DEVELOPMENT OF INNOVATIVE GEL SUNSCREEN PRODUCTS FROM *Momordica cochinchinensis* (LOUR.) SPRENG. EXTRACT

ABSTRACT

A study on the development of sunscreen gel products from Momordica cochinchinensis(Lour.) Spreng. extract aimed to study phenolic content, inhibitory effect of Elastase and Tyrosinase,product stability, toxicity,astringent effect of M. cochinchinensis extract, skin elasticity value, suitable product formula calculation for preparing sunscreen gel products from M.cochinchinensis extract, and irritation test.The process started fromthe selection of raw materials, preparation of extracts for determining the total phenolic content, development of suitable formula, test of safety and product physical characteristics, and then test of the anti-allergic effect of 10 volunteers to get efficient and safe sunscreen gel from M. cochinchinensis extract. The study result indicated that M. cochinchinensisaril extract had antioxidant activity DPPH of 1.51±0.05 mg/ml, compared to standard substance - Vitamin C, and total phenolic content of 13.18±0.18 *(mg equivalent of gallic acid per 100 g - dry weight). RegardingCytotoxicityat a concentration of 0.0001-1 mg/ml, it revealed that M. cochinchinensisaril extract was not toxic to human skin cells with the cell survival percentage at a concentration of* 1 *mg/ml equaled to* 95.35±1.86 *and* 88.15±4.73%, *respectively. M. cochinchinensisaril extract concentration of* 1 *mg/ml had astringent effect which can stimulate human skin cells to move together faster than the control group but showed effect slower than Vitamin C concentrate of* 1 *mg/ml. and did not have inhibitory effect on Elastase and Tyrosinaseenzymes. Regarding M. cochinchinensis seed oil extract, it did not toxic to human skin cells at the concentration of* 0.0001-1 *mg/ml with the survival percentage equaled to 105.67-*111.46%, *and had a few antioxidants activity of unsaturated fatty acids with an* IPC_{50} *more than 1000 mg/ml. This study was only the development of sunscreen gel products from M. cochinchinensis extract.*

Keywords: Momordica cochinchinensis extract, Sunscreen gel, Elasticity

INTRODUCTION

This research study aimed to develop sunscreen gel from *M. cochinchinensis*(Lour.) Spreng. extract, which is an herb that can be easily found and is avegetable that its fruit is normally used to make drinking water for nourishing eyesight and reducing blood sugar. In traditional Thai medicine, its root is used for treatmentby helping eliminate toxic fever, expectorate, and reduce inflammation. Lycopene, the important substance in *M. cochinchinensis*, helps reduce inflammation, and *M. cochinchinensis* also has 10-time higher beta-carotene than carrot, which helps nourish eyes. In addition, these important substances are extracted for using as the significant ingredient in making face cosmetics to decelerate wrinkles on the face because these essential substances possess high antioxidant property (Leelamanit, 2014) and also helps protect skin from harmful sunlight. Therefore, researcher brought *M. cochinchinensis* to develop sunscreen gel products to help society according to the sufficiency economy, keep generatinghigher incomes, expanding tourist attractions, and increasing community potential.

MATERIALS AND METHODS

Purchased herbs and also found them naturally, washed them, choppedthem into small pieces, baked them by using oven at 50 °C, weighed each type of herbs as required, then grinded them roughly with a repeat plate grinder, fermented with 95% Ethanol for 3 days, filtered with filtering paper, brought the residue for further fermentation and filtered them 2 times, mixed the extracts fromall 3 filterings, then made them more concentrated by using Rotary Evaporation, calculated for %yield of extract, examined antioxidant effect by using ,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which was the method for analyzing abilities of anti-oxidation. This analysis used reagent of DPPH for examining total phenolic content, testing toxicity, testing inhibitory effect of elastase and tyrosinase enzymes, testing astringent effect in human skin cells, testing product stability by using freeze and thaw cycle, and testing irritation.

Formulations for calculating the % yield of plant extracts

This is a comparison of the extracted amount with the amount of the reactants. Hence, the production cost can be calculated each time (Phrompittayarat et al., 2007).

% yield = weight of plant extracts / dry weight of medicinal plants × 100

Antioxidant activity with DPPH Assay

DPPH• radical is the stable nitrogen radical with purple color. The analysis was the measurement of the ability of the test substance in eliminating free radicals by providing hydrogen atom. The measurement was made by usingspectrophotometer in measuring color reduction when anti-oxidized substance was added. The light absorbance was measured at the wavelength of 517 nanometer. Antioxidant activity test of *M. cochinchinensis* aril extract by using DPPH Assay method started from dissolving *M. cochinchinensis* aril extract with distilled water to obtain concentrations of 0.001, 0.01, 0.1, 1 and 10 mg/ml, then testing the free radical scavenging activity by using 2,2-Diphenyl-1-picrylhydrazylDPPH) radical method in microwell plate (Manosroi, et al., 2010). The concentration value that can capture free radical DPPH equaled to 50% (SC_{50}).

Determination of total phenolic compound content

Total phenolic compound content was determined by using Folin-ciocalteu (FC reagent) of extract. The extracted substance sample was used for determining total phenolic compound content by the method of Folin-ciocalteu (FC reagent). The values of concentration and light absorbance were used to make equation derived from standard graph y = Ax + B. Y value substituted light absorbance value of sample substance to determine x value which equaled to C value (concentration of total phenolic compounds from standard curve (mg/L)). Then all values were used to substitute in the below formula to get A value which was total phenolic compound content (mg/ 1 g of herb sample). The concentration of sample substance (C value) which derived from reading compared to standard graph was brought to substitute in the formula to calculate total phenolic compound content (Miliauskas et al., 2004).

Cytotoxicity test

Dissolved Mc(A) and Vitamin C with culture medium, brought a test sample to sterilize by filtering it through a membrane with a 0.2-micron pores, diluted test sample solution to get the desired concentration with sterilized culture medium, cytotoxicity was used by sulforhodamine B dyeing method (SRB) (Vichai & Kirtikara, 2006), calculated the cell survival percentage compared to the control group, selected maximum concentration that was not toxic for cells to test wound healing activity by analyzing from the space of lines occurred after cell movement when receiving test substance compared tothe control group and the group that received standard substance - Vitamin C from photos derived from microscope at several point of times (Muhammad et al., 2013).

Anti-tyrosinase activity test

Dissolved *M.cochinchinensis* aril extract with distilled water to obtain concentrates of 0.001,0.01,0.1,1 and 10mg/ml, brought them to test anti-tyrosinase activity by the method of Dopachrome in the microwell plate (Chang, 2009; Manosroi et al., 2011), concentration value that can inhibit tyrosinase enzyme equaled to 50% (IC_{50}).

Anti-elastase enzyme activity test

Prepared Elastase enzyme solution by dissolving in Tris-HCL buffer pH 8.0 in the amount of 100 ml. Dissolved sample to get desired concentrate with Tris-HCL buffer pH 8.0. Pre-incubated enzyme and sample solution by mixing enzyme 900 ul with sample 1 ml, then shook well. Incubated at 25 C for 20 minutes and added Substrate solution (0.8 mM succ-(Ala)-3-nitroanilide) in the amount of100 µl and thenincubated at 25 C for 20 minutes. Measured light absorbance value at the wavelength of 410 nm (Lee andChoi, 1999)

Calculated % Inhibition = [(OD control – OD sample)/ OD control] X 100

Stability and physical characteristics of products

Evaluate physical stability by observing gel texture, stratification, sedimentation,and smell, testing pH balance by using pH meter of products that was just newly prepared, then stored them at room temperature for 1 week.Evaluate the stability of at accelerated conditions by using Freeze and thaw cycle 5 times. The products werestored in temperature and humidity-controlled cabinet which set the cycle at 4 °c for 24 hours. After 24 hours, set the temperature to 45 °c for 24 hours. It counted as 1 cycle. Made 5 cycles totally(Chuayprom, 2010)

SKIN IRRITATION TEST

Testing of skin irritation and allergic reactions on human skin for *M. cochinchinensis* extract and products with the concentrate of 5% relied on closed patch test under occlusion to observe irritant and allergic reaction. The test results will be evaluated according to the scoring system recommended by International Contact Dermatitis Research Group (ICDRG) (Traisut et al., 2016). The inclusion criteria consisted of 10 normal people with good health, aged 20-35 years, 5 males, 5 females, not under other research studies, and willing to participate in the study, not have itching from severe infection, no abscess and no pus, or not have itching from skin disease caused by infection or immune system disease, not have wounds or skin diseases in the upper back. *Skin* areas were 3 points of upper back. Each point had the size of 2x2 $cm^2$with 3 cm apart**.** When starting the test, washed the upper back with saline, waited until dry, used a gauze pad with the substances that want to test such as saline, sunscreen gel base, and sunscreen gel from *M.cochinchinensis* extract to apply on the 3 desired points of upper back. Each point used 3 substances and each substance was used in the amount of 0.3 ml. Then evaluated irritation symptom by researcher such as irritation, itching, blisters, and rashes instantly after use and after 12 hours of use.

Table 1Development of sunscreen gel product made from *M.cochinchinensis*extract

Ingredients/Formula	Amount(%w/w)				
	1	2	3	4	5
Deionized Water	87.153%	85.653%	84.173%	82.953%	81.453%
Carbomer	0.5%	0.8%	1%	1.2%	1.5%
Glycerin	0.5%	0.7%	0.98%	1%	1.2%
Disodium EDTA	0.04%	0.04%	0.04%	0.04%	0.04%
Propylene Glycol	1.47%	1.47%	1.47%	1.47%	1.47%
M. cochinchinensis (Aril) extract	5%	5%	5%	5%	5%
Triethanolamine	0.24%	1.24%	2.24%	3.24%	4.24%
Sodium Polyacryloyldimethyl Taurate	0.49%	0.49%	0.49%	0.49%	0.49%
Niacinamide	1.47%	1.47%	1.47%	1.47%	1.47%
Alpha-Arbutin	0.098%	0.098%	0.098%	0.098%	0.098%
Fragrance	0.049%	0.049%	0.049%	0.049%	0.049%
Phenoxyethanol (and) Chlorphenesin (and) Glycerin	0.49%	0.49%	0.49%	0.49%	0.49%
DisodiumPhenyl Dibenzimidazole Tetrasulfonate	1%	1%	1%	1%	1%
Phenylbenzimidazole Sulfonic Acid	1%	1%	1%	1%	1%
Benzophenone-4	0.5%	0.5%	0.5%	0.5%	0.5%

RESULTS

Sunscreen gel product made from *M. cochinchinensis* extract was developed according Table 1. Characteristics of each sunscreen gel product formula was present in Table 2 and best formula was in Table 3.Yield *of crude* extract was shown in Table 4.According to the test result, Mc(A) extracthad Free radical scavenging activity DPPH with SC_{50}value equaled to1.51±0.05 mg/ml or 0.026 times of Vitamin C standard substance which provided SC_{50}value equaled to 0.04±0.01mg/ml. Mc(S) did not have free radical scavenging activityDPPH and did not possess free radical scavenging activity NO whereas Vitamin C had free radicals scavenging activity DPPH and free radical scavenging activity NO with SC_{50}value equaled to 0.03±0.01 and 0.21±0.04 mg/ml, respectively (Table 5). Mc(A) extract was possessed total phenolic compound content of 13.18±0.18 equivalent mg of gallic acid per 100 g dry weight (Table 6). Regarding the test, it can be summarized that Mc(S) at the concentration of 0.0001-1 mg/ml did not have any effect on human fibroblast skin cells and had a survival percentage between 105.67-111.46% whereas sodium lauryl sulfatehad toxic effect on cells at the concentration of 0.1 and 1mg/ml with survival percentage equaling to 12.03±1.82 and 9.13%, respectively.Mc(A) extract and vitamin C did not have toxic effect on human skin cells at the concentrate of 0.0001 - 1mg/ml with cell survival percentage equaled to 99.35±1.86 and 88.15±4.73%, respectively at the concentrate of 1mg/ml (Table 7).*M. cochinchinensis* aril extract did not possess tyrosinase and elastase inhibitory effects when compared with positive controls (Table 8). It can be summarized that *M. cochinchinensis* aril extract at the concentration of 1 mg/ml possessed astringent effect which can stimulate human skin cell to move together faster than control group but slower than Vitamin C at the concentration of 1 mg/ml (Table 9). This product was stabled at accelerated condition by using five rounds of freeze and thaw cycle method and no stratification observed. all 10 volunteers were not allergic, itching, or irritated when applied saline, massage oil base, and massage oil fromMc(A) within 12 hours.

DISCUSSION

According to the development of innovative sunscreen gel from *M. cochinchinensis* extract to inhibit free radicals and the establishment of community spa, the researcher tested 2 extracts from M. cochinchinensis, which wereMc(A) (*M. cochinchinensis* aril extract) andMc(S) (*M. cochinchinensis* seed oil extract) with %yield of 7.81 and 0.42, respectively. The test revealed that *M. cochinchinensis* aril extract) possessed antioxidant activity DPPH of 1.51±0.05mg/ml compared to standard substance - Vitamin C which had total phenolic compound content of 13.18±0.18 (Equivalent mg of gallic acid per 100 g dry weight). Regarding Cytotoxicity,*M. cochinchinensis* aril extract was not toxic to human skin cells with cell survival percent at the concentration of 1 mg/ml equaled to 95.35±1.86 and 88.15±4.73%, respectively. M. cochinchinensis extract at the concentration of 1 mg/ml possessed astringent effect which helped stimulate human skin cell move together faster than the control group but provided effect slower than standard substance, vitamin C concentration of 1 mg/ml. In addition, this extract did not possess inhibitory effect for elastase and tyrosinase enzymes. *M. cochinchinensis* seed oil extract was not toxic to human skin cells at the concentration of 0.0001-1 mg/ml with survival percentage between 105.67-111.46%.

An experiment on the development of innovative sunscreen gel products from *M. cochinchinensis*extract to inhibit free radicals and the establishment of a community spa used *M. cochinchinensis*aril extract to develop a formula of sunscreen gel product. The best formula result of sunscreen gel development was formula 3. The amount of *M. cochinchinensis*extract in propylene glycol used for the preparation of sunscreen gel was 100 g, consisting of *M. cochinchinensis*extract 5 g., distilled water 86.9 g, EDATA B 0.04 g, Glycerin 0.98 g, Propylene 1.47 g, Capopol 1 g, TEA 2.24 g, Viscolam AT 0.49 g, Vitamin B3 1.47 g, Alpha-Abutin 0.098 g, HH-CP 0.049 g, Microcare 0.49 g, Ensulizole 1 g, Bisdi Sulisoldisodium 1 g, and Benzophenone 0.5 g.After the experiment on product development, product was tested for instability and the test revealed that the product texture was not separated. Regarding irritation test with 10 volunteers, no allergic effect was found. Because this study was the only development of innovative sunscreen gel from *M. cochinchinensis*extract, it needs further study and development.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Table 2 Characteristics of sunscreen gel made from *M. cochinchinensis* extract

Characteristic	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Color	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
Smell	Not rancid, Not sour	Not rancid, Not sour	Not rancid, Not sour	Not rancid, Not sour	Not rancid, Not sour
Clarity	Very clear	Very clear	Very clear	Very clear	Very clear
Sunscreen gel texture	Smooth Very liquid	Smooth Very liquid	Smooth Slightly viscous	Smooth Sticky Viscous	Smooth Sticky Very viscous

Table 3 Sunscreen gel formula containing *M. cochinchinensis* extract

Ingredients	Amount(%w/w)
Deionized Water	84.173%
Carbomer	1%
Glycerin	0.98%
Disodium EDTA	0.04%
Propylene Glycol	1.47%

M. cochinchinensis extract	5%
Triethanolamine	2.24%
Sodium Polyacryloyldimethyl Taurate	0.49%
Niacinamide	1.47%
Alpha-Arbutin	0.098%
Fragrance	0.049%
Phenoxyethanol (and) Chlorphenesin (and) Glycerin	0.49%
Disodium Phenyl Dibenzimidazole Tetrasulfonat	1%
Phenylbenzimidazole Sulfonic Acid	1%
Benzophenone-4	0.5%

Table 4 Yield of *M. cochinchinensis*extracts

Extracts	Weight before Extraction (Gram)	Extract Weight (Gram)	% Yield
Mc(A)	500	39.05	7.81
Mc(S)	3,000	12.68	0.42

NOTE: Mc(A) = *M. cochinchinensis* aril, Mc(S) = *M. cochinchinensis* seed oil

Table 5 Antioxidant activity of*M. cochinchinensis* extracts

Antioxidant Activity (SC_{50} = mg/ml)	**Mc(A)**	**Mc(S)**	Vitamin C
DPPH radical scavenging activity	1.51±0.05	NA	0.03±0.01
NO radical scavenging activity	NA	NA	0.21±0.04

REMARK: Mc(A) = *M. cochinchinensis* aril; Mc(S) = *M. cochinchinensis* seed oil

Table 6 Total phenolic compound of *M. cochinchinensis* extract

Crude Extract	Total phenolic compound content ± SD (Equivalent mg of gallic acid per 100 g dry weight)
Mc(A)	13.18±0.18

Table 7 Cell survival on cytotoxicity test

	Percentage of cell survival				
Sample (mg/ml)	**0.0001**	**0.001**	**0.01**	**0.1**	**1**
Mc(S)	105.67±5.77	106.11±0.89	108.15±4.15	110.98±5.34	111.46±5.77
Mc(A)	106.99±4.54	107.43±6.31	105.84±3.76	103.25±3.98	99.35±1.86
Sodium lauryl sulfate	105.33±4.31	103.33±4.31	98.05±1.01	12.03±1.82	9.13±0.23

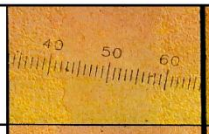
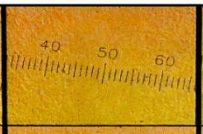
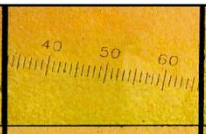
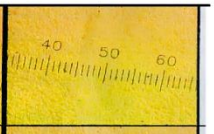
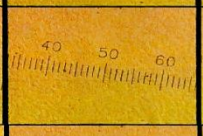
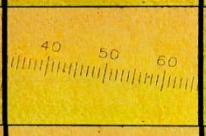
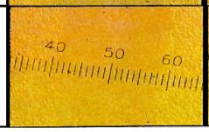
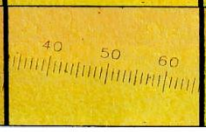
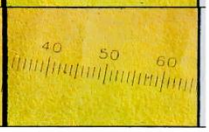
REMARK: The shown value derived from 4 repeated experiments (Mean ±S.D.), Mc(S) =*M. cochinchinensis* seed oil; Mc(A) = *M. cochinchinensis* aril;

Table 8 Tyrosinase and elastase inhibitory activity of *M. cochinchinensis* aril, Mc(A)

Sample/Test (IC_{50} ,mg/ml)	**Anti-tyrosinase**	**Anti-elastase**
Mc(A)	NA	NA
Kojic acid	0.08±0.00	-
Epigallocatechin gallate (EGCG)	-	0.03±0.01

REMARK: The shown value derived from 3 repeated experiments (Mean ±S.D.), NA = No effect

Table 9 The characteristics of human skin cell movement in the line area when receiving test samples

Time Period [Hours]	0	6	24	48
Control group DMEM				
Mc[A] 1 mg/ml				
Vitamin C 1 mg/ml				

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