THE EFFECTS OF *Clinacanthus nutans* LEAF EXTRACT TOWARDS TYROSINASE AND COLLAGENASE ACTIVITIES

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ABSTRACT

Clinacanthus nutans is a plant popular among Southeast Asia countries as a traditional medicine to treat various diseases. *C. nutans* is believed to have potential of growth in different industries due to its anti-diabetic, anti-viral and anti-cancer activities. The undesired formation of black or brown pigmentation on human skin involves tyrosinase while the breakdown of collagen in dermis of skin cell involves collagenase. The effects caused by these two enzymes indicate the aging of skin cells, hence this problem remains as a hot issue. To date, there are not much research done on the anti-tyrosinase and anti-collagenase potential of *C. nutans*. Thus, this study aimed to quantify total flavonoid content (TFC) found in *C. nutans* crude leaf extract and to determine the tyrosinase and collagenase activities of *C. nutans* crude leaf extract. Aluminium chloride colorimetric technique was performed to quantify TFC. Inhibition % of various compounds such as *C. nutans* crude leaf extract, kojic acid and ascorbic acid toward tyrosinase was calculated. The binding affinity of tyrosinase was determined by using both tyrosine and *C. nutans* crude leaf extract whereas the binding affinity of collagenase was examined by using FALGPA and also *C. nutans* crude leaf extract. Their K_m values were identified from the same Lineweaver-Burk plot. *C. nutans* crude leaf extract contained 28.70 ± 0.93 mg QUE per gram of fresh leaf. The % inhibition of tyrosinase of *C. nutans* crude leaf extract, ascorbic acid and kojic were 15.11%, 84.46% and 87.01% respectively. For tyrosinase activity, the K_m value of tyrosine and *C. nutans* crude leaf extract was 0.833 mg/mL and 2.000 mg/mL respectively. Both compounds have the same V_{max} value of 1.7×10^{-2} (mg/mL).min^{-1}. For collagenase activity, the K_m values of FALGPA and *C. nutans* crude leaf extract was 0.083 mg/mL and 0.330 mg/mL respectively. Both compounds have the same V_{max} value of 2.78×10^{-3}(mg/mL).min^{-1} as well. In conclusion, *C. nutans* crude leaf extract has low % inhibition toward tyrosinase, high K_m value toward tyrosinase and collagenase might suggest lack of its certain structural group towards both enzymes.

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LIST OF ABBREVIATIONS

%	Percentage
×	Times
°C	Degree Celcius
µL	Micolitre
ºC	Degree Celsius
<	Less than
$AlCl_3$	Aluminium chloride
ANOVA	One-way analysis of variance
Ca	Calcium
C. nutans	*Clinacanthus nutans*
C-	Carbon-
cm	Centimetre
DHI	Dihydroxyindole
dH_2O	Deionised water
EC	Enzyme Commission
EDTA	Ethylenediaminetetraacetic acid
EGCG	Epigallocatechin gallate
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
FALGPA	Furylacryloyl-Leucine-Glycyl-Propyl-Alanine
g	Gram
g/mol	Gram per mole
Gly	Glycine
HeLa	Human cervical cancer cell line
HepG2	Human liver hepatocellular carcinoma

IMR32	Human neuroblastoma cell line
Ile	Isoleucine
K_m	Michaelis-Menten constant
K562	Human erythroleukemia cell line
LS-1724T	Human colon adenocarcinoma cell line
L-DOPA	L-3,4-dihydroxyphenylalanine
Leu	Leucine
L	Litre
m	Metre
MMP	Metalloproteinase
mL	Millilitre
min	Minute
mg/mL	Milligram per milliliter
mM	Millimolar
mg	Milligram
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
QUE	Quercetin equivalents
QU	Quercetin
Raji	Human Burkitt's lymphoma cell line
RCF	Relative centrifugal force
SNU-1	Human gastric cancer cell line
SPSS	Statistical Package for the Social Sciences
UV	Ultraviolet
w/v	Weight per volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

Tyrosinase and collagenase are two enzymes involved in human aging process. Tyrosinase is responsible for dark pigmentation on skin while collagenase is known to cause reduction of skin elasticity by excessive collagen degradation (Solano& Solano, 2014; Sibilla & Borumand, 2015). Nowadays, anti-aging products have become indispensable as skin care products in cosmetic industry. Skin care products which use natural ingredients are getting more popular in the market because of its easy absorption properties and hence reduce the problem of skin allergy (Wahab, Rahman, Ismail, Mustafa & Hashim, 2014). Plants which contain phytochemicals are excellent sources for getting natural ingredients and they have been widely used in the cosmetic industry. *Clinacanthus nutans* is not an exception as it was proved to have different kinds of phytochemicals (Alam et al., 2016).

C. nutans Lindau (common name: snake grass) primarily called as "belalai gajah" in Malay or "phaya yo" in Thai is used traditionally because of its medicinal potential (Aslam, Ahmad & Mamat, 2014). This plant is commonly used for treating various diseases such as snake and insects bites, pruritus caused by skin rashes, gout and lesions caused by herpes simplex virus in Southeast Asia(Alam et al., 2016). *C. nutans* remains popular due to its anti-cancer, anti-diabetic, anti-viral, anti-oxidant and immune-modulatory activities, and to date, no toxic effect of using the recommended amount which is less than 1.8 g of *C. nutans* leaf as traditional medicine has been found (Yahaya, Dash, Abdullah & Mathews, 2015).

Tyrosinase is an enzyme that is involved in biosynthesis of melanin. Melanin responsible for human skin pigmentations as well as acts as human skin protector against UV radiation (Solano& Solano, 2014; Uchida, Ishikawa & Tomoda, 2014). However, over accumulation of melanin caused by over expression of tyrosinase can lead to many

dermatological related problems such as age spots, freckles and melasma (Nerya, Vaya, Musa, Izrael, Ruth & Tamir, 2003). According to Taherkhani and Gheibi (2014) and Rao et al. (2013), inhibition of tyrosinase may be clinically useful in treating skin cancer by controlling the proliferation of melanocytes. Thus, melanin accumulation which causes skin pigmentation or decoloration in addition to tumor formation can be prevented. Inhibitors of tyrosinase can be used as skin whitening agents in cosmetic application.

Collagenase is an enzyme that responsible for collagen degradation by degrading native collagen helix under non-denatured form and at physiological level of temperature and neutral pH (Eisen, Jeffrey & Bauer, 1970; Kafienah, Buttle, Burnett & Hollander, 1998). Collagen played a vital role in skin and bone tissues by providing integrity and rigidity which contribute to the shape, organization and mechanical properties of tissues (Ricard-Blum, 2011). Excessive breakdown of collagen in dermis which surpasses its synthesis can lead to the occurrence of fine lines in human skin that caused aged skin (Sibilla & Borumand, 2015). Therefore, research in reducing collagen breakdown by using collagenase inhibitor gives a high market value in pharmaceutical and cosmetic applications. Through the inhibitory effect, it may have advantageous effects to maintain healthy skin by avoiding the degradation of dermal matrix (Ghimeray et al., 2015).

Phenolic compounds are often produced by plants for protection purpose. The example of phenolic compounds includes phenolic acids, xanthones, coumarins, flavonoids (Kasote, Pawar, Sadgir, Bharati, Jagtap & Hegde, 2013). Among them, flavonoids and phenolic acids are popular for its health promoting effects as well as its pharmaceutical potential especially in anti-oxidant properties (Sarega et al., 2016). According to Alam et al. (2016), various types of phytochemicals were isolated from *C. nutans* such as flavonoids, cerebrosides and glycosides. Flavonoid is one of the known inhibitors under polyphenol compound which has inhibitory effect towards tyrosinase and collagenase (Chang, 2012; Kasote et al., 2013). To date, there is no research done on the inhibitory effects of total flavonoids content (TFC) in *C. nutans* towards the activities of tyrosinase and collagenase. As mentioned, *C. nutans* is a traditional medicine plant which demonstrates a lot of beneficial properties. It is an easy-to-get and highly valuable plant

used for research on its medicinal potential. Thus, research on this plant gave advantages as its medical potential can be understood and people can take advantages of it.

The aims for this study were:

1. to quantify TFC found in *C. nutans* crude leaf extract.
2. to determine the inhibitory effects of *C. nutans* crude leaf extract towards tyrosinase by calculating % inhibition.
3. to identify the binding affinity, K_m of *C. nutans* crude leaf extract towards tyrosinase and collagenase.
4. to determine the binding selectivity of collagenase towards FALGPA or *C. nutans* crude leaf extract.

CHAPTER 2

LITERATURE REVIEW

2.1 *Clinacanthus nutans*

Clinacanthus burmanni var. *robinsonii* Benoist and *Clinacanthus burmanni* Nees are the alternative names for *C. nutans* (Alam et al., 2016). The taxonomic classification for this plant are Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Subclass: Asteridae; Order: Lamiales; Family: Acanthaceae; Genus: *Clinacanthus Lindau*; Species: *Clinacanthus nutans* (Burmann.f.) *Lindau* (Encyclopedia of Life, 2014). *C.nutans* is a perennial plant with taper, glabrescent and striated stems (SCBG Checklist, n.d.). It can grow up to 1 m tall with branches which are pubescent (SCBG Checklist, n.d.). It has a linear-lanceolate leaf blade normally with length 7 cm - 12 cm and width 1 cm - 4 cm, both surfaces of leaf are pubescent when young and then become glabrescent as shown in Figure 2.1 (a) (SCBG Checklist, n.d.). The petiole is 0.3 cm - 2.0 cm long with sulcate and pubescent appearance (SCBG Checklist, n.d.). It has a flower with dull red corolla and green base, apically greenish yellow or sordidly yellow (Panyakom, 2006). Flowers always terminate at drooping horizontal branches but the flower itself is erected and combined into a leafy and loose panicle as shown in Figure 2.1 (b) (Panyakom, 2006).

(a) (b)

Figure 2.1 The leaf of *C. nutans* (a). The flower of *C. nutans* terminates at the horizontal branch (b).