The inhibition effects of melon on mushroom tyrosinase activity

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Abstract- The inhibition effects seed and peel of melon on diphenolase activity of mushroom tyrosinase were investigated. The IC50 values and Ki values of seed and peel of melon were evaluated. Increased the Km value and decreased the Vm value so it show mixed type inhibition on mushroom tyrosinase when L-DOPA was used as a substrate.

Introduction

Melanogenesis is a physiological process resulting in melanin production [1]. Melanin is one biopigment that widely distributed in nature [2]. This process dependent on activity of melanogenic enzymes, such as tyrosinase(EC.1.14.18.1) [3]. Another name of tyrosinase, which is also known as polyphenol oxidase(ppo) [4]. PPO is a copper-containing glycoprotein widely distributed in microorganisms, animals, plants and insects. and accept many catechols and phenols as substrate [3].

Tyrosinase catalyses two steps of melanogenesis, the hydroxylation of monophenolic compounds to o-diphenols, monophenolase activity, and oxidation of the o-diphenols to o-quinones, diphenolase activity [5].

The common tyrosinase inhibitors for example Kojic acid [6]. This inhibitor is one of the metabolites produced by various bacterial or fungal strains such as penecillium and aspergillus [6]. Today, natural resources, for example, plants have a role in inhibiting tyrosinase. In this study, inhibitory effect of melon of Khuzestan was evaluated on mushroom tyrosinase.

Materials and methods:

Plants materials
Seed and peel of melon were used in this study.

**Chemicals**

Mushroom tyrosinase (EC 1.14.18.1) was purchased from sigma Chemical Co. Kojic acid, DMSO and L-Dopa(Dihydroxy phenilalanin) were products of Aldrich.

**Extraction**

The seed and peel of melon were extracted by maceration method. Filtered, extracts, were concentrated at 45°C temperature on a rotary evaporator and lyophilized. 0.1 g of extracts were solved in 3 ml DMSO. Then the yields were diluted with 25mM phosphate Buffer (pH 6.8).

**Enzyme assay of tyrosinase**

The tyrosinase activity was determined according to Kubo and Kinst-Hori method 1998 with some modification. First 50μl of tested sample(8.3-0.26 mg/mL) was mixed with 100μl of mushroom tyrosinase (9.63U/ml).After incubated at 25°C for 5 min. Then 100μl of 5mM L-Dopa solution added to the mixture [7].The amount of Dopachrom in reaction was immediately determined against blank in optical density at 475 nm in microplate reader (Tecan sunrise, Germay) during 35 min [8].

DMSO and Kojic acid(positive control) were used. inhibitory effects of the tested samples on the mushroom tyrosinase activity were expressed as % inhibition.IC50 values were defined as. The concentration of inhibitor that inhibited 50% of tyrosinase activity under experimental conditions was named IC50 value [7].

Percent inhibition of tyrosinase activity was calculated as:

\[
\text{%Inhibition} = \left\{ \frac{\text{(A-B)-(C-D)}}{\text{(A-B)}} \right\} \times 100
\]

A: optical density at 475 nm without test sample
B: optical density at 475 nm without test sample and enzyme
C: optical density at 475 nm with test sample
D: optical density at 475 nm with test sample, but without enzyme
Measurement of kinetic parameters

100 μL of mushroom tyrosinase solution, and different volume of L-Dopa (10-100μl) and potassium phosphate buffer (pH 6.8) with or without 50 μL of tested samples were added to a 96-well plate. Using a microplate reader, the initial rate of Dopachrome formation from the reaction mixture was determined by Linear increase in absorbance at 475 nm. Kinetic parameters, Michaelis constant ($K_m$) and maximal velocity ($V_m$) of the tyrosinase activity were determined using a Lineweaver-Burk plots. The inhibition constant ($K_i$) was measured by the dixon plots.

Results and Discussion:

In this study, inhibitory effects of peel and seed of melon on diphenolase activity of mushroom tyrosinase were evaluated, so L-Dopa is used as substrate of tyrosinase. Both extracts showed antityrosinase activity weaker than kojic acid. IC$_{50}$ values of extracts seed and peel of melon are expressed 1.5127, 1.2117 mg mL$^{-1}$, respectively (figure 1).

![Figure 1 - IC$_{50}$ values for tested samples and positive control (kojic acid)](figure1.jpg)

The results indicated both extracts mixed-type inhibited tyrosinase activity. Lineweaver-Burk plots for inhibition of tyrosinase by seed and peel of melon are shown in Figure 2,3. Kojic acid exhibited mixed-type of inhibition on tyrosinase.

![Figure 2 - Lineweaver-Burk plots for inhibition of tyrosinase by seed and peel of melon](figure2.jpg)
Figure 2- Lineweaver–Burk plot for inhibition of different concentrations of seed on mushroom tyrosinase for the catalysis of L-Dopa.

Figure 3- Lineweaver–Burk plot for inhibition of different concentrations of peel on mushroom tyrosinase for the catalysis of L-Dopa.

so in their plots increased the $K_m$ and decreased the $V_m$ value. In other words, they binded to the active site of enzyme. $K_m$ and $v_m$ values are shown in table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>seed of melon</td>
<td>0.1987</td>
<td>0.00179</td>
</tr>
<tr>
<td>peel of melon</td>
<td>0.2065</td>
<td>0.002099</td>
</tr>
<tr>
<td>Non inhibitor</td>
<td>0.1815</td>
<td>0.002634</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>0.2934</td>
<td>0.001879</td>
</tr>
</tbody>
</table>

The inhibition constant ($K_i$) of seed and peel of melon were estimated to be 1.499, 1.2189 mg/mL, respectively.

REFERENCES


