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Original Research Article

Diversity of curcuminoids, bioactive compounds and antioxidant activities in three species of Curcuma

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Abstract

Purpose: *To investigate the physical characteristics, bioactive compounds and antioxidant activities of curcuminoids, phenolic acids, and flavonoid compounds in three Curcuma species (C. mangga, C. zedoria, and C. longa).*

Methods: *Rhizomes of three Curcuma species (C. mangga, C. zedoria, and C. longa) were collected, and then curcuminoids, phenolics and flavonoid acids were determined using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy. Antioxidant activities were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.*

Results: *Variations in colour metrics such as lightness (L*), red-green (a*), and yellow-blue (b*) indicated different bioactive compounds. C. longa exhibited significantly higher concentration of curcuminoids (333.2 µg/g DW), total phenolic content (TPC, 116.7 mg GAE/g DW) and total flavonoid content (TFC, 16.1 mg RE/g DW) compared to C. mangga, and C. zedoria ($p < 0.05$). Furthermore, C. longa exhibited significantly higher antioxidant activity than C. mangga, and C. zedoria ($p < 0.05$).*

Conclusion: *The results indicate significant variations in bioactive composition between the three species of the genus Curcuma. Curcuma longa shows significantly higher concentration of curcuminoids, TPC, and TFC as well as antioxidant activity compared to C. mangga and C. zedoria. Future studies are required to examine the impact of natural variables, growing conditions, and processing techniques on the prevalence of bioactive chemicals and correlation with biological activities.*

Keywords: *Curcumin, Phenolic acids, Flavonoids, Zingiberaceae*

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INTRODUCTION

The genus *Curcuma* is widely recognized for its medicinal and nutritional properties with distribution covering South and Southeast Asian Cambodia, China, the Philippines, Malaysia, Laos, Vietnam, India, Indonesia, and Thailand, as well as Madagascar, and some countries in tropical Africa [1,2]. More than 100 species of *Curcuma* have been recorded in Thailand, with

approximately 30 species used for food additives, cosmetics, and traditional medicine [1]. *Curcuma* spp. have been the extensively studied for their anti-inflammatory, antioxidant, and anticarcinogenic characteristics resulting from their potent bioactive compounds, most especially, curcuminoids. The genus encompasses diverse cultivars with unique phytochemical profiles which contribute to health benefits. However, comparative analyses of these cultivars, especially curcuminoid contents, phytochemical compositions, and antioxidant activities are scarce [2,3].

Various species within the *Curcuma* genus are valued for their health benefits including *C. zedoaria* (zedoary or white turmeric), *C. aeruginosa* (pink and blue ginger), *C. alismatifolia* (Siam tulip or summer tulip), *C. petiolata* (jewel of Thailand), *C. amada* (mango ginger) including others such as *C. caesia* (black turmeric) and *C. aromatica* (wild turmeric). Among these, turmeric, white turmeric, and mango ginger have gained popularity for their culinary uses, medicinal properties, and health benefits [4].

Species in the genus *Curcuma* contains abundant phytochemicals such as curcuminoids, phenolics, alkaloids, diarylheptanoids and essential oils [5]. Curcumin, a polyphenolic compound obtained from *Curcuma* spp., was recognized for its diverse biological and pharmacological advantages, such as its ability to act as an antioxidant, stimulate the immune system, reduce inflammation, fight against microbes, protect the heart, kidneys, and liver, combat cancer, alleviate rheumatic conditions, and slow aging process [6-8].

Earlier investigations have documented the ethnobotany of various *Curcuma* spp., which are *C. longa*, *C. alismatifolia*, *C. aeruginosa*, *C. mangga*, and *C. zedoria* from Thailand. Numerous publications have also documented the bioactive substances found in *Curcuma* plants but little is known about the phytochemicals and biological activities of *Curcuma* spp (*C. mangga*, *C. zedoria*, and *C. longa*) in Thailand.

Therefore, this study investigated the physical characteristics, bioactive substances, and antioxidant activity of *C. mangga*, *C. zedoria*, and *C. longa*. The concentrations of flavonoid acids, phenolics, and curcuminoids were quantified and the relationships between antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) were investigated.

EXPERIMENTAL

Plant identification and sample preparation

Rhizomes of the three *Curcuma* species (*C. mangga*, *C. zedoria*, and *C. longa*) were collected in December 2021 during the plants' dormancy period, after they had been cultivated for nine months. The rhizomes were cleaned, cut into smaller sections, and freeze-dried for preservation (Scanvac ColSafe, model 100-9Pro, LaboGene ApS, Denmark). A 1.0 g sample was extracted with 10 mL (80 % methanol) at 37 °C, shaken at 150 rpm for 12 h, filtered and analyzed for phenolics, flavonoids, and antioxidant activities [9].

Microscopic examination

Fresh *Curcuma* rhizomes were taken, cross-sectioned using a plant microtome (MT-3, J08001, Japan), and examined under a light microscope (Carl Zeiss Inc., Toronto, Germany).

Colour determination

Colour variations in fresh tissue of the three *Curcuma* species were examined by applying a Minolta CR-300 Chroma meter (Konica Minolta, Osaka, Japan). The L*, *a**, and *b** colour measurements were verified by applying a white reference standard. Each treatment was assessed using ten samples, and the average calculated.

Determination of curcuminoids

A 200 mg sample was combined with 10 mL 80 % methanol, and sonicated using a probe at 40 % amplitude for 5 min., centrifuged for 10 min at 25 °C, and 19,000 ×g. The liquid portion was passed through a 0.22 nylon membrane and concentration of curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) were quantified using a Shimadzu LCMS-8030 Triple Quadrupole Liquid Chromatography-Mass Spectrometer (LC/MS/MS) (Shimadzu, Kyoto, Japan) [10]. The values were presented in micrograms per gram of dry weight (µg/g DW).

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The determination was done using the Folin-Ciocalteu assay [9]. The obtained sample solution (20 µL) was introduced onto a 96-well plate, and mixed with 10 % Folin-Ciocalteu reagent (100 µL) for 1 min. Thereafter 75 µL of 10 % sodium carbonate (Na_2CO_3) was added

and dark-incubated for 2 h. The TPC measurement was conducted using a microplate reader set to a wavelength of 750 nm, with TPC reported as milligrams of gallic acid equivalent (GAE) per gram dry weight (mg GAE/g DW). The TFC was determined using a microplate reader. The samples were combined with 25 µL of the extract, and supplemented with 100 µL of deionized (DI). Then, 10 µL of a 5 % sodium nitrite solution ($NaNO_2$) solution was mixed in a shaker incubator for 5 min, and 15 µL of 10 % aluminum chloride ($AlCl_3$) was added and shaken for 6 min. Thereafter, a 1 M sodium hydroxide solution was applied to each well plate along with 50 µL deionized water. The absorbance was measured by a microplate reader set at 750 nm, and values presented in milligrams of rutin equivalent per gram of dry weight (mg RE/g DW).

Determination of phenolic acids and flavonoid profile

Samples (1 g) were extracted using a solvent mixture of methanol and hydrochloric acid solution (100:1 v/v), shaken in an incubator for 12 h at 35 °C and 150 rpm. The extracts were filtered by Whatman No. 1 filter paper. The extract solutions were again filtered with a 0.45 µm filter before HPLC analysis using a Shimadzu LC-20 AC series HPLC system (Tokyo, Japan) with a diode array [10]. The phenolics were identified by a photodiode array detector, specifically at 280 nm and 320 nm. Flavonoids were detected at 370 nm. The results were reported as microgram per hundred gram of dry weight (mg /100g DW) for phenolic acids and milligram per gram of dry weight (µg/g DW).

Antioxidant activity

The antioxidant activity was determined using DPPH and FRAP assay [10].

2,2-diphenyl-1-picrylhydrazyl (DPPH)

Each extract or control (20 µL) was combined with 180 µL DPPH solution (60 µM), incubated in the dark for 30 min and read with a microplate reader at 517 nm. Results were expressed as mg Trolox equivalents per gram of dry weight (mg TE/g DW).

Ferric reducing antioxidant power (FRAP)

The sample (5 µL) of each extract was combined with 180 µL of FRAP reagent and incubated for 15 min at 37 °C. The absorbance was measured at 593 nm, and FRAP values were expressed as mg $FeSO_4$ per gram of dry weight (mg $FeSO_4$/g DW).

Statistical analysis

Data was analysed using Statistical Packages for Social Sciences (SPSS) version 29.0 (IBM, Armonk, NY, USA). Measurement data were presented in average ± standard deviation (SD), one-way analysis of variance (ANOVA) was used for comparisons, and the least significant difference (LSD) test was used to identify significant variations among the samples. Pearson's correlation test was employed to assess correlations among the means. $P < 0.05$ was considered statistically significant.

RESULTS

Oleoresin cells in fresh rhizome tissue

The microstructure of fresh *Curcuma* rhizome was compared among the species, and the results revealed the presence of Oleoresin oil cells. Oleoresin oil cells were round to ovoid and globular in fresh tissue.

Colour of the rhizome

A colorimetric analysis of the three *Curcuma* species revealed significant differences in L* (lightness), *a** (red-green), and *b** (yellow-blue) values. *C. longa* displayed intermediate lightness (L* = 57.0), with the lowest a* value (27.9) indicating a slightly red component and a moderate b* value (54.5) indicating a moderate yellow component. *C. mangga* exhibited the highest lightness (L* = 73.4), lowest red-green component (a* = -4.9), and lowest yellow-blue component (b* = 23.2), indicating a less yellow hue compared to *C. longa* and *C. zedoaria*. *C. zedoaria* showed moderate lightness (L* = 65.9), with a* and b* values of 14.4 and 51.4, respectively suggesting a balanced red-yellow hue (Table 1).

Curcuminoid levels

A quantitative analysis of the curcuminoids (Cur, DMC, and BDMC), was performed in the three *Curcuma* species and the results revealed that *C. longa* exhibited significantly higher Cur, DMC, and BDMC compared to *C. zedoaria* and *C. mangga* ($p < 0.05$; Table 2).

Total phenolic content (TPC) and total flavonoid content (TFC)

C. longa showed significantly higher TPC (Figure 2 A) and TFC (Figure 2 B) compared to *C. zedoaria* and *C. mangga* ($p < 0.05$).

Phenolic acids and flavonoid compounds

C. longa showed significantly higher concentration of total pheloic acids (gallic acid, vanillic acid, caffeic acid, *p*-courmaric acid, ferulic acid, sinapinic acid, and cinnamic acid) ($p < 0.05$). Furthermore, *C. longa* showed significantly higher concentration of flavonoids (rutin, apigenin, kaempferol and quercetin) ($p < 0.05$, Table 3).

Antioxidant activities

Antioxidant activities of *C. longa*, *C. mangga* and *C. zedoaria* were evaluated using DPPH radical scavenging and FRAP methods, and the results revealed that *C. longa* exhibited significantly higher antioxidant activity with DPPH and FRAP values of 18.3 mg TE/g DW and 30.1 mg $FeSO_4$/g DW, respectively compared to *C. zedoaria* and *C. mangga* ($p < 0.05$, Figure 3).

Correlations

Total phenolic content (TPC) demonstrated significant positive correlations with TFC (0.986), DPPH (0.990), and FRAP (0.997) ($p < 0.05$). Similarly, TFC showed significant positive correlations with DPPH (0.975) and FRAP (0.977) ($p < 0.05$). Also, there was a significant correlation between DPPH and FRAP results (0.992) ($p < 0.05$).

C. longa *C. mangga* *C. zedoaria*

Figure 1: Oleoresin oil cells of *C. longa*, *C. mangga,* and *C. zedoaria*

Table 1: Colour metrics (mean ± SD, n = 3)

Sample	L*	a*	b*
C. longa	57.00±0.83$^{\&}$	27.93±0.75*	54.50±0.79*
C. mangga	73.47±0.43*	-4.99±0.08$^{\&}$	23.28±0.30$^{\&}$
C. zedoaria	65.98±0.77$^{\#}$	14.41±0.36$^{\#}$	51.46±0.73$^{\#}$

*$^{\#\&}$*P* < 0.05 compared to each specie

Table 2: Curcuminoids contents (mean ± SD, n = 3)

Sample	Curcuminoids contents (µg/g DW)			
	CUR	*DMC*	*BDMC*	*Curcuminoid*
C. longa	190.29±3.56*	35.25±0.46$^{\#}$	107.68±1.32*	333.22±5.34*
C. mangga	17.35±1.52$^{\&}$	1.9±0.02$^{\&}$	2.69±0.10$^{\&}$	21.94±1.64$^{\&}$
C. zedoaria	41.12±0.29$^{\#}$	50.22±0.78*	29.76±0.12$^{\#}$	121.1±1.19$^{\#}$

CUR: Curcumin, DMC: didesmethoxycurcumin, BDMC: bisdemethoxycurcumin, Curcuminoid: (CUR + DMC + BDMC). *$^{\#\&}$*P* < 0.05 vs each specie

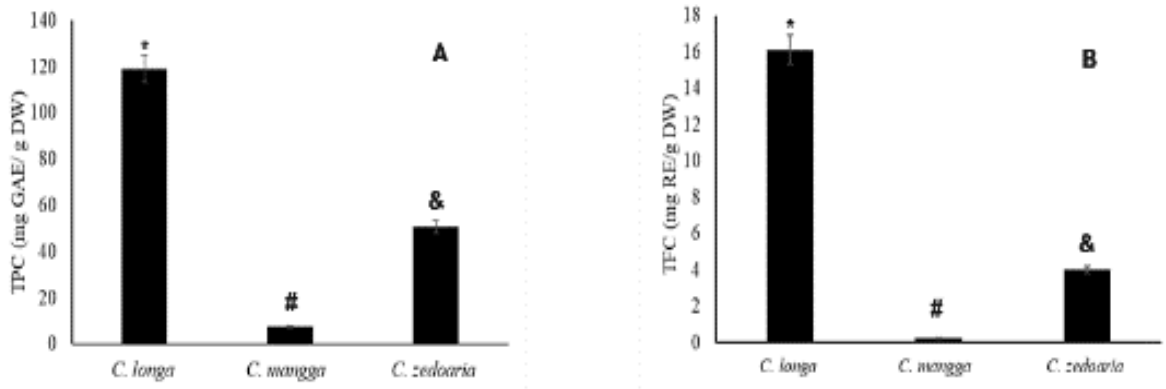

Figure 2: A: Total phenolic content. B: Total flavonoid content in the three *Curcuma* species. *$^{\#\&}$*P* < 0.05

Table 3: Concentration of phenolic and flavonoid compounds (mean ± SD, n = 3)

Parameter	*C. longa*	*C. mangga*	*C. zedoaria*
Phenolic acids (µg/g DW)			
Gallic acid	4.84±0.26$^{\#}$	3.02±0.03$^{\&}$	16.79±0.34*
Protocatechuic acid	92.15±1.88$^{\#}$	148.23±4.40*	58.68±0.82$^{\&}$
Vanillic acid	126.08±2.09*	23.44±0.25$^{\#}$	19.27±0.72$^{\&}$
Caffeic acid	25.84±0.24*	ND	12.47±0.36$^{\#}$
p-coumaric acid	72.16±0.79*	15.22±0.06$^{\&}$	35.35±0.85$^{\#}$
Ferulic acid	122.64±1.05*	5.77±0.06$^{\&}$	29.08±0.78$^{\#}$
Sinapinic acid	57.62±2.43*	19.67±0.42$^{\#}$	16.55±0.24$^{\&}$
Cinnamic acid	5398.30±56.33*	106.50±1.18$^{\&}$	3982.00±26.67$^{\#}$
Total phenolic acids	5899.63±65.07*	321.85±6.40$^{\&}$	4170.19±30.78$^{\#}$
Flavonoid compounds (mg/100 g DW)			
Rutin	3.93±0.08$^{\#}$	4.01±0.14*	2.67±0.13$^{\&}$
Apigenin	7329.66±50.42*	25.09±0.41$^{\&}$	1118.06±7.08$^{\#}$
Kaempferol	184.67±1.09*	7.27±0.07$^{\&}$	101.71±1.68$^{\#}$
Quercetin	192.87±1.61$^{\#}$	10.07±0.22$^{\&}$	305.37±3.61*
Total flavonoid *compounds*	7711.13±53.20*	46.44±0.84$^{\&}$	1527.81±12.5$^{\#}$

: $^{*\#\&}P < 0.05$ compared to each specie, ND = Not detected

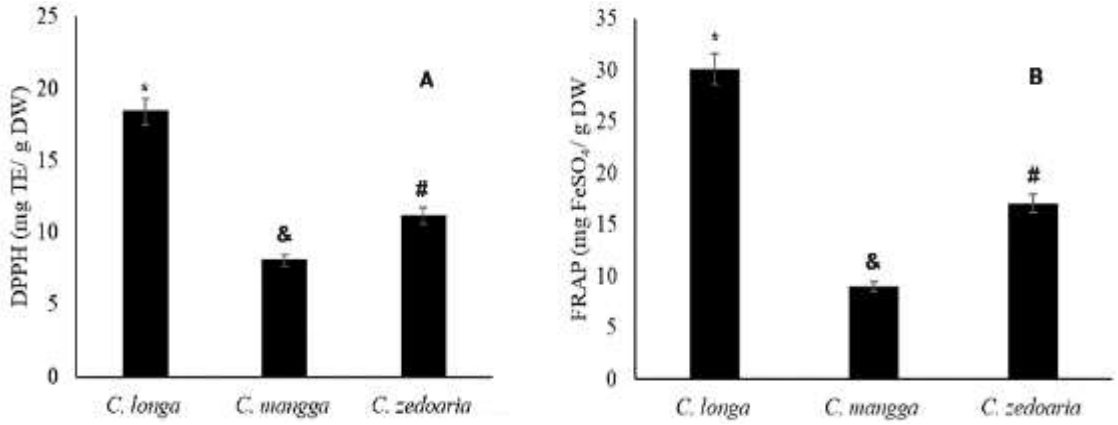

Figure 3: A: DPPH radical scavenging activity. B: Ferric reducing antioxidant power (FRAP). $^{*\#\&}P < 0.05$

Table 4: Correlations between TPC, TFC, DPPH, and FRAP

Parameter	TPC	TFC	DPPH	FRAP
TPC	1	0.986**	0.990**	0.997**
TFC	-	1	0.975**	0.977**
DPPH	-	-	1	0.992**
FRAP	-	-	-	1

TPC: Total phenolic content; TFC: Total flavonoid content; DPPH radical scavenging activities FRAP: Ferric reducing antioxidant activity. **Correlation is significant at 0.01 level (2-tailed)

DISCUSSION

Species in the genus *Curcuma* contains several phytochemicals including curcuminoids, phenolics, alkaloids, diarylheptanoids and essential oils [5]. These are responsible for eliciting diverse biological and pharmacological effect including antioxidant and anti-inflammatory activities [6]. This study investigated the distribution of phytochemicals, anti-inflammatory and antioxidant effect of *C. longa*, *C. mangga*, and *C. zedoaria.* The three *Curcuma* species revealed similar physical characteristics such as an outer shell, an outer zone complete with curcumin cells and oil cells, and an inner zone with starch deposits in vascular bundles. However, *C. longa*, *C. mangga*, and *C. zedoaria* differed in colour and density of oleoresin per area. *C. longa* exhibited an orange color, with the center area on the oil cells ranging from dark orange to black on outer boundary of the oil cells. By contrast, *C. zedoaria* had a yellow oleoresin distribution similar to *C. longa* but with a lower density. *C. mangga* displayed a lower density of light yellow oleoresin oil, and exhibited a white colour [11,12].

Number of oil cells, curcumin, starch, and pectin varied by species [10], with colour differences depending on environmental edaphic and climatic factors [13]. Colour of the genus Curcuma tissue was found to be curcumin in all samples. Curcumin generally appears as a bright yellow pigment but the colour may change from yellow-orange to reddish-brown depending on pH, temperature, and presence of other compounds or impurities [14,15] which influence applications from food colouring to the production of traditional medicine. The yellow oil cells in *C. longa* are used as natural food colouring while the unique colour characteristics of *C. mangga* and *C. zedoaria* influence their selection in pharmaceutical and cosmetic formulations. Previously reported relationships between colour in *Curcuma* species and curcuminoid content suggested that the characteristic yellowish or orange colour was due to the presence of polyphenolic pigments [15,16].

Predominance of curcuminoids in *C. longa* contributes to its strong anti-inflammatory and antioxidant activities, supporting its therapeutic potential [17], while higher DMC content in *C. zedoaria* suggests a different curcuminoid (CUR, DMC, and BDMC) which influences specific pharmacological actions. Curcuminoids are the major chemical compounds found in the genus *Curcuma* [7]. The lower curcuminoid content in *C. mangga* highlights the diversity within *Curcuma* species and suggests that *C. mangga* may contain bioactive compounds of interest beyond curcuminoids. These findings highlight the phytochemical variability among *Curcuma* species, emphasizing the importance of species identification in the application of *Curcuma*-based product [7].

Correlation between colour and curcuminoid content among the three *Curcuma* species was attributed to their distinct phytochemical compositions leading to various potential applications. The strong red and yellow hues in *C. longa* are consistent with its high curcuminoid content, particularly curcumin, which is known for its bright-yellow colour [18]. Colour differences related to varying quantities of bioactive compound such as phenols and flavonoids in *Curcuma* species are utilized in nutritional or medicinal applications [19]. The findings revealed that *C. longa* exhibited high antioxidant capacity, as indicated by elevated levels of TPC and TFC, consistent with previous studies detailing its anti-inflammatory, anticarcinogenic, and antimicrobial properties for potential applications in health and medicine [7].

The three species exhibited significantly different phenolic acid contents, underscoring the diversity in their phytochemical profiles and potential health benefits. The high concentration of cinnamic acid in *C. longa* suggests that cinnamic acid is a precursor of some phenolic acids such as caffeic acid and ferulic acid [20]. Caffeic acid was detected in both *C. longa* and *C. zedoaria* but not found in *C. mangga* suggesting different pathways responsible for phenolic acid synthesis or degradation among these species. Understanding the genetic and environmental factors that influence phenolic acid synthesis or degradation among these species. Understanding the genetic and impacts of natural variables that influence phenolic acid production in these plants will lead to optimized cultivation practices for enhanced phytochemical content, with *C. longa*, *C. zedoaria,* and *C. mangga* used in traditional medicine and as dietary supplements [17].

Flavonoid compounds were found in all three *Curcuma* species as detected by HPLC [10], with rutin, apigenin, kaempferol, and quercetin in *C. longa* (Thailand), *C. longa* (Indonesia), *C. zedoaria* (Indonesia), *C. mangga* (Indonesia) and *C. aeruginosa* (Indonesia). Flavonoids have a wide variety of biochemical and pharmacological antioxidant, anti-inflammatory, antiplatelet, antihypertensive, and anti-ischemic properties. *C. longa* exhibited significantly higher antioxidant activity compared to other species which is attributed to varying phytochemical compositions, particularly curcuminoids, phenolics and flavonoids similar to previous studies [10]. Similar result was observed for TPC, and TFC using DPPH and FRAP tests in *Curcuma* genus [21]. Results suggested a positive relationship between TPC, TFC, and antioxidants activity, consistent with previous studies [22]. Total phenolics and flavonoid content in *Curcuma* genus are influenced by climate, environmental factors, growth conditions and species [23]. These findings highlighted the potential for developing natural antioxidant formulations for health supplements, functional foods and cosmetic ingredients.

CONCLUSION

Curcuma longa exhibits significantly higher concentration of curcuminoids, TPC, and TFC with higher antioxidant activity. Furthermore, TPC and TFC shows significant positive correlation with antioxidant activity. This study provides valuable insights into the potential application of *Curcuma* plants as a functional additive in foods and cosmetics. Future studies should examine the effect of natural variables,

growing conditions, and processing techniques on the prevalence of bioactive chemicals and correlation with biological activities.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chanakran Papayrata, Theeraphan Chumroenphat, Piyapron Saensouk and Surapon Saensouk performed study concept, experiments, data collection, analysis, manuscript handling and manuscript writing. All authors read and approved the final draft of the manuscript for publication.

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