INVESTIGATION OF IN VITRO ANTIOXIDANT ACTIVITY AND CYTOTOXICITY ON HUMAN SKIN FIBROBLASTS OF PLUCHEA INDICA (L.) LESS. LEAVES EXTRACT AND DEVELOPMENT TO MICROEMULSION FOR SKIN COSMETIC PRODUCT

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ABSTRACT

Objectives: The aim of this study was to determine the antioxidant activity and cytotoxicity on normal human skin fibroblasts of Pluchea indica (L.) Less. leaves extract and to prepare the extract into microemulsions for application in skin cosmetic ingredient.

Methods: The total phenolics and total flavonoids content in ethanolic (30%, 70%, and 95% ethanol) and aqueous extracts of P. indica leaves were determined by Folin-Ciocalteu assay and aluminum chloride method. The quantification of 4,5-O-dicaffeoylquinic acid in the extracts was determined by an ultra-high performance liquid chromatography method. The antioxidant activity of extracts was examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays. The cytotoxicity on human fibroblast cell was tested by sulforhodamine B assay. The oil in water microemulsion was prepared by pseudoternary phase diagram. The characterization of prepared-microemulsion including morphology, particle size, and polydispersity index (PDI) was studied and the physicochemical stability of microemulsions was evaluated.

Results: All P. indica leaves extracts contain a phenolic, flavonoid, and 4,5-O-dicaffeoylquinic acid contents. The 70% ethanolic extract possessed higher the antioxidant activities than 30% ethanolic, aqueous, and 95% ethanolic extracts, respectively, followed by DPPH, ABTS, and FRAP assay. Thus, the 70% ethanolic extract had no cytotoxicity on dermal human skin fibroblasts cell. The microemulsion loaded extract provided a mean particle size of 24.397±1.20 nm with minimum PDI of 0.397±0.05 which shown a spherical shape. The microemulsion loaded extract showed a good stability.

Conclusion: From all results, it has concluded that the 70% ethanolic extract of P. indica leaves contain phenolic, flavonoid, and 4,5-O-dicaffeoylquinic acid contents which possessed DPPH, ABTS, and FRAP activities. In addition, this extract had no cytotoxic to normal human skin fibroblasts. Finally, the microemulsion loaded extract showed a good characterization and physicochemical stability.

Keywords: Pluchea indica leaves, Antioxidant activity, Cytotoxicity. Microemulsion.

INTRODUCTION

Free radicals are atoms or molecules which have an unpaired electron, at least 1 electron. Free radicals are unstable and reactive with neighboring molecules to make themselves more stable. As chain reaction, the neighboring molecules which lose or accept electron to become a new free radicals and then they will further react with another molecule to produce the new free radicals [1]. According to the ability to oxidize compounds, the free radicals are known as reactive species (RS). The RS are classified into two types: reactive oxygen species (ROS) including superoxide anion and hydroxyl radical and reactive nitrogen species (RNS). ROS are produced by both intrinsic and extrinsic factors effecting on skin disorder such as skin aging and skin hypigmentation. Under normal conditions, ROS are eliminated by the antioxidant defense systems such as antioxidant enzymes (gluthathione [GSH] peroxidase, superoxide dismutase, and catalase) and non-enzymatic factors (Vitamin E, Vitamin C, GSH, uric acid, and ubiquinol) [2]. Whereas under abnormal conditions, the balance between the generation and elimination of ROS is broken, as a results of biomacromolecules; DNA, membrane lipids, and proteins are damaged by ROS-mediated oxidative stress [3]. These event can suppress by antioxidants [4].

In recent years, there are many researches investigation of new and safe antioxidants from natural sources to inhibit free radicals. Therefore, the extract from natural products with an anti-oxidant properties has been searched to treat a variety of skin conditions. Flavonoids are which mostly found in natural products. Many research found that flavonoids exhibit strong antioxidant activity in several mechanisms [5]. Pluchea indica (L.) Less., namely, Khu in Thai, belongs to Asteraceae family. This plant is an evergreen large shrub found abundantly in salt marshes and mangrove swamps in several countries in Asia including Thailand. P. indica has been used as a traditional medicine to treat diabetes, gallsstone, hemorrhoids, fever, and to heal wounds. Moreover, the dried leaves are prepared in herbal tea as a health-promoting drink available in Thailand [6]. According to the previous studied found that leaves extract of P. indica are also showed a natural antioxidant [7-9] because they contain high amount of flavonoids and phenolic compounds; caffeoylquinic acid derivative (3-O-caffeylquinic acid, 5-O-caffeylquinic acid, 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid, and 4,5-O-dicaffeoylquinic acid) [10-11], and flavonol aglycones (quercetin, kaempferol, and myricetin) [12]. Others pharmacologically, they have been shown anti-inflammatory [13], hypoglycemic and anti-hyperglycemic activities [14], antibacterial [9], anti-alpha glucosidase [10] and anti-collagenase, MMP-2, and MMP-9 [15].

Microemulsions are one of the cosmetic delivery systems which can be defined as being optically isotropic and transparent in oil or water dispersions with diameters slightly greater than swollen micelles. The mean particle sizes of microemulsions are in the range of 10–200 nm. A microemulsion system consisted of water, oil, surfactant, and...
co-surfactant that all are thermodynamically stable with low viscosity and Newtonian behavior. These components are usually optimized using pseudoternary phase diagrams which present the suitable amount of oil, water, and surfactant mixtures. Then, the microemulsion region can be identified in the phase diagram. Microemulsion has been reported to enhance the solubility and oxidation stability of ascorbyl palmitate. Therefore, microemulsion systems present attractive skin and hair formulations that can be used not only to investigate improvements in the stability of extract but also the rate of delivery of its active compounds through the skin barrier, thus obtaining better biological results [16,17].

Therefore, this present study was reported the investigation of the antioxidant activities including the cytotoxicity data from *P. indica* leaves extract used as a natural active ingredient for skin cosmetic industry. The antioxidant activities; 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS+), and ferric reducing antioxidant power (FRAP) assays of the *P. indica* leaves extract were investigated. In addition, the cytotoxicity of the extract was tested on normal human skin fibroblasts. Afterward, microemulsion loaded extract of *P. indica* leaves was prepared and all characterizations were also studied. Finally, the physicochemical stability of prepared microemulsion was determined.

**METHODS**

**Chemicals and reagents**

The chemicals used were analytical reagent grade: ABTZ (ABTZ, Sigma-Aldrich USA), DPPH (DPPH, Sigma-Aldrich USA), Trollos (Sigma-Aldrich USA), Folin-Ciocalteu phenol reagent (Sigma-Aldrich USA), Sodium carbonate (Na CO₃, Univar Ajax Finechem New Zealand) Aluminum chloride (AlCl₃, Univar Ajax Finechem New Zealand), Potassium acetate (CH₃COOK, Univar Ajax Finechem New Zealand), Hydrochloric acid (Lab Scan Thailand), Ethanol (Lab Scan Thailand). HPLC grade: 4,5-O-dicaffeoylquinic acid (Sigma-Aldrich USA), Quercetin (Sigma-Aldrich USA), Gallic acid (Sigma-Aldrich USA), Methanol (Lab Scan Thailand), Phosphoric acid (Lab Scan Thailand), and Cosmetic grade: Tween 80 and propylene glycol (Namsiang Thailand).

**Preparation of *P. indica* less. leave extract**

The mature leaves of *P. indica* were collected in June 2018 from Chantaburi Province, Thailand and authenticated by the Department of Agriculture, Kasetsart University, Thailand. All fresh leaves were cleaned with tap water and dried in a hot air oven at 60°C for 24 h. The dried leaves were ground using a grinder. The powdered leaves were kept in a sealed containers protected from light until use. Extracts of *P. indica* leaves were prepared by a maceration method in various concentrations of ethanol including 0% (DI water), 30%, 70%, and 95%. Briefly, 10 g of dried leaves powder were macerated in 100 ml of each solvent and then kept at room temperature for 24 h. The resulting solvent was then filtered through a Whatman No.1 filter paper. The marc was re-extracted again in the same method and done in triplicates. The pooled filtrate was concentrated using a vacuum rotary evaporator at 45°C. Each crude extract was weighed and kept in a tight container protected from light in refrigerator. The yield of each extract was calculated using the following equation:

\[ \text{Yield} \% = \left( \frac{W1 \times 100}{W2} \right) \]

where *W1* is the weight of *P. indica* leaves extract, and *W2* is the weight of the dried *P. indica* leaves powder.

**Quantitative assays**

**Total phenolic content**

Total phenolic content of *P. indica* leaves extracts was determined by a Folin-Ciocalteu method [18], with slightly modification. Briefly, 500 µl of extract solution was mixed with 500 µl of Folin-Ciocalteu reagent and 10 ml of 7% sodium carbonate (Na CO₃) were added and vortex. After incubation for 60 min at room temperature, the absorbance of the incubated solution was measured at 760 nm. The amount of total phenolic was expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g extract) from the calibration curve. This assay was performed in triplicate.

**Total flavonoid content**

Total flavonoid content of *P. indica* leaves extracts was assessed based on the formation of a complex flavonoid aluminum as previously studied [19], with slightly modification. Briefly, 100 µl of 2% aluminum chloride (AlCl₃) was mixed with the same-volume of extract solution. Absorption measuring at 415 nm was read after incubation for 10 min at room temperature. The amount of total flavonoids was expressed as mg of quercetin equivalent per gram of extract (mgQE/g extract) from the calibration curve. This assay was done in triplicate.

**4,5-O-dicaffeoylquinic acid content**

The quantification of 4,5-O-dicaffeoylquinic acid in the *P. indica* extracts was determined using a ultra-high performance liquid chromatography (UHPLC) method. The separation was done in a HypURITY C18 column (150 × 3 mm, 3 µm), mobile phases consisted of (A) 0.1% phosphoric acid in water and (B) 0.1% phosphoric acid in methanol using a gradient elution and the flow rate was set at 0.8 ml/min with the controlled temperature at 30°C. DAD 190–800 nm detector was set at the wavelength of 330 nm and injection volume was 2.0 µl for sample and reference standard (4,5-O-dicaffeoylquinic acid).

**In vitro antioxidant activities of *P. indica* leaves extracts**

**DPPH assay**

The free-radical scavenging activity of *P. indica* leaves extracts was determined using DPPH assay as previously described [20,21]. Briefly, 150 µl of 0.2 mM DPPH solution was then mixed with 75 µl of various concentrations of the extract solution. The mixture was kept at room temperature for 30 min in the dark, and then the absorbance at 515 nm was measured using a microplate reader. This study was compared with the positive controls, L-ascorbic acid, and trolox. The radical scavenging activity was calculated as a percentage of DPPH decoloration using the following equation:

\[ \text{DPPH radical scavenging activity} \% = \left[ 1 - \left( \frac{A_{S}}{A_{B}} \right) \right] \times 100 \]

where *Aₜ* was an absorbance of DPPH with the tested sample and *Aₜ* was an absorbance of DPPH without the tested sample. The concentration providing 50% inhibition (ICₕ) was calculated from the graph of inhibition percentage plotted against the samples concentration. The study was run in triplicate.

**ABTS⁺ assay**

The radical scavenging activity of *P. indica* leaves extracts was assessed spectrophotometrically by ABTS radical cation decolorization assay as previously studied [22], with slightly modification. Briefly, 7 mM of ABTS solution was mixed with 2.45 mM of potassium persulfate to produce an ABTS radical cation. After that, the mixture was kept in the dark at room temperature for 12–16 h. Then, the ethanol was used to dilute the ABTS+ solution to an absorbance of 0.70±0.02 at 734 nm. The aliquot (25 µl) of various concentrations of the extract solution was added to 2 ml of ABTS+ solution. The absorbance was taken at 734 nm using a microplate reader. Trolox and L-ascorbic acid was used as a reference compounds. The radical scavenging activity was calculated as a percentage of ABTS+ decolorization using the following equation:

\[ \text{ABTS⁺ radical scavenging activity} \% = \left[ 1 - \left( \frac{A_{S}}{A_{B}} \right) \right] \times 100 \]

where *Aₜ* is an absorbance of ABTS+ with the tested sample and *Aₜ* is an absorbance of ABTS+ without the tested sample. The concentration providing 50% inhibition (ICₕ) was calculated from the graph of inhibition percentage plotted against the samples concentration. The study was done in triplicate.
**RESULTS**

**Yield of crude extract, total phenolic, and flavonoid contents of extracts**

All *P. indica* leaves extracts were dark-brown semisolids. The yields, total phenolic, and total flavonoid contents of each extract are shown in Table 1.

The yield results showed that the maximum value was obtained by maceration with 30% (v/v) ethanol which was 25.41% (w/w). The yield then tended to decrease when the ethanol concentration was 70% and 95%, respectively.

The content of total phenolic compound in the extracts was calculated with the calibration curve equation of gallic acid (0.025–0.5 mg/ml, Y = 5.2234x + 0.1618, R² = 0.9976). The highest total phenolic content was obtained from a 30% ethanolic extract followed by 70% ethanolic extract, aqueous extract and 95% ethanolic extract as 175.91±4.06, 174.92±8.46, 126.58±3.59, and 54.61±0.53, respectively.

The amount of total flavonoid compounds of *P. indica* leaves extract was calculated with the calibration curve equation of quercetin (0.039–0.625 mg/ml, Y = 1.8012x + 0.1369, R² = 0.9995) which found that 95% ethanol had a higher amount of total flavonoids (355.19±3.78 mgGAE/g extract), followed by 70% ethanolic extract (344.83±10.19 mgGAE/g extract), aqueous extract (271.73±9.87 mgGAE/g extract), and 30% ethanolic extract (270.99±12.50 mgGAE/g extract), respectively.

**4,5-Dicaffeoylquinic acid content**

Caffeoylquinic acid (CQ) derivatives; 3-O caffeoylquinic acid (3-CQ), 4-O caffeoylquinic acid (4-CQ), 5-O caffeoylquinic acid (5-CQ), 3,4-D caffeoylquinic acid (3,4-CQ), 3,5-D caffeoylquinic acid (3,5-CQ), and 4,5-D caffeoylquinic acid (4,5-CQ) were previously reported as the major constituents in *P. indica* leaves which quantified in *P. indica* leaves extracted using HPLC. They studied found that *P. indica* leaves extract had higher 4,5-D caffeoylquinic acid content than others caffeoylquinic acids [11]. Thus, this present study focused on the quantification of 4,5-D caffeoylquinic acid in *P. indica* leaves extracts according to UHPLC. The chromatograms of 4,5-D caffeoylquinic acid standard compound and *P. indica* leaves extracts are shown in Fig. 1.

From the chromatograms, the content of 4,5-D caffeoylquinic acid in *P. indica* leaves extracts was in the range of 2.202–9.359 µg/ml which was calculated with the calibration curve equation of 4,5-D caffeoylquinic acid (500–1500 µg/mL, Y = 637.1±6.95, R² = 0.9995) at 330 nm. Results found that 70% ethanolic extract (9.359 µg/ml) showed the higher amount of 4,5-D caffeoylquinic acid than 30% ethanolic extract (5.205 µg/ml), aqueous extract (2.213 µg/ml), and 95% ethanolic extract (2.202 µg/ml), respectively. This result was similar to total phenolic content resulting (Table 1).

**Table 1: Yield of crude extracts, total phenolic, and flavonoid contents of extracts**

| Extract             | Yield (w/w) | Total phenolic content (mgGAE/g extract) | Total flavonoid content (mgGAE/g extract) |
|---------------------|-------------|----------------------------------------|-----------------------------------------|
| Aqueous extract     | 11.99       | 126.58±3.59                            | 27.17±3.98                              |
| 30% ethanolic extract | 25.41       | 175.91±4.06                            | 27.09±12.50                             |
| 70% ethanolic extract | 14.70       | 174.92±8.46                            | 344.83±10.19                            |
| 95% ethanolic extract | 11.87       | 54.61±10.53                            | 355.19±3.78                             |

The total phenolic and total flavonoid contents values are given as mean±standard deviation (n=3). Superscript (*) in the same column indicate significant differences (p<0.05; Student’s t-test).
Antioxidant activity

The concentration providing 50% inhibition (IC_{50}) values from DPPH and ABTS•+ radicals scavenging and FRAP value of P. indica leaves extracts and positive controls are presented in Table 2.

The DPPH and ABTS•+ assays are widely used methods for the determination of the antioxidant activity of natural products, they both are spectrophotometric techniques based on quenching of stable colored radicals (ABTS•+ or DPPH) and show the radical scavenging ability of antioxidants even when present in complex biological mixtures such as plant extracts [25]. IC_{50} is the concentration of the samples required to inhibit 50% of free radical. Thus, the lower in IC_{50} value indicated that the higher of antioxidant activity. In this study, the 70% ethanolic extract possesses strong antioxidant activities based on DPPH and ABTS•+ assays as IC_{50} value of 0.17±0.01 mg/ml and 2.04±0.03 mg/ml respectively. This extract showed higher activities than other extracts.

The antioxidant activity of P. indica leaves extracts was accordingly estimated by FRAP assay, which is presenting of the sample reduction of Fe(III)/TPTZ complex to the ferrous form (blue color). This study, the 70% ethanolic extract showed the highest amount of ferric reducing power expressed as trolox (263.31±4.39 mgTE/g extract), followed by 30% ethanolic extract (250.17±6.06 mgTE/g extract), aqueous extract (157.62±2.47 mgTE/g extract), and 95% ethanolic extract (42.26±1.76 mgTE/g extract), respectively.

Table 2: Antioxidant activities of Pluchea indica leaves extracts

| Samples            | DPPH radical IC_{50} (mg/ml) | ABTS•+ radical IC_{50} (mg/ml) | FRAP assay (mgTE/g extract) |
|--------------------|------------------------------|-------------------------------|----------------------------|
| Aqueous extract    | 0.21±0.08                    | 2.76±0.04                     | 157.62±2.47*               |
| 30% ethanolic extract | 0.18±0.01                  | 2.28±0.05*                    | 250.17±6.06*               |
| 70% ethanolic extract | 0.17±0.01                  | 2.04±0.03*                    | 263.31±4.39*               |
| 95% ethanolic extract | 0.81±0.05*                  | 10.68±0.51*                   | 42.26±1.76*                |
| L-ascorbic acid    | 0.0178±0.0001*               | 0.57±0.02*                    | -                          |
| Trolox             | 0.023±0.01*                  | 0.0065±0.0005*                | -                          |

Values are given as mean±S.D of triplicate. Superscript (*) in the same column indicate significant differences (p<0.05; Student’s t-test)

Cytotoxicity to normal HDFS cell

From all the results as above, the 70% ethanolic extract was selected for investigation of cytotoxicity to normal HDFS cell. The obtained results are expressed as percentage of cell viability according to SRB assay and are shown in Fig. 2.
The result indicated that the P. indica extract at all concentrations used (0.001–1.0 mg/ml) had no effect on the viability of the human skin fibroblasts which was the percentage of in the range of 96.84–97.13. This demonstrated that the P. indica leaves extract was not toxic to human skin fibroblast in vitro, whereas sodium lauryl sulfate, positive control, showed statistically significant (p < 0.05) toxicity on normal human skin fibroblast at 0.1 and 1.0 mg/ml and it gave the percentage of cell viability were 32.28 and 8.10, respectively.

**Microemulsion preparation**

Pseudoternary phase diagram was used to prepare oil in water microemulsion. The composition of microemulsion was selected from the pseudoternary phase diagram that gave the largest microemulsion area as shown in Fig. 3. Optimal microemulsion formula consisted of oleic acid (1%w/w), tween 80 (20%w/w), propylene glycol (5%w/w), and deionized water (74%w/w).

**Physicochemical characterization of prepared microemulsion**

The physical appearance of free microemulsion was a yellowish transparent liquid and dark green transparent liquid for microemulsion loaded 0.085%w/w of P. indica leaves extract as shown in Fig. 4. Table 3 shows the color (L*, a*, and b*) and pH values of free microemulsion and microemulsion loaded the P. indica leaves extract. The result found that after loaded the extract into microemulsion, the 

**DISCUSSION**

The effective extraction and proper assessment of antioxidants from medicinal plants are crucial to explore the potential antioxidant sources and promote the application in pharmaceuticals and/or cosmetic fields. In this study, P. indica leaves extracts were obtained using water and ethanol with different concentrations; aqueous (100% water), 30% ethanol, 70% ethanol, and 95% ethanol. The extraction resulting

**Fig. 3: Pseudoternary phase diagram of microemulsion**

**Fig. 4: The physical appearance of (a) microemulsion loaded extract and (b) free microemulsion**

L* and b* values were significantly changed (p < 0.05), excepted a* value which was not significantly changed (p > 0.05), similar to the evaluating by visual as Fig. 4 which showed that the color of microemulsion loaded extract appeared dark green. The pH values of both formulations are suitable for topical application when their pH values are in the range of 5–8 [26].

The obtained results of droplet size, PDI, and viscosity of free microemulsions and microemulsion loaded extract are shown in Table 4. After loaded the extract into microemulsion, the results showed that the mean droplet sizes, PDI, and viscosity were significantly increased (p < 0.05).

Fig. 5 presents the mean droplet sizes and distribution of free microemulsions and microemulsion loaded extract determined by nanoparticle analyzer. This results showed the increasing tendency of the mean droplet size and distribution after the extract was loaded into microemulsion.

The morphology of microemulsion loaded extract assessed by TEM. The result indicated the spherical shape of internal droplet sizes of microemulsion loaded P. indica leaves extract as shown in Fig. 6.
showed the crude extract obtained by a 30% ethanol found the highest percent yield. This might due to the combination between water and ethanol serve the extraction of compounds that are soluble in water and ethanol in *P. indica* leave such as phenolic acid and flavonoid compound. Other compounds may have been extracted and contribute to higher yield.

The content of total phenolic compound in the extracts was calculated with the calibration curve equation of gallic acid based on Folin–Ciocalteu assay. The extract obtained by a 30% ethanol had a highest amount of total phenolic. This demonstrated that the total phenolic content in *P. indica* leaves extract might contained mainly of caffeoylquinic acid (CQ) derivatives; 3-O-caffeoylquinic acid (3-CQ), 4-O-caffeoylquinic acid (4-CQ), 5-O-caffeoylquinic acid (5-CQ), 3,4-dicaffeoylquinic acid (3,4-CQ), 3,5-dicaffeoylquinic acid (3,5-CQ), and 4,5-dicaffeoylquinic acid (4,5-CQ) [11]. This studied extract had higher content of total phenolic compound than previously reported [7,8,27,28].

The amount of total flavonoid compounds of *P. indica* extracts were calculated with the calibration curve equation of quercetin determined by aluminum chloride assay. Obtained resulted found that 95% ethanolic extract had the highest amount of total flavonoids. These implied that this ethanol-water (5%) solvent could extracted the flavonoid compounds from *P. indica* leaves such as quercetin, kaempferol, and myricetin more than other solvents which contain higher percentage of water. These resulted were similar to previously reported [12]. In addition, this present showed a higher a flavonoids contents than previous studied [27,28].

The present report concluded that *P. indica* leaves extract had higher amount of total phenolic and flavonoids than previous studied may...
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Fig 6: Transmission electron microscope of microemulsion loaded the Pluchea indica leaves extract

be attributed to differences in extraction or the aforementioned pre-harvest factors such as climate, geography, or agronomic practices.

The quantification of 4,5-O-dicaffeoylquinic acid in P. indica leaves extracts according to UHPLC. Results found that the extract obtained by 70% ethanol had the highest content of the major compound, 4,5-O-dicaffeoylquinic acid. This result was similar to total phenolic content resulting.

Antioxidant properties of P. indica leaves extracts were determined through different methods including DPPH, ABTS, and FRAP methods. Our results found that the 70% extract showed a higher DPPH and ABTS’ radical scavenging activity than other extracts as well as FRAP values, and all extracts showed lower than reported previously [27,28]. However, free-radical scavenging activity of the extract that obtained in this study may result from high phenolic and flavonoid contents. Thus, the antioxidant properties of natural extract may be attributed to the main components of herbal extract which may act as an antioxidant [29]. All finding together indicated that the 70% ethanolic extract contained high level of phenolic, flavonoids and 4,5-O-dicaffeoylquinic acid, and it demonstrated the highest antioxidant activities. Therefore, this extract was selected to further study for applied in skin cosmetic products.

The cytotoxicity of 70% ethanolic extract to normal HDGF cell was investigated by SRB assay. Our study showed that the P. indica extract had no effect on the viability of the human skin fibroblasts. This demonstrated that the extract was not toxic to human skin fibroblast in vitro.

The physiochemical characterization of free microemulsion and microemulsion loaded extract; the color, pH values, viscosity, droplet size, and PDI were studied. Resulted the \(L^*, a^*, b^*\) values and \(\Delta \text{E}\) values were similar to evaluating by visual which showed that the color of microemulsion loaded extract appeared dark green. The extract did not affect the \(pH\) values. The \(pH\) values of both formulations were suitable for topical application when their \(pH\) values are in the range of 5-8 [26]. The extract incorporation did significantly affect the viscosity of unloaded microemulsion (\(p < 0.05\)) but it was found that both prepared-microemulsion exhibited Newtonian behavior since their viscosity values were not changed by increasing the shear rate (rpm).

The extract incorporation affected the droplet size and PDI of microemulsion (\(p < 0.05\)). This results based on the solubility of the extract, it might mainly be localized in the external phase and surfactant layer between internal phase and external phase of the microemulsion. This might have resulted in larger droplet size. PDI value describes the homogeneity of the droplet size. This resulted were similar to the previous studied [30]. Both PDI values were smaller than 0.5. Therefore, these results indicate that droplet sizes have appropriate homogeneity.

The homogeneity of the microemulsions remained clear; the sedimentation and phase separation did not occur due to the high stable of microemulsions after stability testing. The physicochemical stability of microemulsion loaded the extract was performed under tested conditions. The viscosity value did not significantly change under test conditions (\(p > 0.05\)). This implies that the microemulsion loaded extract exhibited Newtonian flow behavior. The mean droplet size and PDI were a tendency of decreasing; however, it was still in the range of microemulsions formation. The color did significantly change in HC and room temperature conditions (\(p < 0.05\)), but it did not affect to microemulsion form. The \(pH\) values significantly decreased (\(p < 0.05\)). This might be affect from the acidic compounds in the extract released to external phase. For free microemulsion was stable at all storage conditions (data not show).

CONCLUSION

The P. indica leaves extract which extracted by 70% ethanol found that it contained high a phenolic, flavonoid and 4,5-O-dicaffeoylquinic acid contents which possessed DPPH, ABTS, and FRAP activities. In addition, this extract also showed a complete lack of any toxicity toward normal human skin fibroblasts. The optimal microemulsion formula consisted of the 70% ethanolic extract of P. indica leaves (0.085%), oleic acid (1.0%), tween 80 (20.0%), propylene glycol (5.0%), and deionized water. The microemulsion loaded the extract showed a good stability under all conditions tested. All finding indicated that P. indica leaves extract can be prepared in microemulsions as a carrier for the herbal extract delivery system. Further investigation will evaluate skin penetration and skin irritation of microemulsion loaded P. indica leaves extract along with the performance of clinical trials in skin cosmetic products.

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AUTHORS’ CONTRIBUTIONS

Khunjit Itsarasook analyzed the laboratory work, analyzed the data, and wrote the manuscript. All authors read and approved the manuscript and especially edited and final revised by Dr. Piyanuch Prompamorn.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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