

## Short communication

**Anti-inflammatory activity of *Curcuma cf. amada* Roxb. 'Wan en Lueang'**

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**KEYWORDS:**

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**ABSTRACT**

The rhizome of *Curcuma cf. amada* Roxb. 'Wan en Lueang' (CWeL) was added to the list of Thai household remedy herbal drugs since 2013. It is recommended as one of the main ingredients in recipes for external use to treat muscle pain and physical trauma, which is a complex biological response of body tissues known as inflammation. However, the anti-inflammatory activity of CWeL has not been scientifically proven yet. Thus, the objective of this study was to investigate the anti-inflammatory activity of CWeL using ethyl phenylpropiolate (EPP)-induced ear edema in rats. The hexane, deep fried in coconut oil (DF) and the essential oil were prepared. In addition, the chemical constituents of the extracts were investigated by GC-MS. Epicurzerenone, curdione and germacrone are the major constituents in essential oil. The screening analysis of the curcuminoid in the extracts was also evaluated by TLC. Only the DF extract possessed all the bands of the curcuminoids. For the anti-inflammatory test, the results showed that the hexane extract (0.5, 1 and 2 mg/20 $\mu$ l/ear), DF extract (20  $\mu$ l/ear) and essential oil (50% and 100% /20 $\mu$ l/ear) reduced the rat's ear edema. Among of all extracts, the DF extract showed the highest anti-inflammatory effect with percent inhibition of 67.92, followed by 100% essential oil and 50% essential oil with percent inhibition of 61.53 and 60.35, respectively. In conclusion, the DF extract exhibited a topical anti-inflammatory activity which could be scientific evidence for the traditional use of this plant and the list of the Thai household remedy drug.

**1. INTRODUCTION**

The plants of genus *Curcuma*, Zingiberaceae family, are used for many purposes, such as spices, food dye and medicinal herbs<sup>1</sup>. Some species of *Curcuma* have been reported to show anti-inflammatory activity, e.g. *Curcuma aromatica* Salisb., *Curcuma comosa* Roxb. and *Curcuma longa* L.<sup>2</sup>. *Curcuma cf. amada* Roxb. 'Wan en Lueang' (CWeL) is an herb that is widely found and can also be cultivated in many parts of Thailand. In Thai traditional medicine, CWeL has been used to treat sprains, strains and muscular and joint pain for a long time.

In Thai traditional medicine, CWeL has been used to treat sprains, strains, muscular and joint pain for a long time. The rhizome of CWeL is categorized to be one of the major ingredients in recipes used for muscle pain relief notified in the list of the Thai household remedy drug for over eight years<sup>3</sup>. After that, CWeL become generally used in oil and balm products. Most topical products called “Namman Wan” or “Namman Lueang” available in market were prepared by deep frying the thin sliced pieces in coconut oil with other herbs of Zingiberaceae<sup>4</sup>. Moreover, the essential oils of many *Curcuma* species have been reported to inhibit edema formation<sup>5</sup>. However, no scientific data on the efficacy of the CWeL rhizome has been shown. The aim of this study is to investigate the anti-inflammatory activity and chemical constituents of the essential oil and extracts of CWeL rhizome.

## 2. MATERIALS AND METHODS

### 2.1. Collection and authentication of plant material

The rhizomes of CWeL were purchased from a cultivation field in Bueng Kan Province, Thailand. Then the rhizomes of CWeL were verified by using morphological characteristic by Mr. Suwat Tangchitcharoen, Mr. Boonyuen Phongphaew and Mr. Wut Wutithamawech, licensed Thai Traditional Medical doctors. A crude drug voucher specimen (No. 1000461) of CWeL has been deposited at the Thai Traditional Medicine Herbalium, Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand.

### 2.2. Animals

Male Sprague-Dawley rats (100-120g), 5-8 weeks old, from the National Laboratory Animal Center, Mahidol University were used. The animals were housed in groups of three per cage. They were kept in a temperature controlled room (25±1°C) under a 12 hour light/dark cycle for one week before the experimental study. The experiment protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University (No. PYT002/2557). The experiments were conducted according to the ethical care of laboratory animal guidelines.

### 2.3. Preparation of extracts

#### 2.3.1. Preparation of hexane extract

Ten kilograms of CWeL rhizomes were sliced and then dried in an oven at 50 °C for 24 hours. The dried pieces were collected and then ground into powder. Five hundred grams of powder was extracted by hexane using the percolation method. The solution was then filtered. Hexane was removed from the filtrate using a rotary evaporator at 45 °C under a reduced pressure. The extracts were stored at 10°C in amber glass bottles until use. The concentrated extract was dissolved in coconut oil at three concentrations (0.5 mg/20 µl, 1 mg/20µl and 2 mg/20 µl) for anti-inflammation tests.

#### 2.3.2. Preparation of DF extract

Coconut oil was prepared by heating 5 l of coconut milk to 80 °C until the water was removed and it showed a clear oil solution with some suspended brown materials. The coconut oil was then collected after filtration. Two kilograms of CWeL rhizomes were sliced into thin pieces, and deep fried in 1 l of hot coconut oil until the pieces of the rhizome turned crispy brown<sup>4</sup>. The pieces of rhizome were removed and filtered using cheesecloth, and then the extract was collected and stored at 10°C in amber glass bottles.

#### 2.3.3. Preparation of essential oil extraction

Three hundred grams of CWeL rhizome were sliced and subjected to hydro-distillation in 1000 ml distilled water for six hours using a Clevenger type apparatus. The essential oil was separated and anhydrous sodium sulfate was added for three minutes to absorb the small amount of water remaining in the oil, and the oil was collected by a micropipette. The oil was stored at 10°C in amber glass bottles.

### 2.4. Quality of chemical constituents analysis

#### 2.4.1. Quality of curcuminoid analysis by TLC

The screening of the curcuminoid constituents was performed using the TLC technique. The essential oil extract and hexane extract were dissolved in ethanol (1:1). The DF extract was dissolved in dichloromethane (1:1). A silica gel 60 GF 254 plate was used as the stationary phase,

hexane:dichloromethane:95% ethanol (41:49:10) and dichloromethane:95% ethanol (9:1) were used as the mobile phase<sup>6</sup>. The three extracts, three extracts mixed with curcuminoid standard 1:1) and curcuminoid standard were used and analyzed.

#### 2.4.2. Essential oil analysis by GC-MS

The chemical constituents of the essential oil was determined using a Shimadzu GC-17 gas chromatography together with a Shimadzu QP5000 mass spectrometer. The column DB-5MS with length 30m x inner diameter 0.25mm x film thickness 0.25  $\mu$ m was used. Helium gas was used as the carrier gas at a flow rate of 1.0mL/min. The mass spectrometer was operated in the electron-impact (EI) mode with detection at 1.2 kV in the scan range of 10-400 amu. The temperature program was set as follows: essential oil extract held for 2 minutes at 50 °C, then heated to 290 °C at 4 °C/minute, 40:1 split ratio, 250 °C injection temperature and 230 °C ion source temperature. The temperature of the DF extract, coconut oil and hexane extract program were set as follows: extracts were held for 5 minutes at 150 °C, then heated to 160 °C at 2 °C/minute; held for 10 minutes, then heated to 200 °C at 2 °C/minute; held for 30 minutes, then heated to 220 °C at 2 °C/minute; held for 20 minute, then heated to 240 °C at 2 °C/minute; and held

for 10 minute, 40:1 split ratio, 275 °C injection temperature and 250 °C ion source temperature. The temperature was modified from the method of Mishra et al. (2018)<sup>7</sup>. The components of the essential oil were identified by a comparison of their retention index and mass spectra with the NIST 2014 spectral library.

#### 2.5. Anti-inflammatory test with ethyl phenylpropionate (EPP)-induced ear edema in rats

The topical anti-inflammatory effect of the CWeL extracts was examined in EPP-induced ear edema in rats by modifying the methods of Brattsand et al. and Young et al.<sup>8,9</sup>. Twenty-seven male Sprague-Dawley rats were randomly divided into nine groups, with three rats per group as shown in Table 1. EPP was dissolved in acetone and topically applied to the inner and outer surfaces of the rat ears at a concentration of 1  $\mu$ g/20 $\mu$ l/ear using an automatic microliter pipette. One hour after EPP induction, either distilled water (20  $\mu$ l/ear), coconut oil (20  $\mu$ l/ear), hexane extract (0.5, 1 and 2 mg/20 $\mu$ l/ear), 50% essential oil in coconut oil (50% EO) and 100% essential oil (20 $\mu$ l/ear), DF extract (20  $\mu$ l/ear) or indomethacin (1 mg /20 $\mu$ l/ear) were topically applied to the same area as the EPP. The ear thickness was measured initially and then every hour for four hours after inflammation using a micrometer. The percentages of the ear swelling and the inhibition were calculated.

**Table 1.** Experiment Groups

Number	Group Name	Applied Solution
Group I	Control group	Distilled water
Group II	Vehicle group	Coconut oil
Group III	Reference group	Indomethacin
Group IV	0.5 mg hexane group	0.5 mg hexane extract
Group V	1 mg hexane group	1 mg hexane extract
Group VI	2 mg hexane group	2 mg hexane extract
Group VII	50% essential oil in coconut oil group	50% essential oil in coconut oil extract
Group VIII	Essential oil group	100% essential oil extract
Group IX	DF group	DF extract

#### 2.6. Statistical analysis

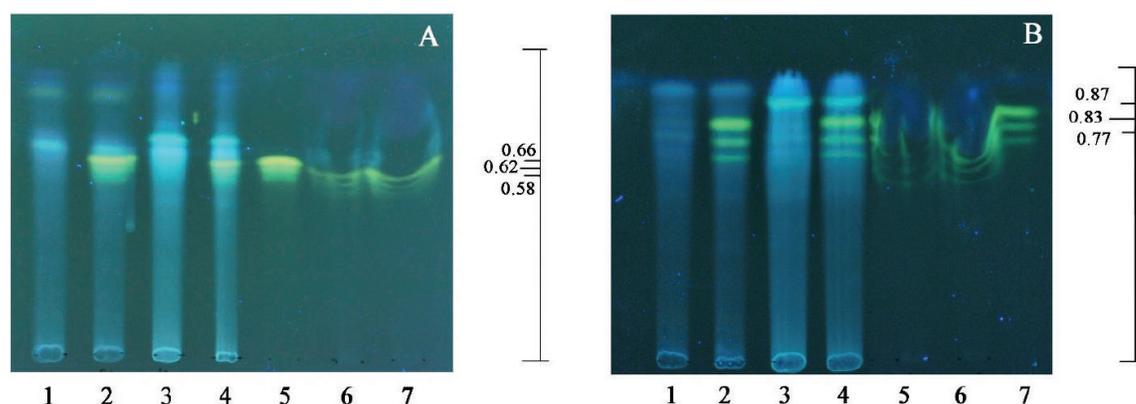
All values were expressed as mean  $\pm$  standard error of the mean (S.E.M.) and statistically significant differences between the treated groups and vehicle or control group were evaluated by

one way analysis of variance (ANOVA) followed by a Tukey Honestly test (HSD) for multiple comparisons using the SPSS computer program 17.0. Differences with *P-values* of less than 0.05 were considered statistically significant.

### 3. RESULTS

From hexane:dichloromethane:95% ethanol (41:49:10) mobile phase, the standard curcuminoids showed the three typical bands of curcumin at RF 0.66, demethoxycurcumin at RF 0.60 and bis-demethoxycurcumin at RF 0.52. From dichloromethane:95% ethanol (9:1) mobile phase, the standard curcuminoids showed the three typical bands of curcumin at RF 0.87, demethoxycurcumin at RF 0.83 and bis-demethoxycurcumin at RF 0.77.

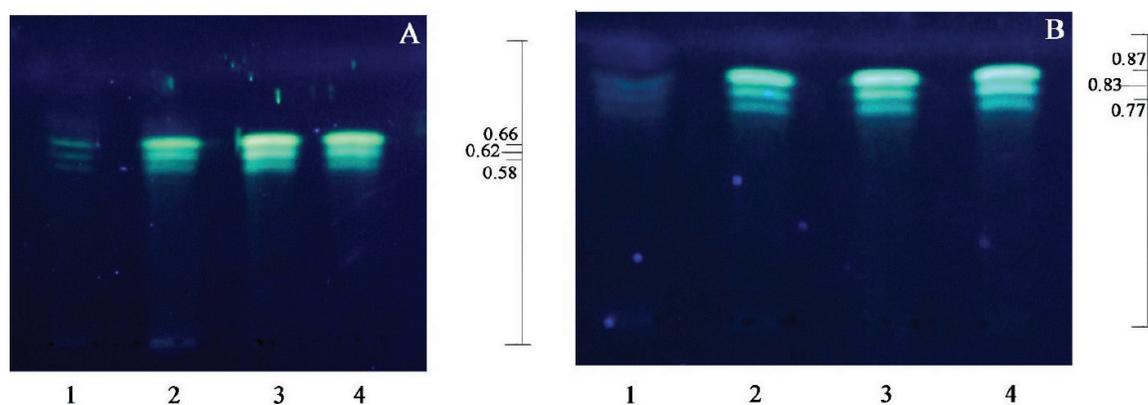
Neither the essential oil nor the hexane extracts showed any bands of curcuminoid, but the DF extract showed some similar bands close to the standard curcuminoid in comet like shape, as shown in Figure 1. So the experiment was modified by reduced the concentration of the DF extract and mixed the extract with the standard, then the experiment was conducted again. The result showed that the DF extract possessed all bands of the curcuminoids, as shown in Figure 2.



**Figure 1.** The TLC fingerprint of WeL rhizome extracts;  
Solvent system: (A) Hexane : dichloromethane : 95% ethanol (41 : 49 : 10)  
(B) Dichloromethane : 95% ethanol (9 : 1)

Detectors: UV 366

1 = Essential oil, 2 = Essential oil : Curcuminoid standard (1:1), 3 = Hexane extract,  
4 = Hexane extract : Curcuminoid standard (1:1), 5 = Curcuminoid standard, 6 = DF extract,  
7 = DF extract : Curcuminoid standard (1:1)



**Figure 2.** The TLC fingerprint of WeL rhizome extracts;  
Solvent system: (A) Hexane : dichloromethane : 95% ethanol (41 : 49 : 10)  
(B) Dichloromethane : 95% ethanol (9 : 1)

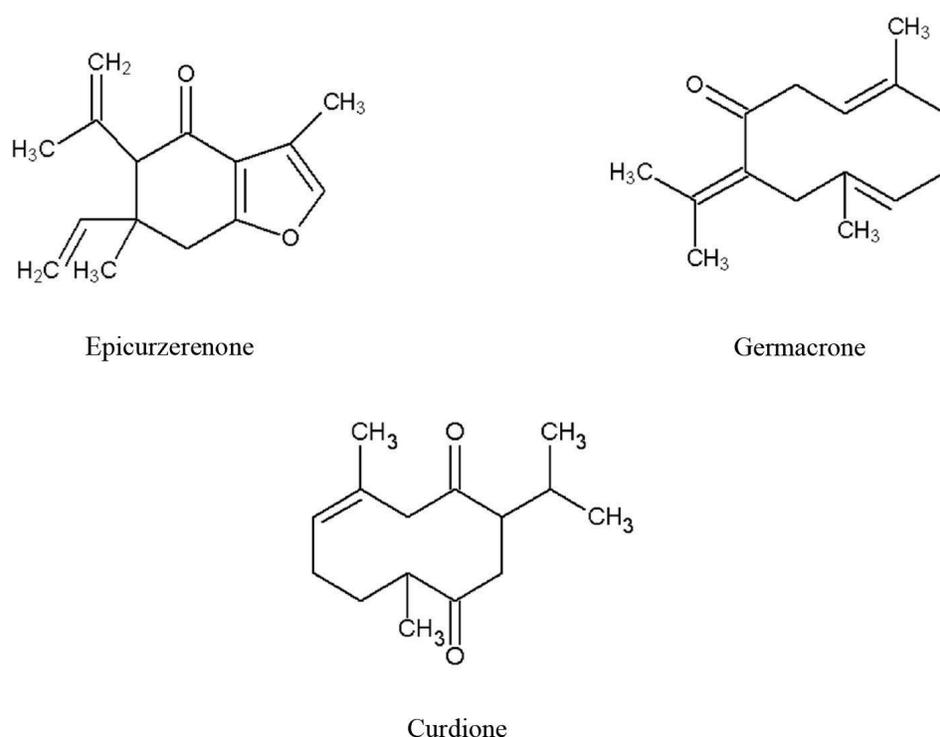
Detectors: UV 366

1 = DF extract dissolved in dichloromethane (1:9)  
2 = DF extract dissolved in dichloromethane (1:9) : Curcuminoid standard (1:1)  
3 = DF extract dissolved in dichloromethane (1:9) : Curcuminoid standard (1:9)  
4 = Curcuminoid standard

The major constituents of the extracts are shown in Table 2. The major compounds of the essential oil from the rhizomes of CWeL were epicurzerenone (25.33%), curdione (16.47%) and germacrone (14.42%) (Figure 3). Coconut oil, which was used as a vehicle, contained dodecanoic acid (56.73%) and trioctanoin (19.64%). The main constituents in the CWeL extract by DF were curcumol (0.63%), 2-nonadecanone (0.17%) and epicurzerenone (0.16%). The main constituents in the CWeL hexane extract were curdione (43.31%), curcumenol (29.81%) and epicurzerenone (9.45%).

**Table 2.** Major constituents of extracts

Compound	Major constituents (%)			
	Coconut oil	Essential oil extracts	WeL extract by DF	WeL hexane extract
Azulene	-	0.61	0.08	-
Benzene	-	-	-	3.38
Beta-elemenone	-	0.5	0.06	-
2-Bromododecane	-	-	-	3.98
Camphor	-	3.75	-	0.89
1,8-Cineole	-	2.74	-	-
Curcumol	-	-	0.63	-
Curcumenol	-	-	-	29.81
Curcumenone	-	-	-	3.76
Curdione	-	16.47	-	43.31
Curzerene	-	2.02	-	-
Cyclohexene	-	2.05	-	1.64
Delta-decalactone	0.14	-	0.38	-
Dodecanoic acid	56.73	-	40.35	-
Epicurzerenone	-	25.33	0.16	9.45
Germacrone	-	14.42	-	-
Isoborneol	-	2.31	-	0.77
Isovelleral	-	2.71	-	-
2-Nonadecanone	-	0.45	0.17	-
2-Octenoic acid	-	-	-	3.01
Squalene	4.92	-	-	-
Tetradecanoic acid	4.62	-	0.34	-
Trioctanoin	19.64	-	43.4	-
Zingiberene	-	2.63	-	-



**Figure 3.** Major compounds of essential oil from WeL rhizome

All CWEL extracts as well as coconut oil (the vehicle group) suppressed the ear edema throughout the period of the study ( $p < 0.5$ ) when compared to the control group, as shown in Table 3 and Figures 4 and 5. These effects were similar to the effect of indomethacin, a reference anti-inflammatory drug. Although the essential oil had anti-inflammatory activity, it produced a reddish skin area on the ear surface, which may indicate

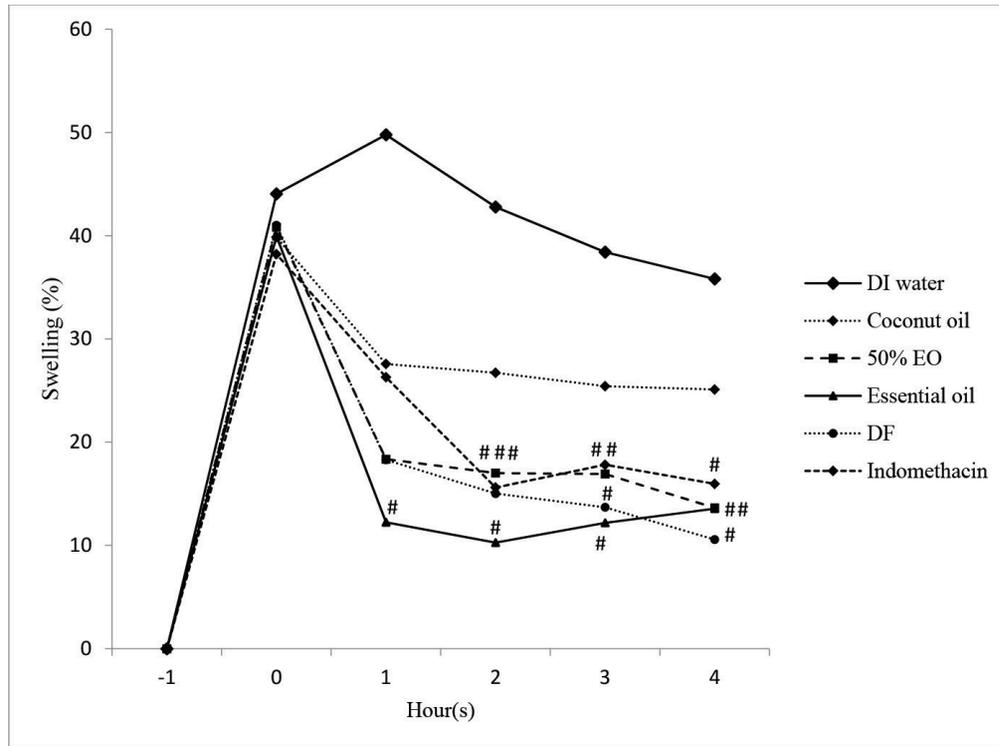
skin irritation. However, the 50% EO exhibited anti-inflammatory activity without skin irritation. For the results of the hexane extract, hexane extract reduced the ear edema in a dose dependent manner and showed less potency than the essential oil or DF extract. Among the extracts in this recent study, the DF extract had the best anti-inflammatory activity on the EPP-induced ear edema in rats.

**Table 3.** The percent inhibition of WeL extracts on EPP-induced ear edema in rats

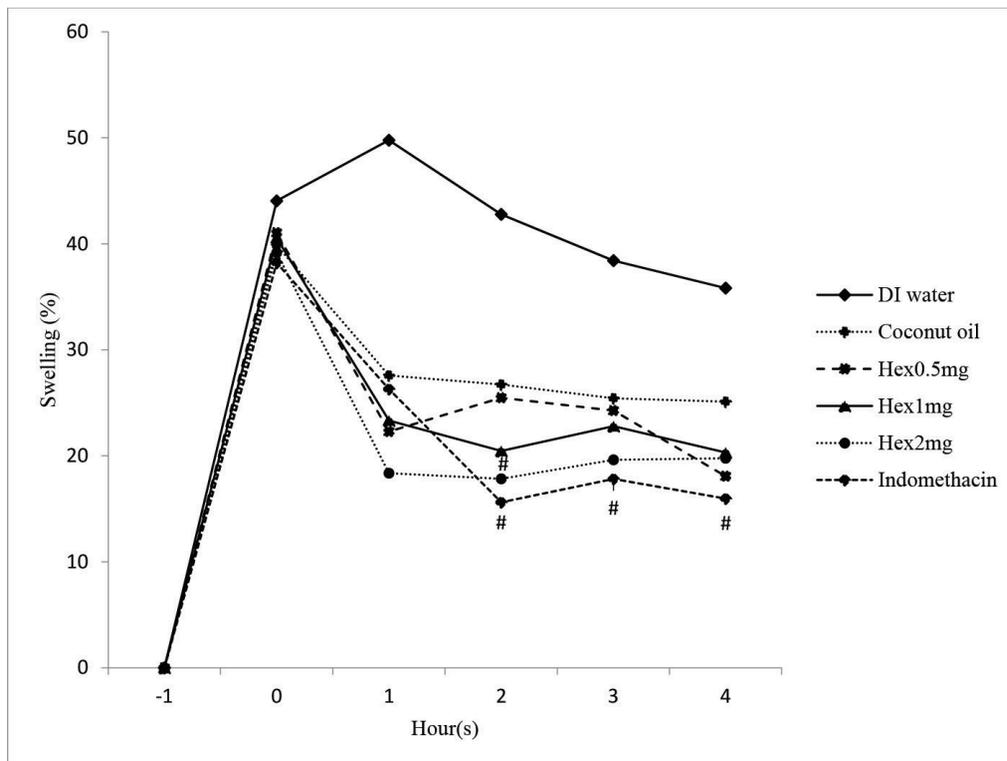
Drug	Inhibition (%)				
	H <sub>0</sub>	H <sub>1</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>
Distilled water	-	-	-	-	-
Coconut oil	-	41.73 ± 4.74	34.42 ± 8.80	30.43 ± 9.951	26.15 ± 11.91
Indomethacin	-	43.05 ± 4.67	60.99 ± 3.11 <sup>#</sup>	50.00 ± 4.82	52.44 ± 6.36 <sup>#</sup>
Hexane 0.5 mg	-	52.09 ± 1.87	36.99 ± 9.12	33.95 ± 11.99	39.17 ± 8.20
Hexane 1 mg	-	49.70 ± 4.76	49.16 ± 8.65	36.93 ± 12.93	39.52 ± 8.55
Hexane 2 mg	-	54.92 ± 6.16 <sup>#</sup>	49.85 ± 6.01	38.36 ± 8.33	33.37 ± 6.60
50% EO	-	61.35 ± 2.16 <sup>#</sup>	58.45 ± 3.03 <sup>#</sup>	53.69 ± 8.30	60.35 ± 3.49 <sup>#</sup>
Essential oil	-	74.80 ± 5.62 <sup>#</sup>	75.56 ± 6.26 <sup>#</sup>	67.80 ± 4.63 <sup>#</sup>	61.53 ± 4.24 <sup>#</sup>
DF	-	60.06 ± 4.07 <sup>#</sup>	62.21 ± 5.11 <sup>#</sup>	61.41 ± 3.75 <sup>#</sup>	67.92 ± 7.06 <sup>#</sup>

Values are expressed as Mean ± S.E.M. (N=5)

Statistically significant from Coconut oil group : <sup>#</sup> P < 0.05



**Figure 4.** The percentage of ear swelling of WeL oil extracts on EPP-induced ear edema in rats at 1, 2, 3 and 4 hours after EPP application. Statistically significant from Coconut oil group: # P < 0.05



**Figure 5.** The percentage of ear swelling of WeL hexane extracts on EPP-induced ear edema in rats at 1, 2, 3 and 4 hours after EPP application. Statistically significant from Coconut oil group: # P < 0.05

#### 4. DISCUSSION

EPP-induced ear edema in rats was selected for the testing of the topical anti-inflammatory effect of the extracts as this model is suitable for topical testing of anti-inflammatory compounds and EPP causes the release of many inflammatory mediators, such as PGs, bradykinin and serotonin, which increase the vascular permeability and produce edema<sup>10</sup>. Indomethacin, a commonly used NSAID, was selected as the standard drug.

The results showed that the DF extract from the CWeL exerted the greatest inhibition of the EPP-induced ear edema in rats. The DF extract contained azulene, curcumol and curcuminoid compounds, which have been reported to demonstrate anti-inflammatory activity<sup>11-14</sup>. It was considered that azulene, curcumol and curcuminoid of DF should be the compounds that demonstrated the anti-inflammatory effect, especially the curcuminoids, which were proven to be potent anti-inflammatory effect compounds. The present results suggested that the DF extract should be studied further to determine the quantity of the curcuminoid. Otherwise, there is no data of anti-inflammatory activity of epicurzerenone which was the major compound in the essential oil, so anti-inflammatory activity of it should be investigated more in the future.

#### 5. CONCLUSIONS

All of the extracts reduced the rats ear edema starting one hour after the topical application and was maintained throughout a period of four hours, in which the DF extract exhibited the most potent inhibition of the inflammation and was comparable to indomethacin, which was the standard anti-inflammatory drug. The coconut oil could suppressed the ear edema with a lower potency than the CWeL extract. The essential oil of CWeL also significantly suppressed ear edema, but the reddish skin showed dilated blood vessels beneath the ear surface, whereas the 50% EO showed a similar inhibitory effect to the essential oil but with no sign of the skin irritation.

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#### Conflict of interest

No conflicts of interest.

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#### Ethical approval

None to declare

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