ANTI-TYROSINASE AND CYTOTOXIC ACTIVITIES OF SYNTHETIC COMPOUND 2'-HYDROXY-3,4,3',4'-TETRAMETHOXYCHALCONE

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Dedicated to:

My beloved parents,

Haji Ahmad bin Haji Ramli and Hajah Awa binti Othman

who give me strength and full support,

my family,

my lecturers,

and my friends.

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ABSTRACT

It has been demonstrated by several studies that synthetic chalcone compound products having antioxidant and anti tyrosinase properties. In this study, the novel synthetic 2'-hydroxy-3,4,3',4'-tetramethoxychalcone (HTMC) was evaluated for their antityrosinase, antioxidant, and cytotoxicity properties. In anti tyrosinase activities, HTMC showed an activity and is postulated as potential tyrosinase inhibitor. The antityrosinase activity of HTMC, however, was lower than Kojic Acid (positive control). It was due to the structure of HTMC that have been modified synthetically at position 4 by inserting methoxy group. For that reason, the potency of HTMC as tyrosinase inhibitor was reduced and this statement supports our finding that is HTMC have less active compared to kojic acid. In contrast, HTMC demonstrate low antioxidant activities for both DPPH and FRAP assays. The highest percentage of inhibition for HTMC was 37% in DPPH assay at the concentration of 242 μM whilst the highest FRAP value of HTMC was 2 351 μmol/L. It is due to their chemical structure that contains only 1 group of hydroxyl in A-ring at position 2 that reflected to its ability to scavenge free radicals. HTMC showed negligible toxicity effect on Chinese Hamster Ovary (CHO) cell at the concentration of (0, 0.01, 0.1 and 1) μM. As it considered as preliminary report on cytotoxicity effect of HTMC, this data is valuable to be further investigated on human cell line.

ABSTRAK

Beberapa kajian penyelidikan telah menunjukkan bahawa produk kalkon sintetik mempunyai aktiviti sebagai antioksidan dan anti-tyrosinase. Dalam kajian ini, sebatian sintetik novel iaitu 2'-hydroxy-3,4,3’,4 '-tetramethoxychalcone (HTMC) telah dinilai untuk sifat-sifat antityrosinase, antioksidan, dan sitotoksisiti mereka. Dalam aktiviti anti-tyrosinase, HTMC menunjukkan aktiviti dan diandaikan berpotensi sebagai perencat enzim tyrosinase. Walau bagaimanapun, aktiviti antityrosinase HTMC adalah rendah berbanding asid kojik (kawalan positif). Ini disebabkan oleh struktur kimia HTMC telah diubah suai secara sintetik pada kedudukan 4 dengan memasukkan kumpulan metoksi. Atas sebab itu, potensi HTMC sebagai perencat tyrosinase dikurangkan dan kenyataan ini disokong oleh keputusan yang diperolehi bahawa HTMC adalah kurang aktif berbanding dengan asid kojic. Sementara itu, HTMC menunjukkan aktiviti antioksidan yang rendah untuk kedua-dua jenis assay iaitu DPPH dan FRAP. HTMC mencatatkan peratus tertinggi perencatan sebanyak 37% dalam DPPH assay (pada kepekatan 242 μM) manakala nilai FRAP pula adalah 2 351 μmol/L. Ia adalah disebabkan oleh struktur kimia sebatian ini yang mengandungi hanya 1 kumpulan hidroksil dalam cincin A yang mempengaruhi keupayaan untuk mengumpul radikal bebas. HTMC menunjukkan kesan toksik yang kecil terhadap sel Chinese Hamster Ovary (CHO) pada kepekatan (0, 0.01, 0.1 and 1) μM. Memandang ini merupakan laporan pertama untuk kesan toksik oleh HTMC, data ini penting untuk dilanjutkan pada sel manusia.

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LIST OF ABBREVIATIONS AND SYMBOLS

et al.,	:	and others
EtOH	:	ethanol
NaOH	:	sodium hydroxide
HCl	:	acid chloride
UV	:	Ultraviolet
g	:	gram
µM	:	Micromolar
%	:	percent
s	:	seconds
min	:	minutes
v/v	:	volume per volume
˚C	:	degree Celsius
ml	:	millilitre
mg	:	milligram
sp.	:	species
DMSO	:	Dimethyl sulfoxide
HTMC	:	2'-hydroxy-3,4,3',4'-tetramethoxychalcone
DMEM	:	Dulbecco's Modified Eagle Medium
FBS	:	fetal bovine serum
DPPH assay	:	1,1-Diphenyl-2-picrylhydrazyl
FRAP assay	:	Ferric Reducing Ability of Plasma
MTT assay	:	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
L-DOPA	:	L-3,4- dihydroxyphenylalanine,
ANOVA	:	Analysis Of Variance
IC_{50}	:	50% inhibitory concentration
nm	:	nanometer
OH	:	Hydroxyl group
UV	:	Ultraviolet
v/v	:	Volume per volume
ºC	:	Degree of Celsius

MeOH	:	Methanol
CV	:	Coefficient of Variations
e.g	:	example
mg	:	Miligram
µl	:	Microliter
µg	:	Microgram

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Natural antioxidants, mostly in fruits and vegetables have grown a great concern among consumers as epidemiological studies have signified that frequent consumption of natural antioxidant is associated with lower risk of cardiovascular disease and cancer (Renaud *et al.*, 1998; Temple *et al.*, 2000). Three major groups of defensive effects of natural antioxidant in fruits and vegetables are vitamins, phenolics and caratenoids (Halliwell, 1996). Phenolics groups is identified by the presence of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Variety of phenolic compounds has been discovered previously to contain secondary structure called flavonoids. One of six skeleton structure of the main classess of flavonoids is chalcone. Avila *et al.*, (2008) reported that hydroxylation at position 2' in A-ring of chalcone is indirectly promoting structural stability.

In chemistry, organic synthesis specifically becomes core of attraction for scientists due to the ability to construct beneficial products artificially for human goods. It have been demonstrated by several studies about synthetic chalcone product including 2',5'-dihydroxy-4-chloro-dihydrochalcone and 2',5'-dihydroxydihydrochalcone that inhibit the iNOS protein expression and the former

compound also inhibits cyclooxygenase-2 (COX-2) activity in RAW 264.7 cells (Ko *et al.,* 2003). The antioxidant activity of some synthetic 2'-hydroxy-chalcone and its derivatives has been investigated by Detsi and her colleagues (2009). They suggested that among tested compounds with methoxy group on positions 2' and 4' of the A-ring, possess combined antioxidant-LOX inhibitory profile (Detsi *et al.,* 2009). Besides, Nerya *et al.* (2004) showed the relatedness between structure of chalcone and tyrosinase inhibition activity. These findings implied that some chalcone may be promising as anti-inflammatory, antioxidant and antityrosinase agents and have potential in medical perspective.

Tyrosinase is a copper-containing enzyme that extensively distributed in nature. This enzyme is also known as polyphenol oxidase (Whitaker, 1995). Two major reactions that catalyzes by tyrosinase is relating molecular oxygen in the melanin biosynthesis pathway: a) the hydroxylation of monophenols to *o*-phenols (monophenolase activity), and b) the oxidation of the *o*-phenols to *o*-quinones (diphenolase activity). These quinones are reactive compound and have a tendency to polymerize spontaneously to form melanin (Seo *et al.,* 2003). This scenario can be determined by various dermatological disorders or the production of abnormal pigmentation, for instance age spot, freckles, melasma and liver spot that can be a serious aesthetic setback (Briganti *et al.,* 2003). Therefore, tyrosinase inhibitors have become progressively vital in medication and in cosmetic as it can block melogenic pathway by inhibiting enzymatic oxidation. On top of that, natural tyrosinase inhibitors are in general believed to be fewer side effects and can be produced at reasonable low cost (Saewan *et al.,* 2011).

1.2 Problem statement

Synthetic compounds of chalcone namely 2'-hydroxy-3,4,3',4'-tetramethoxychalcone in this study have been synthesized via classical methods of Claisen-Schmidt condensation reaction. Their physicochemical properties are reflected to their extent bioactivities such as anti-tyrosinase and antioxidant

activities. Previous research supports that this plant-derived polyphenolic compound is believed to possess a potential as whitening agents. It is based on its chemical structure that contains hydroxyl group in A-ring at position 2 and methoxyl groups in A- and B-ring of this compound. Thus far, the 2'-hydroxy-3,4,3',4'-tetramethoxychalcone synthetic compound, however, is not discovered precisely in term of their bioactivities. Therefore, this study is performed to explore pharmacologically effects of this synthetic compound to overcome some of the limitations associated with constraint in the production of natural pure compound.

1.3 Objectives

a) To investigate an anti-tyrosinase effect of synthetic compound namely 2'-hydroxy-3,4,3',4'-tetramethoxychalcone (HTMC)
b) To evaluate antioxidant property of HTMC by using DPPH and FRAP assay
c) To determine cytotoxicity effect of HTMC on the growth of Chinese Hamster Ovary (CHO) cell.

1.4 Scope of work

In this study, synthetic compound namely 2'-hydroxy-3,4,3',4'-tetramethoxychalcone was obtained from Dr. Farediah binti Ahmad, Department of Chemistry, Faculty of Science, UTM. Anti-tyrosinase assay was done in order to determine the ability of this synthetic compound to inhibit tyrosinase enzyme in the synthesis of melanin. The antioxidant activity of this compound was determined in terms of their anti radical power as assessed by DPPH radical scavenging assay and FRAP assay. Furthermore, IC_{50} (half maximal inhibitory concentration) of this synthetic compound is also can be demonstrated from these assays. MTT assay was performed so as to assess the cell viability for evaluation of different concentration of synthetic compound that are introduced to CHO cell line.

1.5 Significant of research

To date, biological activities explicitly anti-tyrosinase, antioxidant and cytotoxicity effects of this synthetic compound is not revealed by previous researcher. Therefore, this study hypothesized that synthetic compound namely 2'-hydroxy-3,4,3',4'-tetramethoxychalcone have antioxidant properties and tyrosinase inhibition activity. The cytotoxicity effect of this compound can serve an overview that reflected to their ability to be toxic to human cell at certain concentration. These 3 indicators will be manipulated for further study to support their applications in modern and traditional medicine.

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