International Journal of Heterocyclic Chemistry, Vol. 1, No. 4, pp. 38-42 © Ahvaz Branch, Islamic Azad University

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 mashroom tyrosinase were investigated. The IC_{50} values and K_i 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 rsulting in melanin production [1]. Melanin is one biopigment that widly 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 pholyphenol oxidase(ppo) [4] .PPO is a copper-containig glycoprotein widly distributed in microorganisms, animals, plants and insects. and accept many cathhochols 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 mashroom 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^{o}c temperatore 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 25ºC 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 mashroom 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:

$\%Inhibition=\{[(A-B)-(C-D)]/(A-B)\}\times100$

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

Measurment 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 Linewear-Burk plots. The inhibition constant (K_i) was measured by the dixon plots.

Results and Disscution:

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, respectively(figure 1).

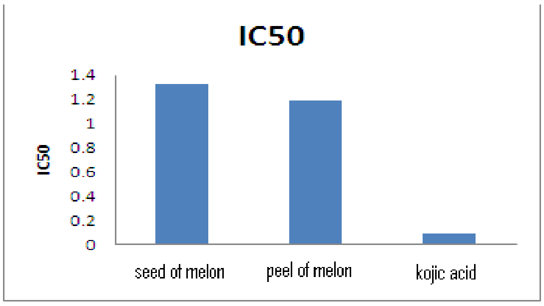

Figure 1- IC_{50} value for tested samples and positive control (kojic acid)

The results indicated both extracts mixed-type inhibited tyrosinase activity. Linewear-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.

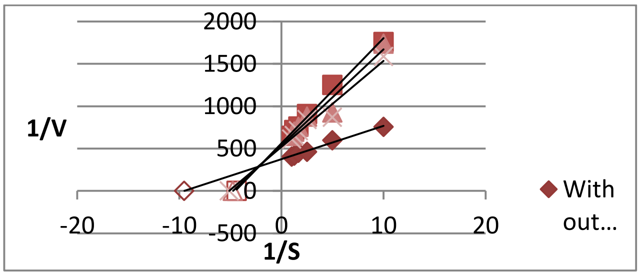

Figure2- Lineweaver–Burk plot for inhibition of different concentrations of seed on mushroom tyrosinase for the catalysis of L-Dopa.

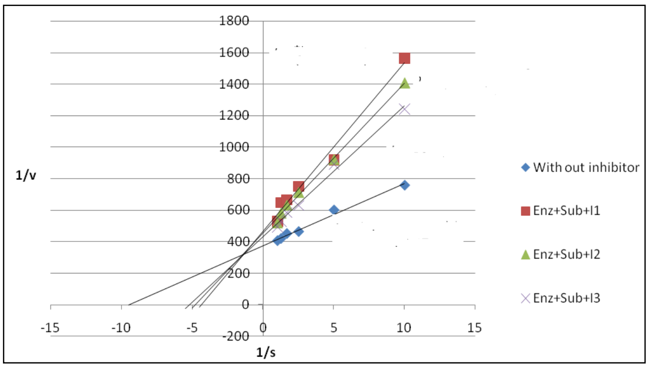

Figure3- Lineweaver–Burk plot for inhibition of different concentrations of peel on mushroom tyrosinase for the catalysis of L-Dopa.

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 table1.

Table1- kinetic parameters in presence of extracts and kojic acid

Name	K_m	V_{max}
seed of melon	0.1987	0.00179
peel of melon	0.2065	0.002099
Non inhibitor	0.1815	0.002634
Kojic acid	0.2934	0.001879

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

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