Original article

In vitro bioactivities and preparation of nanoemulsion from coconut oil loaded Curcuma aromatica extracts for cosmeceutical delivery systems

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1. Introduction

Curcuma aromatica (CA) is a herbaceous plant in the Zingiberaceae family. It has antioxidative activity and anti-inflammatory properties. The purpose of this study was to investigate the effect of solvents and extraction methods on CA rhizomes. The crude extracts were tested for phenolic and flavonoid contents, antioxidative activity by DPPH and lipid peroxidation assay, and protein denaturation inhibition. The crude extract with 95% ethanol by maceration technique showed good results. It had phenolic content at 99.28 ± 1.09 mg GAE/g extract, flavonoid content at 397.00 ± 27.54 mg QE/g extract, antioxidative activity by DPPH assay and lipid peroxidation assay at IC50 value of 0.55 ± 0.02 mg/ml and 0.60 ± 0.10 mg/ml, respectively. The percentage of protein denaturation inhibition was 65.97 ± 4.68%. The crude extract with 95% ethanol by maceration technique was selected to formulate nanoemulsion. Nanoemulsion formulation consisted of DI water, Tween 80, CA extract, coconut oil and Span 80 at 72.50, 12.93, 7.07, 5.00 and 2.5%w/w, respectively. Its appearance was an opaque yellow liquid with no precipitation and no phase separation at room temperature. The particle size, pH, and viscosity were 70.20 ± 0.38 nm, 5.87 ± 0.01 and 3.56 ± 0.24 cP, respectively. Nanoemulsion loaded CA extract had bioactivities and highly stable characteristics after heating–cooling test for 6 cycles. This study has demonstrated the potential of nanoemulsion from coconut oil loaded CA extract for further development to novel cosmetic products.

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Nanoemulsions are very useful in the application of chemical, medical, pharmaceutical, food and cosmetic industries. This drug delivery is especially suited to entrap functional compounds because it enhances the formulation stability (Silva et al., 2012; Solans and Solé, 2012). This study was aimed to determine active contents and bioactivities of CA extracts. The preparation, characteristic determination, bioactivities and stability of nanoemulsion from coconut oil loaded CA extract were further investigated to develop the novel skin care products.

2. Material and methods

2.1. Materials

L-(-)-ascorbic acid, quercetin, gallic acid, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and Folin-Ciocalteu reagent were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade. CA was collected from Lampang province, Thailand.

2.2. CA extracts preparation

A portion of CA was extracted by non-heating and heating with water or ethanol. For the non-heating extraction, the sample was macerated in the solvents at room temperature for 24 h. For heating extraction, the samples extracted with 50% ethanol and 95% ethanol were refluxing at 50 ± 2 °C for 2 h while the samples extracted with distilled water were boiled for 2 h on the electric hot plate. The mixture was filtered using Whatman No.1 filter paper, evaporated under reduced pressure by a rotary evaporator (R-124 Buchi, Switzerland) until dried extracts and kept at 4 °C.

2.3. Total phenolic content determination

The total phenolic content was measured in a 96-well plate by Folin-Ciocalteu assay with slight modification (Sainakham and Mungmai, 2020). 10 μl of CA extracts solution at 1 mg/ml dissolved in the solvent used in the extraction method was mixed in a 96-well plate with 30 μl of 7.5% Na2CO3 and 170 μl of 10% v/v Folin-Ciocalteu reagent, covered the plate in the dark for 30 min at room temperature. The absorbance was measured by the microplate reader at 725 nm (BioTek Synergy H1, VT, USA) at 100 rpm. The pH value was measured by pH meter (Brookfield viscometer H1, VT, USA) at 100 rpm. The particle size of formulation (100 folds dilution) was measured using the

2.4. Total flavonoid content determination

The total flavonoid content was determined by aluminium chloride colorimetry with slight modification (Kim et al., 2003). In a 96-well plate, 30 μl of CA extracts solution was added to 120 μl of distilled water followed by 10 μl of 5% NaNO2. After incubation for 5 min, 10 μl of 10% AlCl3 was added and further incubated for 6 min. The reaction mixture was treated with 60 μl of 1 M NaOH and 10 μl of distilled water. Finally, the absorbance was measured by the microplate reader at 510 nm after incubated for 10 min. The standard curve of total flavonoid content was plotted between the absorbance and quercetin concentrations at 100–500 μg/ml. The total flavonoid content was presented as mg of quercetin equivalents (QE) per gram of the extract.

2.5. Free radical scavenging assay

Free radical scavenging activity was evaluated using DPPH (Sainakham and Mungmai, 2020). In brief, the reaction consisting of 0.25 μl of 0.25 mg/ml DPPH in ethanol and 50 μl of various concentrations of sample was added in a 96-well plate. After incubation for 30 min in the dark at 27 ± 2 °C, an absorbance of 517 nm was measured on the plate. The samples were calculated as follows: % scavenging activity = [(Acontrol-Asample)/Acontrol] x100, where Acontrol was the absorbance of the control and Asample was the absorbance of the sample. The IC50 value was the concentration of the sample causing 50% DPPH scavenging.

2.6. Lipid peroxidation inhibition assay

The lipid peroxidation inhibition activity was evaluated using Ferric-thiocyanate assay (Bhaokam et al., 2020). Briefly, the lipid peroxidation inhibitory reaction was added in a 96-well plate consisting of 50 μl of five serial concentrations of the extracts at 0.001 to 10 mg/ml and 50 μl of linoleic acid in 50% (v/v) DMSO. The reaction mixture was treated with 50 μl each of NH2SCN at 0.39 mg/ml and FeCl3 at 0.25 mg/ml dissolved in 2.4% HCl. The complexation from ferric-thiocyanate measured an absorbance of 490 nm after incubation for 1 h at 37 ± 2 °C. The samples were calculated as follows: % lipid peroxidation inhibition activity = [(Acontrol-Asample)/Acontrol] x100, where Acontrol was the absorbance of the control and Asample was the absorbance of the sample. The IC50 value was the concentration of the sample causing 50% lipid peroxidation inhibition.

2.7. Protein denaturation inhibition assay

The ability of samples to inhibit the egg albumin denaturation was evaluated using the method as previously described (Sharma et al., 2021). The reaction consists of 0.2 ml of fresh hen’s egg white, 2.8 ml of phosphate buffered saline at pH 6.4 and 2 ml of samples. After incubation in the water bath at 37 ± 2 °C for 15 min, the mixtures were heated for 5 min at 70 °C. The absorbances after cooling were measured at 660 nm. The samples were calculated as follows: % inhibition = [(Acontrol-Asample)/Acontrol] x100, where Acontrol was the absorbance of the control and Asample was the absorbance of the sample.

2.8. Preparation of nanoemulsion loaded CA extract

Blank nanoemulsion was formulated as presented in Table 1. CA extract (which gave the high bioactivities) was selected and loaded in this formulation. Oil phase was prepared with mixed coconut oil, the CA extract was homogenized in Tween80 and then mixed with Span80 by continuous stirring. The concentration of CA extracts in all nanoemulsions was at a constant level of 7.5% w/w. DI water was added and mixed with homogenizer at 10000 rpm for 15 min at room temperature.

2.9. Physicochemical characteristic examination of nanoemulsion loaded CA extract

Physicochemical characteristics of the nanoemulsion loaded CA extract were observed at room temperature in phase separation, viscosity, pH value and particle size. Phase separation was investigated by centrifugation at 3500 rpm for 30 min at 25 °C. The viscosity was determined by Brookfield viscometer (BioTek Synergy H1, VT, USA) at 100 rpm. The pH value was measured by pH meter which was calibrated using standard buffer solution. The particle size of formulation (100 folds dilution) was measured using the
dynamic light scattering technique by Zetasizer (Malvern Instruments Ltd., Malvern, UK).

2.10. In vitro bioactivities and stability test of nanoemulsion loaded CA extract

The nanoemulsion loaded CA extract was investigated free radical scavenging activity, lipid peroxidation inhibition and protein denaturation inhibition as previously described. The formulation was observed stability by heating–cooling test for 6 cycles (1 cycle kept at 4 ± 2 °C for 24 h and 45 ± 2 °C for 24 h). The physicochemical characteristics were observed as previously described, and morphology of selected nanoemulsion loaded CA extract was visual by SEM (SEM Quanta 250, USA).

2.11. Data analysis

The separate experiments in triplicate were performed in all assays. The data was presented as mean ± SD (standard deviation) and statistically analysed using the SPSS program for Windows.

3. Results

3.1. Percentage yield, physical characteristics and of CA extracts

The percentage yields and physical characteristics of CA extracts are summarized in Table 2. Most extracts were brown of color and had a semisolid form, whereas the extract by hot extraction with DI water was brown of color and had a solid form. The extracts gave a percentage yield ranging from 10.45 to 17.15%. The extracts with 50% ethanol by hot extraction and maceration technique gave a high percentage yield at 17.15% and 12.50%, respectively.

3.2. Total phenolic and total flavonoid content determination

Total phenolic and total flavonoid content of CA extracts are shown in Table 2. CA extracts gave a total phenolic content from 14.93 to 99.28 mg GAE/g. The highest total phenolic content at 99.28 ± 1.09 mg GAE/g was observed in the extract with 95% ethanol by maceration technique (MTC95). The total flavonoid content of CA extracts was ranged from 5.33 to 397.00 mg QE/g. The extract with 95% ethanol by maceration technique (MTC95) gave the highest total flavonoid content at 397.00 ± 27.54 mg QE/g extract.

3.3. Bioactivities of CA extracts

Free radical scavenging, lipid peroxidation and protein denaturation inhibition activity of CA extracts are shown in Table 3. The highest free radical scavenging activity with IC50 value of 0.46 ± 0.01 mg/ml was observed in the extract by refluxing with 95% ethanol (RFC95) which was 0.02 folds of L-ascorbic acid (IC50 of 0.01 ± 0.01 mg/ml). The extract with 95% ethanol by maceration (MTC95) exhibited the highest potent lipid peroxidation inhibition activity at IC50 value of 0.60 ± 0.10 mg/ml which was 0.08 folds of L-ascorbic acid (IC50 of 0.05 ± 0.01 mg/ml). In the protein denaturation inhibition assay at 1000 μg/ml, diclofenac gave a value at 29.64 ± 3.50%, while RFC95 and MTC95 gave a high value at 80.67 ± 8.16 and 65.97 ± 4.68% which was more potent than diclofenac for 2.72 and 2.22 folds, respectively. Hence, the extract with 95% ethanol by maceration technique (MTC95) was selected and considered as an active ingredient for developed skin care product based on its bioactivities and active contents. The CA extract had further investigated the nanoemulsion preparation, bioactivities and physicochemical stability.

3.4. Preparation of nanoemulsion loaded CA extract

All blank nanoemulsions formulated with various components immediately observed after preparation, were transparent or translucent with single phase system. For initial screening of blank nanoemulsions, the preliminary investigation of particle size and appearance of nanoemulsion kept for 1, 24 and 72 h was observed as shown in Table 4. Particle size and PDI value of blank nanoemulsion kept for 1 h was in the range of 11.55 to 109.10 nm and 0.29 to 0.84, respectively. After kept for 24 h, particle size and PDI value of blank nanoemulsion were slightly changed in the range of 14.59 to 112.50 nm and 0.27 to 0.87, respectively. BN14 showed the small-

![Table 1](data:image/svg+xml)

**Table 1**
The ingredients of blank nanoemulsion formulation.

| Ingredient | INCI name | Function | Formulation (%w/w) |
|------------|-----------|----------|-------------------|
| Part A     |           |          |                   |
| DI water*  | Aqua      | Solvent  | 80                |
| Part B     |           |          |                   |
| Coconut oil| Cocos Nucifera (Coconut) Oil | Emollient | 5 5 5 5 5 5 5 5 |
| Tween 80   | Polysorbate 80 | Emulsifier | 15 15 15 15 20 20 20 20 |
| Span 80    | Sorbitan Oleate | Emulsifier | – 2.5 5 7.5 – 2.5 5 7.5 |

* The amount of water added in the formula was just enough to make the formula equal to 100%w/w.

![Table 2](data:image/svg+xml)

**Table 2**
The percentage yields, total phenolic and total flavonoid content determination of C. aromatica extracts.

| Sample | Appearance and color | Percentage yield (%) | Total phenolic content (mg GAE/g extract) | Total flavonoid content (mg QE/g extract) |
|--------|----------------------|----------------------|------------------------------------------|------------------------------------------|
| HEC95  | Semisolid in yellow  | 11.50                | 94.93 ± 1.65                             | 324.50 ± 26.02                           |
| HEC50  | Semisolid in brown   | 12.50                | 40.43 ± 4.52                             | 97.83 ± 5.77                             |
| HECD   | Solid in dark brown  | 10.60                | 23.91 ± 0.43                             | 7.01 ± 1.44                              |
| MTC95  | Semisolid in light brown | 10.45            | 99.28 ± 1.09                             | 397.00 ± 27.54                           |
| MTC50  | Semisolid in light brown | 17.15            | 40.14 ± 3.26                             | 104.50 ± 14.4                            |
| MTC95  | Semisolid in dark brown | 13.40            | 14.93 ± 3.25                             | 5.33 ± 0.43                              |

Note: HE = Hot extraction, MT = Maceration, C = C. aromatica, 95 = 95% ethanol, 50 = 50% ethanol, D = DI water.
Percentage yield (%) = [Dried extract weight (g)/ Dried C. aromatica weight (g)] × 100.
est particle size at 14.59 nm with PDI value of 0.56. After kept for 72 h, clear dispersibility and no phase separation of nanoemulsion were observed in BN4, BN5, BN13, BN14 and BN15. Based on the stability of blank nanoemulsion including clear dispersibility, no phase separation and small particle size, BN4, BN5, and BN14 contained Tween 80 and Span 80 in the ratio of 15:2.5, 15:5 and 20:2.5%w/w, respectively were selected to load CA extract and represented into WN4, WN5 and WN14, respectively. The solubility of MTC95 was performed in various solvents i.e., distilled water, 95% ethanol, Tween 80 and Span 80. It was found that MTC95 was maximum soluble in Tween 80 at concentration of 500 mg/g and used as active ingredients to load in selected nanoemulsion as shown in Table 5. All nanoemulsions loaded CA extract were homogeneous liquids with brown or dark brown colors (Fig. 1). Three nanoemulsions loaded CA extracts, WN4, WN5 and WN14, showing suitable physical characteristics were further investigated for bioactivities.

3.5. Bioactivities investigation of nanoemulsion loaded CA extract

The bioactivities of nanoemulsion loaded CA extract, WN4, WN5, and WN14, were performed as previously described. All nanoemulsions were diluted with distilled water to obtain the final concentrations of CA extract at 0.001, 0.01, 0.1, 1.0 and 10 mg/ml. Free radical scavenging, lipid peroxidation inhibition and protein denaturation inhibition activity of C. aromatica extracts are shown in Table 3.

| Sample | Free radical scavenging | Lipid peroxidation inhibition | Protein denaturation inhibition |
|--------|--------------------------|-------------------------------|-------------------------------|
|        | IC50 (mg/ml) | Folds of L-ascorbic acid | IC50 (mg/ml) | Folds of L-ascorbic acid (%) | IC50 (mg/ml) | Folds of Diclofenac (%) |
| RFC95  | 0.46 ± 0.01 | 0.02 | ND | – | 80.67 ± 8.16 | 2.72 |
| RFC50  | 0.78 ± 0.06 | 0.01 | ND | – | 16.26 ± 9.07 | 0.55 |
| RFCD   | 1.31 ± 0.17 | 0.008 | 2.16 ± 0.15 | 0.02 | ND | – |
| MTC95  | 0.57 ± 0.02 | 0.02 | 0.60 ± 0.10 | 0.08 | 65.97 ± 4.68 | 2.22 |
| MTC50  | 0.95 ± 0.02 | 0.01 | 0.91 ± 0.24 | 0.06 | 57.86 ± 0.25 | 1.95 |
| MTC8C  | 2.43 ± 0.40 | 0.004 | 1.95 ± 0.23 | 0.03 | 8.87 ± 2.13 | 0.30 |

Note: ND = Not detected.

Table 4

The preliminary investigation of particle size and appearance of nanoemulsion formulation kept for 1, 24 and 72 h.

| Formulation | After 1 h | After 24 h | After 72 h |
|-------------|-----------|-----------|-----------|
|             | Size (nm) | PDI       | Size (nm) | PDI       | Note         |
| BN3         | 105       | 0.57      | 112.5     | 0.48      | Phase separation |
| BN4         | 28.84     | 0.84      | 33.76     | 0.87      | Clear dispersibility and no phase separation |
| BN5         | 93.27     | 0.30      | 87.49     | 0.47      | Clear dispersibility and no phase separation |
| BN6         | 96.61     | 0.30      | 97.92     | 0.32      | No phase separation |
| BN13        | 47.90     | 0.67      | 50.60     | 0.69      | Clear dispersibility and no phase separation |
| BN14        | 11.55     | 0.52      | 14.59     | 0.56      | Clear dispersibility and no phase separation |
| BN15        | 61.31     | 0.55      | 62.02     | 0.54      | Clear dispersibility and no phase separation |
| BN16        | 105.10    | 0.29      | 102.2     | 0.27      | Phase separation |

Note: B = Blank; N = Nanoemulsion.

Table 5

The ingredients of nanoemulsion loaded C. aromatica extract.

| Ingredient                  | INCI name           | Function          | Formulation (%w/w) |
|-----------------------------|---------------------|-------------------|--------------------|
| Part A                      |                     |                   | WN4 | WN5 | WN14 |
| DI water*                   | Aqua                | Solvent           | 70 | 67.5 | 65   |
| Part B                      |                     |                   |                | |
| Coconut oil                 | Cocos Nucifera (Coconut) Oil | Emollient | 5 | 5 | 5 |
| Span 80                     | Sorbitan Oleate     | Emulsifier        | 2.5 | 5 | 7.5 |
| C. aromatica extract        | –                   | Active ingredient | 7.5 | 7.5 | 7.5 |
| Tween 80                    | Polysorbate 80      | Emulsifier        | 15 | 15 | 20   |

* The amount of water added in the formula was just enough to make the formula equal to 100%w/w.
shown in Table 6. When CA extract was loaded in nanoemulsion, free radical scavenging activity of all formulations was significantly more potent than CA extract. The IC50 of free radical scavenging activity of all formulations was significantly ranging from 16.90 ± 1.00 to 22.80 ± 2.00 cP. The particle size and significantly increased in BN4, BN5, BN14 and WN14, the viscosity ranged from 5.74 ± 0.04 to 6.94 ± 0.06. While the viscosity was significantly increased in BN4, BN5, BN14 and WN14, the viscosity ranged from 5.74 ± 0.04 to 6.94 ± 0.06. However, the difference of the extracted yield from various ethan–water extraction techniques is dependent on several factors such as the solubility of plants compositions and solvent polarity (Goli et al., 2005; Li et al., 2009). The yields of phenolic content from natural sources by solvent extraction are strongly related to natural phenolic esters around 100 nm as shown in Fig. 3. These results demonstrated the nanoemulsion from coconut oil loaded CA extract from WN14 was highly stable after stability investigation.

4. Discussion

The highest percentage yield was from the extract by maceration with 50% ethanol (MTC50). From previous study, the crude extracts of Jasminum sambac Ait. from dried leaves were a significantly different extracted yield. The highest extracted yield was achieved by using ethanol at 50% (Poonpaiboonpipat et al., 2011). In this study, 50% ethanolic extraction by maceration technique may be a suitable condition for obtaining the highest percentage yield CA extract which contained polar and semi-polar compounds. However, the difference of the extracted yield from various ethanol–water extraction techniques is dependent on several factors such as the solubility of plants compositions and solvent polarity (Goli et al., 2005; Li et al., 2009). The yields of phenolic content from natural sources by solvent extraction are strongly related to the solvent polarity (Jayaprakasha et al., 2008). Natural phenolic compounds gave a high solubility in intermediate polarity solvents such as acetone and alcohols rather than more polar solvent such as water. The previous reports found that the high molecular weight phenols were able to be extracted by hydro-alcoholic mix-

Table 6
Free radical scavenging and protein inhibition of nanoemulsion loaded C. aromatica extract.

| Sample | Free radical scavenging IC50 (mg/ml) | Folds of CA extract | Folds of L-ascorbic acid | Protein denaturation inhibition % | Folds of CA extract | Folds of Diclofenac |
|--------|-------------------------------------|--------------------|--------------------------|----------------------------------|--------------------|-------------------|
| BN4    | –                                   | –                  | –                        | –0.46 ± 0.44                     | –                  | –                 |
| BN5    | –                                   | –                  | –                        | –5.22 ± 0.10                     | –                  | –                 |
| BN14   | 0.18 ± 0.01*                        | 2.88               | 0.11                     | 16.35 ± 0.75*                    | 1.24               | 0.57              |
| WN4    | 0.12 ± 0.01*                        | 4.33               | 0.16                     | 16.25 ± 0.75*                    | 1.23               | 0.56              |
| WN14   | 0.14 ± 0.01*                        | 3.71               | 0.14                     | 12.05 ± 0.19*                    | 0.91               | 0.42              |
| CA extract in Tween80 | 0.52 ± 0.02 | – | 0.03 | 13.12 ± 0.19 | – | 0.45 |
| 1-ascorbic acid | 0.02 ± 0.01 | – | – | – | – | – |
| Diclofenac | – | – | – | 28.23 ± 0.14 | – | – |

Note: B = Blank; W = Wild turmeric (C. aromatica); N = Nanoemulsion; * = significant difference compared with CA extract in Tween80 (p value < 0.05).

3.6. Physicochemical and stability investigation of nanoemulsion loaded CA extract

Stability investigation of nanoemulsion loaded CA extract is shown in Table 7. Physical appearance after centrifugation showed good dispersibility in BN4, BN14, WN4 and WN14. However, phase separation was observed in BN5 and WN5 which had the same composition and proportion of components. The pH and viscosity values of all nanoemulsions were in the range of 5.87 ± 0.01 to 6.73 ± 0.04 and 3.11 ± 0.25 to 7.86 ± 0.24 cP, respectively. After 6 cycles of heating–cooling testing, the pH value was changed to 6.73 ± 0.04 and 3.11 ± 0.25 to 7.86 ± 0.24 cP, respectively. After stability investigation, all nanoemulsions loaded CA extract were significantly increased in particle size and changed PDI value as shown in Table 7. The percentage of protein denaturation inhibition of WN14, WN5 and WN4 was 12.05 ± 0.19, 16.25 ± 0.75 and 16.35 ± 0.79%, respectively. Based on the bioactivity investigation, nanoemulsion loaded CA extract was further observed physico-chemical characteristics and stability test by heating–cooling test for 6 cycles.

Table 7
Stability investigation of the nanoemulsion loaded C. aromatica extract.

| Sample  | Heating–cooling 6 cycles | Appearance after centrifuge |
|---------|--------------------------|-----------------------------|
|         | pH                      | Viscosity at 100 rpm (cP)   | Particle size (nm) | PDI           | Before | After | Before | After | Before | After | Before | After |
| BN4     | 6.73 ± 0.04             | 6.94 ± 0.06                 | 5.07 ± 0.37        | 22.80 ± 2.00  | 17.23 ± 0.12| 37.43 ± 0.13| 0.34 ± 0.01| 0.18 ± 0.01| Good dispersibility |
| BN5     | 6.66 ± 0.02             | 6.85 ± 0.01                 | 4.95 ± 0.13        | 25.46 ± 4.51  | 126.76 ± 0.64| 162.90 ± 1.21| 0.25 ± 0.01| 0.15 ± 0.01| Phase separation |
| BN14    | 6.70 ± 0.02             | 6.85 ± 0.02                 | 7.86 ± 0.24        | 42.70 ± 1.73  | 12.39 ± 0.27| 56.54 ± 0.32| 0.34 ± 0.01| 0.16 ± 0.02| Good dispersibility |
| WN4     | 6.03 ± 0.02             | 5.94 ± 0.05                 | 3.39 ± 0.18        | 4.30 ± 0.57   | 55.12 ± 0.24| 547.70 ± 24.41| 0.30 ± 0.02| 0.03 ± 0.03| Good dispersibility |
| WN5     | 6.19 ± 0.03             | 6.15 ± 0.02                 | 3.11 ± 0.25        | 5.95 ± 1.00   | 105.10 ± 0.34| 140.30 ± 10.78| 0.19 ± 0.01| 0.35 ± 0.09| Phase separation |
| WN14    | 5.87 ± 0.01             | 5.74 ± 0.04                 | 3.56 ± 0.24        | 16.90 ± 1.00  | 70.20 ± 0.38| 181.17 ± 8.70| 0.17 ± 0.01| 0.56 ± 0.06| Good dispersibility |

Note: B = Blank; W = Wild turmeric (C. aromatica); N = Nanoemulsion.
The largest group of naturally occurring phenolic compounds was flavonoids (Do et al., 2014). Phenolic and flavonoid compounds, contained at least one hydroxyl group in an aromatic ring (Tungmunnithum et al., 2018). It was found that high total flavonoid content was observed with extraction by alcoholic solvents such as methanol and ethanol in previous reports (Shrestha and Dhillion, 2006; Aryal et al., 2019). Moreover, flavonoid contents from plants extracted by aqueous ethanol were higher than extracted by aqueous acetone and aqueous methanol (Wang and Helliwell, 2001). In some plants, hydro-alcoholic and alcoholic extracts were determined total phenolic content using Folin-Ciocalteu’s reagent with gallic acid and total flavonoid content using aluminium chloride with quercetin. It was found that flavonoid content was higher than total phenolic content (Agbo et al., 2015; CI and Indira, 2016; Puangpronpitag et al., 2021). The other active compounds of phenolic and flavonoid compounds may be used and obtained the appropriated the amount of active compounds in their extracts (Vongsak et al., 2013). Therefore, the flavonoid content of CA extracts may be higher than total phenolic content. In this study, CA extract from maceration technique may retain high contents of total phenolic and flavonoid compounds because high temperature extraction might result in decreasing of the phytochemical compounds in some plants (Praychoen et al., 2013). Moreover, when the solvent polarity increased, the extracts decreased the free radical scavenging. A safe solvent for human consumption and frequently used for recovering polyphenolic compounds from plant matrices is ethanol (Dai and Mumper, 2010).

Limnophila aromatica extracted by a pure and aqueous organic solvent exhibited higher free radical scavenging activity than the water extracts (Do et al., 2014). The results of free radical scavenging activity not only depend on the method and conditions of extraction but also on the solvent used for extraction (Robards, 2003; Pinelo et al., 2004). The main compounds in CA rhizome extract isolated by column chromatography are a rich source of sesquiterpenes and curcuminoids which gave powerful antioxidative activity (Pintatum et al., 2020). Unsaturated fatty acids, linoleic acid and arachidonic acid, are abundant in lipids of membrane that are susceptible targets to degrade by lipid peroxidation (Yu, 2001). The lipid peroxidation and antioxidation inhibition of linoleic acid by plant extracts may be due to antioxidation by free radical scavenging (Rajpai et al., 2014). This ability to inhibit lipid peroxidation of plant extracts depends the characteristics of the antioxidant structure and the interaction with lipid bilayer (Salcedo et al., 2014). In various diseases, the inflammatory response has been well correlated with protein denaturation (Mizushima, 1966). Non-steroidal antiinflammatory drugs (NSAIDs) were pharmacological drugs commonly used in clinical management due to their capacity of anti-inflammatory and protein denaturation inhibition (Saso et al., 2001). The extraction with 95% ethanol may be a suitable condition to obtain an extract with...
high protein denaturation inhibition. Overproduced reactive oxygen species provoke an inflammatory response such as chemotactic factors, pro-inflammatory mediators and inflammatory amplification (Mittal et al., 2014). In fact, antioxidants found in phytochemical constituents of the extracts can reduce inflammatory reaction via the increase of the anti-inflammatory mediator and the inhibition of pro-inflammatory mediators (Moura et al., 2015). In previous reports, phytochemical constituents involved in anti-inflammatory effect are polyphenols, flavonoids and tannins. The effects of polyphenolic extracts from pomegranate on the inflammatory mediator production could be controlled through IL-8, PGE2 and NO synthesis, which could be mediated by a modulation of both NF-kB and ERK1/2 activation. The presence of flavonoids as active substances in Anacyclus clavatus extracts exerted anti-inflammatory effect by reduced ear edema and paw edema in rats. In coffee beans extract, the major polyphenolic compound like tannins exhibited concentration dependent anti-inflammatory activity by inhibiting protein denaturation throughout the concentration range of 31.25 to 1,000 μg/ml. For this effect, the presence of these phytochemical constituents probably regulated proteins denaturation, which is related to inflammation (Romier-Crouzet et al., 2009; Chandra et al., 2012; Bouriche et al., 2016). The plant extracts can be attributed in vitro anti-inflammatory activity by synergistic effects from their phytochemical constituents (Bhattacharya, 2011). For nanoemulsion preparation, the increasing concentration of non-ionic cosurfactant widely used in cosmetics, Tween 80 and Span 80, was able to adsorb to the oil–water interface, reduce the average diameter and prevent the aggregation of the internal droplets. During emulsification, the interfacial tension and internal droplet size of nanoemulsion had reduced more by the combination of surfactants. Moreover, the decreased particle size by the increasing amount of surfactant also influenced the stability of the internal droplets during the preparation process and storage (Chuacharoen et al., 2019). Additionally, all nanoemulsions loaded CA extract had an internal droplet size larger than blank nanoemulsions. It was found that the extract was loaded into the internal droplet of the nanoemulsions (Marsup et al., 2020). In bioactivities investigation, The CA extract loaded in nanoemulsions were significantly effective in free radical scavenging activity compared to CA extract in Tween80 at p value < 0.05. In addition, the formulation of CA extracted as a nano-sized delivery system seems to show greater potential in antioxidant activity compared to the crude extract as represented in Table 3 and Table 6. The synergistic antioxidative activity of nanoemulsion loaded CA extract may be enhanced by coconut oil used to form nanoemulsion. Coconut oil containing a high content of a medium-chain fatty acid was extensively used for cosmetic benefits on the skin such as moisturizing and antioxidant effects (Wuttikul and Boonme, 2016). Moreover, the antioxidant activity of the formulation was enhanced by surfactant contained in nanoemulsion by increasing the solubilization of lipid antioxidants into the water phase (Joung et al., 2016). However, all nanoemulsions loaded with CA extract showed no lipid peroxidation inhibition activity. This result might be due to the final concentration of CA extract being insufficient for inhibition reaction. It was related to the composition of the nanoemulsion formula, which had emulsifiers, water, and coconut oil. The saturated fatty acids in coconut oil might be a factor in the ineffectiveness of the lipidperoxidation test because these are typically oxidized. The oxidation of unsaturated fatty acids can inhibit by adding antioxidants into the system. In addition, the efficiency of antioxidant processes depends on the concentration of antioxidants added (Costa et al., 2021). Phase separation was observed in some formulations. It could be described as the dispersed particles in both BN5 and WN5 were moved, collided, and eventually agglomerated to form larger particles with a mechanical force from high-speed centrifugation (Aswathanaryan and Vittal, 2019). The particle size of all nanoemulsions (loaded and unloaded CA extract) in this study increased significantly (p value < 0.05) after completion of the heating and cooling cycles at 6 cycles (Fig. 2). The result is to increase the viscosity of the nanoemulsion. These phenomena might be related to the destabilization of nanoemulsion when kept at different temperatures in heating–cooling testing. The previous study also reported that the different temperatures affected the enlarging of the dispersed particle and resulted in an increasing viscosity via reformation which provided instability including flocculation, coalescence or Ostwald ripening (Nejadmansouri et al., 2018). After completing the stability testing cycle, a significant increase at p value < 0.05 in the mean diameter of the dispersed particles was observed. It was explained by the Ostwald ripening phenomenon. Higher temperatures had induced to dissolve the small particle in nanoemulsion. Then, the reformation to larger droplets began when the temperature cooled. In addition, storage time was another major factor causing Ostwald ripening (Khalid et al., 2017; van Westen and Groot, 2018; Liu and Hu, 2020).

5. Conclusions

This study demonstrated that CA extract from maceration with 95% ethanol (MTC95) exhibited the high phenolic and flavonoid content. The IC50 values of free radical scavenging and lipid peroxidation inhibition and the percentage of protein denaturation inhibition were 0.55 ± 0.02 mg/ml, 0.60 ± 0.10 mg/ml and 65.97 ± 4.68%, respectively. MTC95 was selected to prepare nanoemulsion which consisted of DI water, Tween 80, coconut oil and Span 80. The appearance of nanoemulsion loaded CA extract was an opaque yellow liquid with no precipitation and no phase separation at room temperature. The particle size, pH value and viscosity of WN14 formulation which showed the most stable physicochemical characteristics were 70.20 ± 0.38 nm, 5.87 ± 0.01 and 3.56 ± 0.24 cP, respectively. The formulation gave the IC50 value of free radical scavenging and the percentage of protein denaturation inhibition at 0.14 ± 0.01 mg/ml and 12.06 ± 0.18%, respectively. WN14 formulation also showed small particle size and good dispersibility after stability investigation by heating–cooling test for 6 cycles and centrifugation test. This result has demonstrated the potential of nanoemulsion from coconut oil loaded CA extract for further development to novel cosmetic products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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