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Extraction of *Coccinia grandis* leaf for development of insect bites cream formulation

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Abstract

Coccinia grandis (L.) Voigt, also known as Ivy gourd, is a plant commonly found in Thailand. It has been used in primary health care for relieving inflammation from insect bites. The fresh leaves are crushed and applied on the affected area, that is inconvenient. Therefore, this research aimed to extract the leaf of *C. grandis*, to evaluate the *in-vitro* anti-inflammatory activity of the extracts, and to formulate a topical cream for insect bites treatment. The dried powder of plant sample was extracted with 90% ethanol using ultrasonic bath, then the extract was sent to liquid-liquid extraction with hexane/water. Anti-inflammatory activity of these extracts was evaluated by inhibiting the breakdown of the bovine serum albumin method compared with a drug, diclofenac sodium. Ivy gourd cream was formulated and evaluated for its physical properties and stability. The results showed that hexane fraction exhibited potent anti-inflammatory activity (87.44%) which was more potent than diclofenac sodium (27.23%). Two concentrations (1% and 5%) of Ivy gourd cream were formulated. Both formulas had soft, creamy texture with smooth and shiny surface. The skin was slightly yellow when applied the cream, but it could be easily washed out. The freeze-thaw stability study indicated that these creams had good stability.

Keywords : Coccinia grandis, β-sitosterol, anti-inflammation, Ivy gourd cream, insect bites

1. Introduction

Coccinia grandis (L.) Voigt is known as Ivy gourd. It is a member of the Cucurbitaceae family in the genus Coccinia. The fresh leaf is used as a medicinal plant in Thailand for relieving inflammation from insect bites. Several pharmacological activities of the plant extract have been reported such as antioxidant, antihyperglycemic, anti-ulcer, antimicrobial, and anti-inflammatory activities (Pekamwar, 2013, p.54-58, Amir Hossain, 2014, p.65-71, Varuna, 2018, p.188-200). *β*-sitosterol is a phytosterol compound which was found in many parts of ivy gourd, including leaf (Alagarraja, 2017, p.54-58). The strong anti-inflammatory activity of *β*-sitosterol was observed by membrane stabilization of human red blood cell and inhibition of protein denaturation methods which indicated that *β*-sitosterol promoted the neutralizing lysosomal enzymes or stabilizing the lysosomal membrane (Ushir Yogrsh, 2013, p.85-89). According to Thai traditional medicine, fresh leaves of *C. grandis* are crushed and applied on affected area which is inconvenient. Therefore, Ivy gourd extract was formulated as cream for ease of use and promoting the use of Thai herb.

2. Objectives

The objectives of the study are to evaluate the *in-vitro* anti-inflammatory activity of the leaf extracts of *C. grandis* and to formulate a topical cream for insect bites treatment.

3. Materials and methods

3.1 Chemicals

Anisaldehyde, bovine serum albumin, *β*-sitosterol, and dimethyl sulfoxide were purchased from Sigma-Aldrich. Diclofenac sodium was obtained from Henan Dongtai Pharmaceutical Co., Ltd. Dichloromethane, ethanol, ethyl acetate, hexane, methanol, and sulfuric acid are analytical grade reagents.

3.2 Plant material

Fresh *C. grandis* was purchased from Thipgasorn market, the local market at Bang Phli district, Samut Prakan province, Thailand. The plant was identified and compared to virtual herbarium (TCD0017100) in JSTOR Plant Science.

3.3 Preparation of plant extracts for qualitative analysis

The size of fresh leaves used in the study was 6-13 cm wide and 4-10 cm long. The fresh leaves were washed with tap water and dried at 50°c using hot air oven before grounded into coarse powder by blender. The plant powder (3 g each) was extracted with 60 mL hexane, dichloromethane, absolute ethanol, ethanol-water (9:1), or water using ultrasonic bath for 30 minutes at room temperature, then each extract was filter and dried in vacuum rotary evaporator to obtain CGHe, CGDi, CGEt, CGEW, and CGWa extracts, respectively. Each extract was redissolved to 10 mL volume with its solvent and subjected to qualitative analysis using thin-layer chromatography.

3.4 Condition of thin-layer chromatography

Chromatography was performed on 10 x 10 cm aluminum plates precoated with 0.2 mm layers of silica gel 60 F254 (E. Merck, Germany). Sample and standard solutions, each 10 μL, were applied on the plates as 2 mm wide bands, positioned 10 mm from lower edge of the plate. The mobile phase was dichloromethane-ethyl acetate 9:1 (v/v). Pre-saturated chamber (30 minutes at room temperature) was used for development of the plates with a distance of 95 mm from lower edge of the plate using ascending mode. The developed plates were detected with color reaction using anisaldehyde-sulfuric acid reagent, the color reaction occurred when heat at 105°c for 15 minutes. *β*-sitosterol (1.25 mg/mL) was used as reference standard.

3.5 Preparation of plant extracts for cream formulation

The plant powder (640 g) was extracted with 14.7 L ethanol-water (9:1) using ultrasonic bath for 2 hours at room temperature, then the extract was filtered. 8.7 L of the filtrate was dried in vacuum rotary evaporator at 40°c, 165-72 mbar to obtain CGEW-F. Another 6 L of the filtrate was sent to liquid-liquid extraction with hexane/water. The aqueous phase was separated and dried in vacuum rotary evaporator at 40°c, 65 mbar to obtain CGW-F. While hexane phase was dried in vacuum rotary evaporator at 40°c, 335 mbar to obtain CGH-F.

3.6 Albumin denaturation inhibition assay

Protein denaturation has been correlated with the inflammatory disorders. Several non-steroidal anti-inflammatory drugs (NSAIDs) such as, indomethacin, ibufenac, flufenamic acid, and salicylic acid enhanced the ability to prevent heat denaturation of bovine serum albumin (BSA) at pathological pH 6.2-6.5 (Grant, 1970, p.715-722).

The reaction mixture consisted of 0.9 mL of 5% bovine serum albumin, 2.8 mL of phosphate buffer saline pH 6.3, and 2.0 mL of 100 μg/mL of the extract. Diclofenac sodium was used as reference. The mixture was incubated at 37°C for 15 minutes then heated at 70°C for 5 minutes. Absorbance of the mixture was measured at 660 nm. The percentage inhibition of albumin denaturation was calculated using the equation as shown below.

%inhibition = [1 – (absorbance of sample/absorbance of control)] x 100

3.7 Formulation of *Coccinia grandis* cream

3.7.1 Development of cream bases

Five different cream bases were prepared. The oil phase consisted of stearic acid, cetyl alcohol, liquid paraffin, isopropyl palmitate, and Span 60 was melted at 75°C and stirred. The water phase consisted of propylene glycol, Tween 60, Tween 80, sorbitol, and distilled water was heated at 78°C. The water phase was poured into the oil phase with continuously stirring until the mixture congealed. Then preservative was added, and the mixture was stirred to become homogenous cream. The physical properties (color, odor, consistency, washability, and pH) of these cream bases and their stability using centrifugation test were evaluated. The cream base with good properties and stability was chosen to prepare *C. grandis* cream.

3.7.2 Preparation of *C. grandis* cream

The selected cream base was prepared using the same method described above (3.7.2). *C. grandis* extract was dissolved in liquid paraffin before added into the cream base to produce two concentrations (1% and 5%) of *C. grandis* cream.

3.8 Evaluation of physical properties and stability of *C. grandis* cream

3.8.1 Physical appearance

Physical appearance of cream was evaluated by observing its color, odor, and consistency.

3.8.2 Dye test

Lake blue color was mixed with cream. A drop of cream was examined under a microscope. The cream is o/w type if blue globules are appeared with colorless continuous phase. In contrast, the cream is w/o type if colorless globules disperse in blue continuous phase.

3.8.3 pH measurement

Cream (5 g) was dispersed in 50 mL distilled water and sent to measure pH value using pH meter at room temperature.

3.8.4 Viscosity measurement

The viscosity was determined using Brookfield viscometer model LVDV-II+Pro with T-bar spindle at speed of 12 rpm and 25°C for 1 minute.

3.8.5 Accelerated stability study using centrifugation test

The cream was centrifuged for 30 minutes at 3,500 rpm. Then it was inspected for signs of creaming.

3.8.6 Freeze-thaw stability study

The cream was stored in airtight glass containers and stability tests were performed on 4 cycles of temperature testing, -20°C (48 hours) and 45°C (48 hours). Then physical properties, viscosity and pH of cream were evaluated.

4. Results and discussions

The extraction of *C. grandis* leaves using 5 different solvents obtained CGHe, CGDi, CGEt, CGEW, and CGWa extracts. The chemical compositions of these extracts were investigated by TLC. *β*-sitosterol is a phytosterol compound which was found in many parts of *C. grandis* such as root, aerial parts, fruit, stem, and leaf (Alagarraja, 2017, p.54-58). Paniagua-Pérez (2017) reported a potent anti-inflammatory activity of *β*-sitosterol in acute inflammation in rodents using reversed passive Arthus reaction in the rat (an immune reaction) and mouse ear edema assay (a non-specific reaction). Therefore, *β*-sitosterol was used as a reference compound. TLC chromatograms (fig. 1) showed that *β*-sitosterol was found in CGHe, CGDi, CGEt, and CGEW extracts, except in CGWa extract. The chemical composition of CGEW extract was greater than that of any other extract. In addition, the solubility of *β*-sitosterol in some organic solvents was reported the increasing of solvent's polarity the decreasing of its solubility. However chemical structure of solvent also influenced the solubility. *β*-sitosterol has a hydroxyl group at 3-position of hydrophobic steroidal skeleton which makes it weakly polar. Moreover, hydrogen bond is an interaction between solvent and solute that provides the solvation of solute. The solubility of *β*-sitosterol in ethanol was higher than that in *n*-hexane (Wei, 2010, p.2917-2919). As a result, 90% ethanol was used as solvent for further extraction.

The plant powder (640 g) was extracted with 14.7 L of 90% ethanol using ultrasonic bath for 2 hours at room temperature to yield 124.95 g of CGEW-F extract (19.52% w/w of plant powder). The CGEW-F extract (50 g) was then liquid-liquid extracted with hexane/water to yield 20.64 g of CGW-F extract (7.79%w/w of plant powder) and 14.19 g of CGH-F extract (5.09% w/w of plant powder).

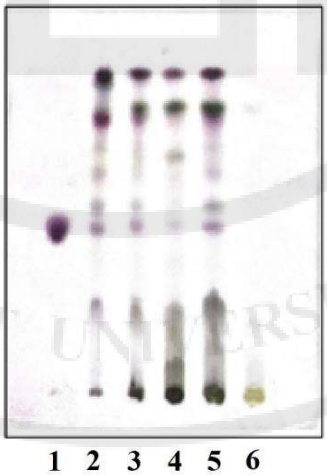

Figure 1 TLC chromatogram of *C. grandis* extracts. (1) *β* - sitosterol, (2) CGHe extract, (3) CGDi extract, (4) CGEt extract, (5) CGEW extract, and (6) CGWa extracts.

Anti-inflammatory activities of CGEW-F, CGW-F, and CGH-F extracts were evaluated by albumin denaturation inhibition assay and diclofenac sodium was used as

reference. At the concentration of 100 µg/mL, CGH-F extract presented 87.44% inhibition of albumin denaturation which was greater than diclofenac sodium (27.23%). Whereas CGEW-F and CGW-F extracts showed 6.39% and 21.06% inhibition, respectively. Accordingly, CGH-F extract was sent to formulate the *C. grandis* cream.

The composition of 5 cream bases (I-V) was shown in table 1. All cream bases were odorless, white, and easily removable by water. Cream base I leave a greasy feeling on the skin while II-V leave a little greasiness. Cream base II and III presented grittiness felt but IV and V did not. Cream base V showed the easiest spread on skin. The pH values of I-V were 5.01, 4.45, 4.43, 5.04, and 5.13, respectively. Phase separation was not observed after the centrifugation test of all cream bases. Cream base V which demonstrated good properties and stability was chosen to prepare *C. grandis* cream.

Table 1 Composition of cream base I -V

Ingredients (g)	Cream base I	Cream base II	Cream base III	Cream base IV	Cream base V
Stearic acid	2.00	14.00	14.00	-	-
Cetyl alcohol	3.00	1.00	3.00	5.00	7.00
Stearyl alcohol	3.00	-	-	7.00	5.00
Liquid paraffin	12.00	-	-	5.00	5.00
Isopropyl palmitate	0.50	1.00	1.00	-	-
Propylene glycol	-	-	-	10.00	10.00
Paraben concentration	0.15	0.15	0.15	1.00	1.00
Span 60	1.00	2.00	2.00	-	-
Sorbitol 70%	3.00	3.00	3.00	-	-
Tween 60	1.50	1.50	1.50	-	-
Tween 80	-	-	-	5.00	5.00
Distilled water q.s.	100.00	100.00	100.00	100.00	100.00

Table 2 presents the composition of *C. grandis* cream. The CGH-F extract was dissolved in liquid paraffin before blended into cream base to produce 1% and 5% *C. grandis* creams. The dye test revealed that both creams were o/w type, and their physical properties are presented in table 3. The results showed that increasing of the extract concentration caused the increasing of pH and viscosity of cream. The centrifugation test indicated the stability of *C. grandis* creams and cream base. The color, odor, consistency, pH, and washability of 1% and 5% *C. grandis* creams did not change after the 4 cycles of freeze-thaw stability study. Nevertheless, the increasing of viscosity of both creams was observed. The viscosity of 1% *C. grandis* cream increased from 872,000 to 948,733 centipoise while viscosity of 5% *C. grandis* cream increased from 978,000 to 1,145,000 centipoise. Accordingly, the extract of *C. grandis* leaf can be formulated as 1% and 5% creams with good physical properties and good stability.

Table 2 Composition of *C. grandis* cream

Ingredients (g)	*C. grandis* Cream 1%	*C. grandis* Cream 5%
CGH-F extract	1.70	8.50
Liquid paraffin	3.57	17.85
Cream base V q.s.	170.00	170.00

Table 3 Physical properties of *Coccinia grandis* creams

Physical properties	*C. grandis* Cream 1%	*C. grandis* Cream 5%	Cream base
Color	Green brown 	Dark green brown 	White
Odor	Characteristic	Characteristic	Odorless
Consistency	Smooth and non-grease	Smooth and non-grease	Smooth and non-grease
pH	6.15	7.09	5.13
Washability	Good	Good	Good
Viscosity (centipoise)	872,000	978,000	863,000
Centrifugation test (phase separation)	No	No	No

5. Conclusion

The hexane fraction which was extracted from the 90% ethanolic extract of *C. grandis* leaves, contained the anti-inflammatory compound, *β*-sitosterol. It presented 87.44% inhibition of albumin denaturation at the concentration of 100 µg/mL. The formulation of 1% and 5% *C. grandis* creams produced good physical property creams with good stability under 4 cycles of freeze-thaw study.

6. Acknowlagement

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