TYROSINASE ACTIVITY AND ANTI-MICROBIAL POTENTIAL OF *Cosmos caudatus* EXTRACT

ABEGAL THEN

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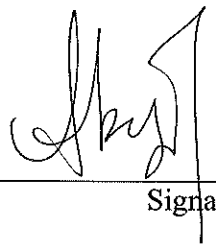
Abegal Then	
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16th December 2016	

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ABSTRACT

Cosmos caudatus, or known locally as "ulam raja" is a plant popular among Malaysians as a local delight. *C. caudatus* is believed to have growing potentials in various industries due to its natural anti-oxidant and anti-microbial properties. The undesirable browning effect on mammalian skin cells involves an enzyme tyrosinase and it indicates the aging of skin cells, thus this problem remains an aesthetic skin issue. To date, no studies had been done on the anti-tyrosinase potential of *C. caudatus*. Thus, this study aimed to determine the total phenolic content (TPC) and total flavonoid content (TFC) in *C. caudatus* crude leaf extract, to quantify the content of thin layer chromatography (TLC)-isolated quercetin (QU) in *C. caudatus* crude leaf extract, and to assess the potential uses of *C. caudatus* crude leaf extract as a tyrosinase inhibitor and as an inhibitory agent against the growth of *Staphylococcuss aureus*. *C. caudatus* leaves (3.0 g) were grinded and water was used as the extraction solvent. Two separate assays using Folin-Ciocalteu reagent and 2% (w/v) methanolic aluminium chloride respectively were performed to quantify TPC and TFC. TLC was used to isolate QU from crude extract and QU concentration was determined. *C. caudatus* crude leaf extract (20 µL) was introduced to tyrosinase in order to assess its inhibition activity. Anti-microbial qualitative screening was done by growing *S. aureus* on *C. caudatus* crude leaf extract (250 µL). *C. caudatus* crude leaf extract contained 12.862 ± 0.04 mg GAE/g of TPC and 5.132 ± 0.04 mg QUE/g of TFC based on fresh leaf weight. Moreover, the TLC-isolated QU from crude leaf extract was 0.110 mg QUE/g based on QU standard curve. The percent (%) tyrosinase inhibition of *C. caudatus* crude extract, kojic acid and ascorbic acid were 71.98%, 98.08% and 98.83% respectively. Michaelis-Menten parameters, K_m and V_{max} of *C. caudatus* crude extract were 1.05×10^{-2} mM and 1.75×10^{-4} mMmin^{-1} respectively, and for tyrosine, were calculated to be 3.04×10^{-4} mM and 1.42×10^{-2} mMmin^{-1} respectively. *S. aureus* colonies grown under presence of *C. caudatus* crude extract were lesser than that grown on QU control. In conclusions, TPC was higher than TFC due to the presence of various phenolic compounds in crude leaf extract of *C. caudatus*. The lowest % tyrosinase inhibition (71.98%) and higher K_m (1.05×10^{-2} mM) of *C. caudatus* crude extract might suggest lack of its selectivity towards tyrosinase compared to other inhibitors such as kojic acid and ascorbic acid as well as tyrosinase's substrate, tyrosine.

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LIST OF ABBREVIATIONS

%	Percent
×	times
°C	degree Celsius
µL	microlitre
µm	micrometre
AEAC	ascorbic acid equivalent anti-oxidant
$AlCl_3$	aluminium chloride
ANOVA	one-way analysis of variance
C. caudatus	*Cosmos caudatus*
cm	centimetre
DHI	dihydroxyindole
DHICA	dihydroxyindole-2-carboxylic acid
DPPH	free radical-scavenging activity
EC	Enzyme Commission
FRAP	ferric ion reducing anti-oxidant potential
g	gram
GA	gallic acid
GAE	gallic acid equivalent
hr	hours
KH_2PO_4	Monopotassium phosphate
L	litre
LB	Luria-Bertani
L-DOPA	L-3,4-dihydroxyphenylalanine
m	metre
mg	milligram

mg/mL	milligram per millilitre
min	minutes
mL	millilitre
mM	millimolar
N	Normality
Na_2HPO_4	Disodium phosphate
–OH	hydroxyl
PPO	polyphenol oxidase
QU	quercetin
QUE	quercetin equivalent
RCF	relative centrifugal force
R_f	retention factors
rpm	revolutions per minute
S. aureus	*Staphylococcus aureus*
SPSS	Statistical Package for the Social Sciences
TFC	total flavonoid content
TLC	thin layer chromatography
TPC	total phenolic content
units/mg	units per milligram
units/mL	units per millilitre
v/v	volume per volume
W	Watt
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Native to Latin America, *Cosmos caudatus* is a plant introduced to the Southeast Asia and has been widely used as a traditional medicine since then (Cheng, Barakatun-Nisak, Anthony & Ismail, 2015a). Local Malaysians are familiar with *C. caudatus*'s common name – ulam raja, literally translated as the 'King's salad" (Cheng et al., 2015a). *C. caudatus* remains popular in Southeast Asia because it demonstrates anti-diabetic, anti-inflammatory, anti-microbial and bone-strengthening properties, and negative side effects of using *C. caudatus* as a traditional medicine has yet to be found (Cheng et al., 2015a; Mohamed, Sahhugi, Ramli & Muhammad, 2013). Because of these properties a single plant yields, the impact of *C. caudatus* can be more than just a plant and because of this, there have been a lot of studies going on involving *C. caudatus* to evaluate the effectiveness of its properties (Cheng et al., 2015a).

To date, there are numerous research efforts done on *C. caudatus.* Mohamed et al. (2013) have demonstrated the effectiveness of using fresh *C. caudatus* extract as an alternative treatment for osteoporosis, a disease that often affects the elderly people. Apart from its proven use against fungal activity, *C. caudatus* made a good bone-restorative agent (Mohamed et al., 2013). Although the anti-tyrosinase activity of *C. caudatus* has never been assessed, *C. caudatus*'s anti-oxidant property has been widely investigated (Cheng et al., 2015a; Mediani, Abas, Khatib & Tan, 2013). According to Mohamed et al. (2013), ethanol-extracted *C. caudatus* was tested to have an ascorbic acid equivalent anti-oxidant (AEAC) of about 2500 milligram (mg), which was classified as extremely high compared to that of apples, oranges, strawberry and so on. Reihani and Azhar (2012) have determined that *C. caudatus* scored the highest in both the free radical-scavenging activity (DPPH) assay and the ferric ion reducing anti-oxidant potential (FRAP) assay. The anti-diabetic properties of *C. caudatus* were further determined in a study by Cheng, Barakatun-Nisak, Anthony and Ismail (2015b), where they found out that after eight weeks of consuming 15.0 gram (g) of *C. caudatus*

daily, the type-2 diabetic patients saw significant improvement to both resistance and sensitivity to insulin.

Even though *C. caudatus* is reputed to be anti-microbial, not much research papers can be found to evaluate this property. However, Yusoff, Noor and Rukayadi (2015) have found out that 10 minutes (min) of exposure to *C. caudatus* was sufficient to significantly reduce the number of microflora found on raw chicken meat. This proved the potential of using *C. caudatus* as an alternative to chemical food sanitizer.

To date, no previous studies have been done on the anti-microbial activity of *C. caudatus* on a specific strain of bacteria – *Staphylococcus aureus*. Without carrying out clinical testing, the hypothesis is that *C. caudatus* may be useful as a natural mouth disinfectant in fighting against transient, opportunistic *S. aureus* found in the oral cavity and the periodontal pockets of individuals who suffer from periodontal diseases or other oral infections (Loberto, Martins, Santos, Cortelli, & Jorge, 2004).

Hence, the objectives of this study are:

1. to determine the total phenolic content (TPC) and total flavonoid content (TFC) found in crude leaf extract of *C. caudatus*, extracted using water as the extraction solvent.
2. to separate quercetin (QU), a flavonoid found in *C. caudatus* crude leaf extract using thin layer chromatography (TLC) and determine its content using spectrophotometer.
3. to assess the anti-tyrosinase potential of *C. caudatus* crude leaf extract by determining its percent (%) inhibition of tyrosinase and the Michaelis-Menten parameters K_m and V_{max}.
4. to screen the effectiveness of using *C. caudatus* crude leaf extract in inhibiting the growth of facultative anaerobe *S. aureus*.

CHAPTER 2

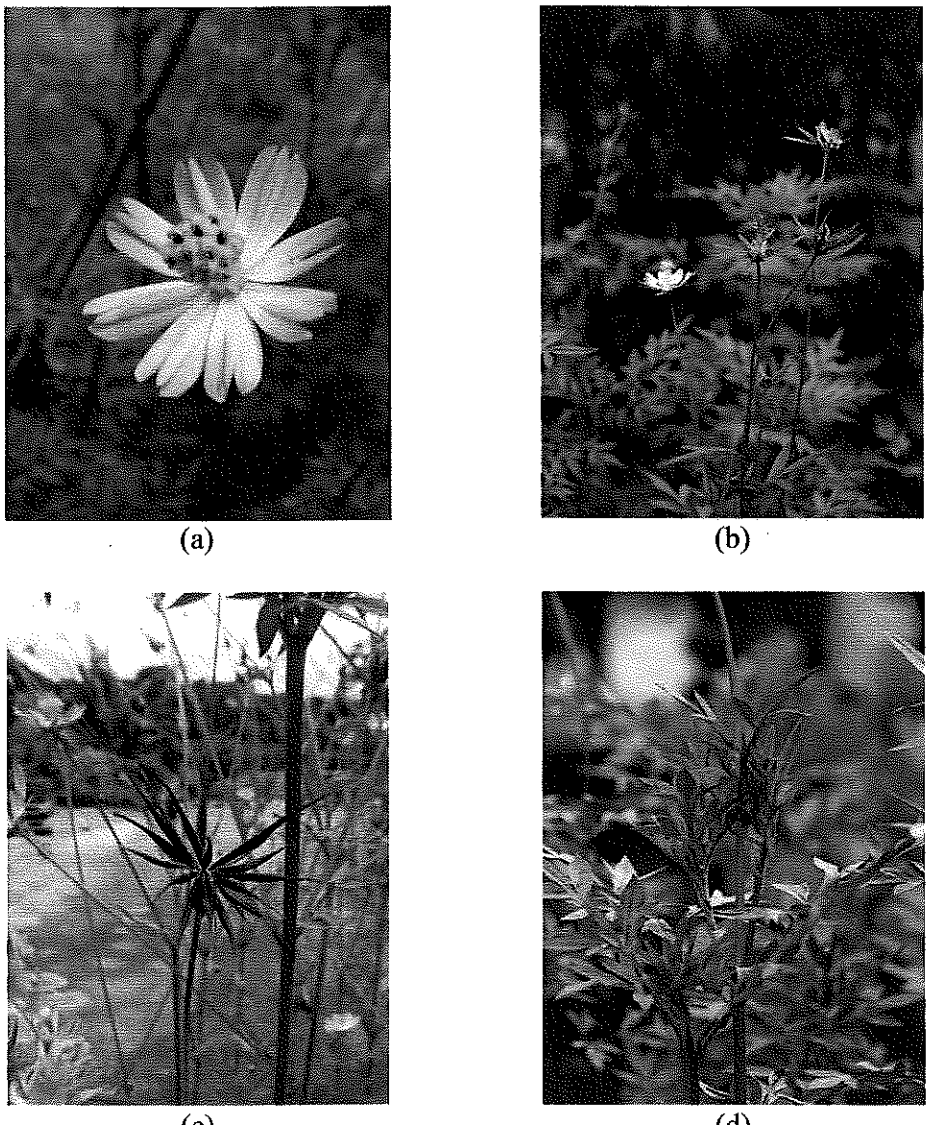
LITERATURE REVIEW

2.1 *Cosmos caudatus*

C. caudatus is a plant with edible parts, such as the leaves and shoots (Cheng et al., 2015a). It can grow up to 3 metre (m) and its flowers are purple or pink in colour as seen in Figure 2.1. According to Cheng et al. (2015a), it belongs to the family *Asteraceae*. According to GLOBinMED (n.d.), *C. caudatus* finishes its life cycle within a year and it will perpetuate itself by seeds to repeat the cycle. The stems are succulent, light-green in colour and normally has a tint of purple (GLOBinMED, n.d.). The leaves of *C. caudatus* vary in sizes but they bear simple morphology – oppositely arranged pairs of either pinnate or bipinnate leaves GLOBinMED (n.d.). The leaves can be divided and lobed into 10-20 centimetre (cm) in length, and it has a strong, pungent smell (GLOBinMED, n.d.). *C. caudatus* has an inflorescent flowering nature (GLOBinMED, n.d.). According to Ferry (2011), inflorescence refers to the specific arrangement and portion that involves the flowers of a plant. However, Castel, Kusters and Koes (2010) explained that a branch or a system of branches that bear flowers, along with other sexually reproductive structures of a plant are referred as inflorescence. Castel et al. (2010) further explained that an inflorescence plant can produce flowers ranging from a single flower to clusters of flowers.

C. caudatus is also referred to as "ulam raja", and in our national language, "ulam" is understood as a collection of Malay vegetables consumed raw, and is popular in Malay tradition (Cheng et al., 2015a). Malaysians are strongly encouraged by the Ministry of Health to consume "ulam", under the Malaysia Dietary Guidelines (Cheng et al., 2015a). This is because from a survey conducted by the National Health and Nutrition Examination, people eating raw vegetables are found out to have higher levels of vitamins (Cheng et al., 2015a). *C. caudatus* has a unique taste and it is popular among Malaysians, usually consumed after dipping the leaves or shoots in either shrimp or chili paste (Cheng et al., 2015a).

Apart from being a unique local delicacy, *C. caudatus* has been used as a medicinal plant, passed down from old traditions (Cheng et al., 2015b). In the past, people used *C. caudatus* to delay aging, to stimulate blood circulation, to lower the glucose level in blood and to treat infectious disease thanks to its anti-microbial and anti-fungi activities (Cheng et al., 2015b).



(a) (b)

(c) (d)

Figure 2.1 Some features of *C. caudatus* includes the (a) flower of *C. caudatus*, (b) *C. caudatus* single stalk flowering-heads, (c) *C. caudatus* fruit achenes and (d) young, edible shoots of *C. caudatus*.