification, the  $\beta$ -ketoester derivatives prepared using tyrosol, whose phenolic hydroxyl group was preferentially protected, had a greater inhibitory effect on  $\alpha$ -glucosidase and  $\alpha$ -amylase, and the inhibition of enzyme activity for 3, 4-dihydropyrimidinone derivatives prepared using this  $\beta$ -ketoester was improved significantly. Meanwhile, fluorine-containing derivatives were considerable inhibitors for both enzymes.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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