ejpmr, 2020,7(5), 707-711

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION AND FORMULATION OF GEL FORM *ANITDESMA VELUTINUM* LEAVES EXTRACT FOR ANTIOXIDANT AND ANTI-WRINKLE ACTIVIES

Supachai Chumchuen*

Thai Traditional Medicine, Thai Charoen Biotechnology Co., Ltd Samut Prakan Province, Thailand, 10540.

***Corresponding Author: Supachai Chumchuen**

Thai Traditional Medicine, Thai Charoen Biotechnology Co., Ltd Samut Prakan Province, Thailand, 10540.

Article Received on 20/03/2020 **Article Revised on 10/04/2020** **Article Accepted on 30/04/2020**

ABSTRACT

Anitdesma velutinum (AV) has been used in Thai traditional medicine. The most antioxidant and anti-wrinkle properties were found on leaves. But the method in ancient has complicated. For ease of used, formulation of gel were the best answer. **Objective**: the study is to formulate and evaluate a topical herbal gel containing AV leaf extracts for their anti-oxidant and anti-wrinkles activities. **Methods:** The varies formulates of gels , concentration of extract. Activities for antioxidant tested DPPH method and anti-wrinkle processed by enzyme inhibitor of collagenase and elastase. Physiologicals were tested by oranoleptic, pH, viscosity etc and confirmed irritation on animal. **Results**: The herbal gel have concentrated 10 (% w/v) of extract was significantly $p < 0.05$, inhibiter enzyme for wrinkles and better stability than other formulation. Non detected irritation on rabbits. **Conclusion**: The gel formula with 10 % w/v has antioxidant activity and inhibit the enzymes for wrinkles, without irritation.

KEYWORD: Gel, Thai traditional medicine, Anti-wrinkle, Anti-oxidant.

INTRODUCTION

People are daily exposed to air pollutants (smoke, ozone, particulatematter, etc.) and to all kinds of blue light sources (fluorescent and LED lighting, flat-screen TV, digital devices such as smartphones, laptops, etc.). They are more and more concerned with the impact of pollution and their hectic lifestyle, on the quality and beauty of their skin.[1] Pollution cause the skin damaged. Collagenase enzyme, which was caused the amount of collagen in the skin to be destroyed.[2] In addition, elastin fibers are factor in maintaining the skin's elasticity, but the fibers are destroyed by enzymes that are stimulated by pollution. Which were gone to cause the elastin function to decrease.

Extracted form Thai herbs, such as AV, have been widely investigated and found to possess free radical scavenging, anti-collagenase and anti-elastase activities[3,4] The AV contains various chemical constituents such as quercetin, kaempferol and gallic acid, which are classified in the groups of catechin, flavonoids and phenolic acid. Plant extracts, containing these structural-related components, therefore, might show anti-aging benefits.

Thai traditional medicine has been used AV for nourishing benefits of the body. But people of the era can't explain properties.[5] In ancient texts, there has been a record of the widespread use of this of herb in women. Nowadays, Thai traditional medicine uses fresh leaves to mask the skin for beauty. By explaining according to Thai traditional medicine theory that it helps to balance the body (earth, fire, windy and water).[6] But the researchers found that the old method is complicated. This the first reason for changed form of treats. Gel is another form of product that is interesting because it is not sticky, suitable for tropical conditions in Thailand. In addition, creating a trade value of Thai herbs. **Figure 1.**

Figure 1: **Show the treatment methods of Thai traditional medicine that use AV leaves to mask the skin**.

Gel is a pharmaceutical form. Which has the characteristics of semi-solid system according to the definition of USP, which is a pharmaceutical product consisting of two phases in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved in the continuous phase. That the basically

characteristics of gels such as swelling ageing rheology and syneresis etc.[7,8]

MATERIALS AND METHODS

Plant material

The plant AV was collected on January 2019 form Dongbung Farm, Prachinburi, Thailand. The plant material were washed and dried at 60°C for 24 hrs in a hot air oven and were reduced to powder.

Plant extraction

The plant extraction method described by Chumchuen S.[3] Soxhlet extraction method was used 20 g of plant powder, leave of AV was packed in Whatmann No. 1 filter paper and placed in extraction chamber. Which was suspended below in the round bottom flask containing the solvent (200 ml of ethanol). The samples were extracted at 73.5 ^{o}C for 36 hrs and then concentrated under reduced pressure at 60 ^{o}C with the rotaryevapolater and stored at 4 ^{o}C for further studies.

Preparation of Gel

Carbopol 940, used as gelling agents. weighed and sprinkled on the surface of pure water for 50 minutes, after which it was continuously stirred, after which it was continuously stirred when the solution felt homogeneous. Add extracts, varies concentrations of 2.5, 5 and 10 (% w/v) and then add propylene glycol, methyl paraben, propyl paraben and distilled water up to 100 ml respectively. Puted triethanolamine, small increment and stirred . The formulae for the gels are given in **Table 1.**

Discoloration of the Extract

Discoloration of the extract is modified from Plainfosse H et al.[9] Discoloration of extraction was performed with 1% (w/w) of activated carbon for 2 hr. The powdered activated carbon is then easily removed by settling and filtration. The finish product were show at **Figure 2**.

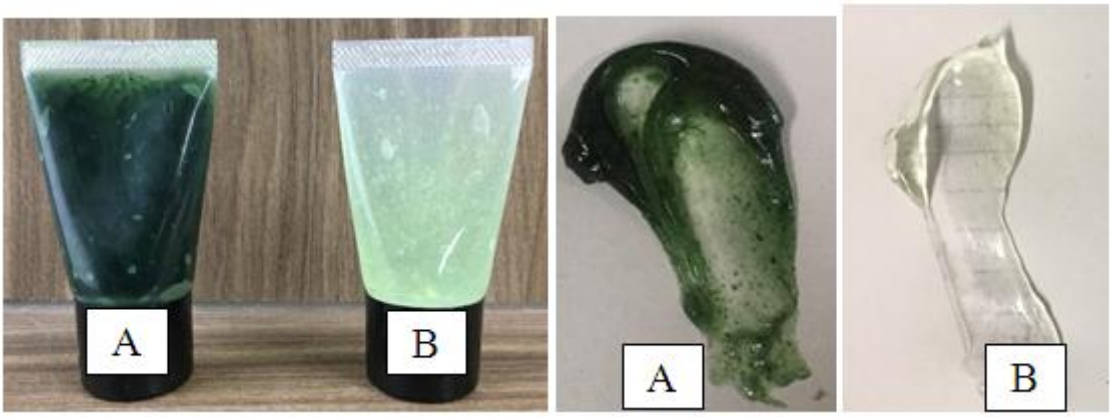

Figure 2: A is the original gel with a dark green color, rough texture. B is the gel that discoloration bright green and smooth texture.

Anti-oxidant activity; DPPH

The radical scavenging activity (RSA) which is modified from Bhagyashree V et al.[10] 3 ml each of B formulations were transferred into tubes, diluted to a concentration 20, 40, 60 and 80 µg/ml respectively. Then mixture of solvent n-hexane, ether and ethanol in proportion of 1:1:1 were mixed with 0.5 ml of DPPH solution (9 µM in absolute ethanol) were added into each. Incubation at the dark room temperature for 50 min. Absorbance were measured at 516 nm using the UV–visible spectrophotometer. The RSA were calculated in the equation here. Gallic acid (GA) was used as positive control.

% of inhibition RSA =
$[(A_{516\ blank} - A_{516\ sample}) / A_{516\ blank}] \times 100$

Anti-collagenase inhibitory activity

Anti-collagenase inhibitory activity which is modified from Rungruang R et al.[11] The assay was followed EnzChek® collagenase/gelatinase assay kit. Briefly, The gel in each B formula was tested by dilution when the extract amount was calculated as minimum as 2.5% w/v on the 96-well plate. And add DQ^{TM} collagen (MMP-1) and DQ^{TM} gelatin (MMP-2), 20 µl. Continuously added Collagenase obtained from *Clostridium histolyticum* about 100 µl. Has been incubated 90 min. Fluorescence intensity was measured with the excitation and the emission wavelength at 485 nm and 538 nm, respectively, using a fluorescent microplate reader and compared with epigallocatechin gallate (EGCG).

Anti-elastase inhibitory activity

Anti-elastase inhibitory activity which is modified from Pientaweeratch S et al.[4] Briefly, The gel in each B formula was tested by dilution when the extract amount was calculated as minimum as 2.5% w/v on the 96-well plate. And add porcine pancreatic elastase (PE), 20 µl was preprad by 0.2 mM Tris-HCL buffer (pH 8.0). Continuously added 1.6 mM N-Succinyl- Ala-Ala-p-nitroanilide (AAAPVN) 250 µL/plate. Measured with microplate reader that wavelength at 485 nm and 538 nm, respectively. Was compared with epigallo catechin gallate (EGCG).

Evaluation of gels

1. Appearance

Appearance was tested by observing texture, separation and color.

2. Contaminated of microbes (CM)

Microbes were tested the plate counting method, according to USP 41. It has been identified. The amount of bacteria, yeast and mold growth using air must not exceed 1000 cfu / 1 g of sample. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Clostridium spp*, must not be found. Detector was done using growth promotion test method and negative control.

3. pH of gel

The sample solution was prepared 10% w/v in water. The pH was measured at a temperature of 25 ^{o}C, the result was limited to a value of 3.5-7.5.

4. Viscosity

Viscosity of gel was determined by Brookfield viscometer with spindle.

5. Spreadibility

Spreadibility of gel which is modified from Das S et al.[12] Briefly, 3 g of gel was transferred into ground slide, like a sandwich between slide and another glass slide. Put 1 Kg weight was placed on the top for 5 min and then excess of the gel was scrapped off from the edges. The top of sample pull of 80 g with string attached to the hook and the time required slide cover a distance of 7.5 cm. Spreadibility of gel were calculated in the equation here.

S = M x L / T

S = Spreadibility of gel, M = Weight in the pan, L = Length moved by the glass slide and T = Time (in sec).

6. Stability Study

The stability is modified form THAI SMEs STANDARD 15-2562.[13] The samples were tested at 4 ^{o}C for 24 hours and then stored at 45 ^{o}C for 24 hours. General characteristics were compared with the product's original condition.

7. Irritation test

Irritation test is modified form THAI SMEs STANDARD 15-2562. The license No.EAR 02/63. Briefly, 12 of healthy white rabbits, New Zealand female weight 2 kg. Each rabbit will be separated. Controlling the laboratory temperature at 20 ^{o}C, relative humidity not less than 30% and not more than 70% and installing the illumination device with a light and dark cycle each for 12 hr, The food and water provided for rabbits must be in accordance with nutrition of rabbits, experiment and prepare clean all the time. Carefully shave the hair on the right ear and use 4 gauze patch, two pads have been applied samples onto 0.5 g. As for the other 2 pads applied sterile of water 0.5 cm^3. The gauzes were covered on the test area on rabbit skin. And wrapped with an elastic bandage. Irritations are measured every 24 and 48 hours.

RESULTS AND DISCUSSION

Anti-oxidant and Anti-wrinkle Activities.

The studied have been antioxidant collagenase and elastase inhibition activities. Cause of wrinkles, have been discovered the extraction of leaves form *Anitdesma velutinum* (AV). Were developed as a gel formula. This may be a research of Chumchuen S. found that the extracts from the leaves of AV, effective for anti free radicals. The gap in previous research has not been explained, position of extracts. Information was distributed in this work that searches for uncomplese data and explanations, the extract has been a lot of hydroxy group, which is the substance that causes the enzyme collagenase paralyze, binded to Zn^{2+} ions at the active site of the enzyme. However, the elastase enzyme has been discovered in previous research by Malesev D et al.[14] the polyphenols will cause the enzyme to unstable. From the experiment **Table 2**, it was found that the gel with the concentration of 10 g/mL of the extract was effective against free radicals with non significant difference p = 0.05 with the standard Gallic acid, IC_{50} 98.01 ± 0.03 µg/mL. But anti collagenase and elastase have significant difference p = 0.05. That were lower concentration of extraction form discoloration with carbon powder. That was absorbed substance. Therefore, the challenge will be revealed in the future by those interested.

Evaluation test

The formula B gel has been color removed by carbon powder. It was tested after the ready-mixed characteristics found that as shown in **Table 3**, the color was light green, Unique smell Clear, translucent, non microorganism detected, pH value is suitable for skin 6.87, has a weak acid. The viscosity is equal to 32325 Cps, which is detected from the gel having the characteristic of the flow type pseudo plastic.That is when the more shear rate dueto the viscosity are reduced, the such properties may be a consideration when choosing a package. The spread should be related to the viscosity in the opposite direction, if the viscosity is spread slowly. However, this gel formula has been tested for stability in hot and cold conditions for 48 hours, found that its characteristics have changed little that showed result at **Table 4**. Which may be an assumption that the gel is durable. But how to do further testing on other topics. Which is obvious that heat affects the viscosity of the gel, probably because heat changes the structure of the gel. This corresponds Sailaja A et al, found that the gel will deteriorate over time because the basic properties of the gel is syneresis, which increases the space of the space after the water in the gel evaporates.

Irritation test

Irritation was tested on animals that ethical permission according number. EAR No. 02/63 with 12 animals, there is a minimum and most efficient use of it. All of animals have experiments follower the requirements for THAI SMEs STANDARD 15-2562. The result was

herbal gel without irritation that show on **Table 5**. Which may be a preliminary experiment on safe gels. In the future, if further development, gels should be tested in a clinic for safe efficacies.

Table 1: Different formulation prepared gels of leave , *Anitdesma velutinum* extract.

Ingredients	Control	F_1	F_2	F_3
Carbopol 934 (g)	2	2	2	2
Methyl Paraben (1 %)	0.3	0.3	0.3	0.3
Propyl Paraben (0.5 %)	0.1	0.1	0.1	0.1
Propylene glycol 400 (5 %)	10	10	10	10
Extraction (% w/v)	0	2.5	5	10
Water	Up to 100	Up to 100	Up to 100	Up to 100
Triethanolamine (q.s)	q.s.	q.s.	q.s.	q.s.

Table 2: The results B formulations gels of *Antidesma velutinum* extracted by leaves for antioxidant and anti-wrinkle activities.

Orders	Anti-oxidant activity DPPH method IC_{50} (µg/mL)	Anti-collagenase inhibitory activity		Anti-elastase inhibitory activity IC_{50} (µg/mL)
		MMP-1 IC_{50} (µg/mL)	MMP-2 IC_{50} (µg/mL)	
control	987.17 ± 0.02 [d]	NT	NT	NT
F1	95.57 ± 0.12 [c]	124.56 ± 0.29 [h]	145.98 ± 0.73 [l]	10.09 ± 0.12 [p]
F2	59.02 ± 0.03 [b]	112.06 ± 0.44 [g]	135.04 ± 0.01 [k]	14.08 ± 0.46 [o]
F3	12.31 ± 0.05 [a]	98.01 ± 0.03 [f]	103.87 ± 0.82 [j]	20.18 ± 0.08 [n]
GA	8.87 ± 0.05 [a]	NT	NT	NT
EGCG	NT	9.97 ± 0.12 [e]	8.94 ± 0.32 [i]	102.98 ± 0.43 [m]

NT = Non test

*The different letters in the same column showed the significant difference p = 0.05

Statistical analysis were performed ANOVA method.

Table 3: The results B formulations fresh gels of *Antidesma velutinum* extracted by leaves for evaluation.

No.	Apperance	CM	pH	Viscosity (Cps)	Spreadability ($gm.cm.sec^{-1}$)
control	clear and transparent	ND	6.98 ± 0.01	28756 ± 0.19	26.36 ± 0.45
F1	pale of green and transparent	ND	6.85 ± 0.03	30012 ± 0.33	24.46 ± 0.38
F2	pale of green and transparent	ND	6.84 ± 0.01	30214 ± 0.46	19.63 ± 0.12
F3	pale of green and transparent	ND	6.87 ± 0.02	32325 ± 0.01	17.32 ± 0.50

ND = not detected

Table 4: The results B formulations gels of *Antidesma velutinum* extracted by leaves for stability.

No.	Apperance	pH	Viscosity (Cps)	Spreadability ($gm.cm.sec^{-1}$)
control	clear and transparent	6.88 ± 0.98	27756 ± 0.39	25.99 ± 2.39
F1	pale of green and transparent	6.65 ± 0.67	26012 ± 0.01	22.12 ± 2.36
F2	pale of green and transparent	6.64 ± 0.91	29214 ± 0.89	18.98 ± 3.21
F3	pale of green and transparent	6.57 ± 0.11	31325 ± 0.65	15.02 ± 1.02

Table 5: Skin irritation study, gel were 10 % w/v of extract.

Formulation	Scores of primary irritation (SPI)	
	24 hr	48 hr
Control	0.10 ± 0.78	0.15 ± 0.98
10 % w/v of extraction gel	0.28 ± 0.08	0.31 ± 0.11

Criterial; less than 0.20 = Non Irritation 0.21 < SPI < 0.50 = Slight Irritation 0.51 < SPI < 1.00 = Moderate Irritation more than 1.01 = Irritation

CONCLUSION

The gel formulas with a concentration of 10(%w/v) can inhibit free radicals and collagenase elastase , enzymes that cause wrinkles. The gel has been tested in animals no irritation.

REFERENCES

1. Krutmann Liu, Pan W, Crawford X, Sore M, and Seite S. Pollution and skin: From epidemiological and mechanistic studies to clinical implications. J. Dermatol. Sci, 2014; 76(1): 163–8.

2. Pan T, Wang W, Aljuffali H, and Fang J. The impact of urban particulate pollution on skin barrier function and the subsequent drug absorption. J. Dermatol. Sci, 2015; 78(2): 51–0.
3. Chumchuen S. Antioxidant Activity of the flower fruit and leaf, *Anitdesma velutinum* (Tulas) - A Comparison. Word Journal of Pharmacy and Pharmaceutical Sciences, 2020; 9(4): 1160-6.
4. Pientaweeratch S, Panapisal V, and Tansirikongkol A. Antioxidant, Anti-Collagenase and Anti-Elastase Activities of *Phyllanthus Emblica*, *Manilkara Zapota* and Silymarin: an In Vitro Comparative Study for Anti-Aging Applications. Pharmaceutical Biology, 2016; 54(9): 1865 -72.
5. Chumchuen S. Wisdom of Folk Medicine at Wat Salut Community, Bang Phli District, Samut Prakan Province. Journal of Humanities and Social Sciences Mahasarakham University, 2019; 38(5): 95-4. 19; 38(5): 95-4.
6. Chumchuen S. Effectiveness of Thai massage with *Derris scandenson* pain in Osteoarthritis Patients. Journal of Traditional Thai Medical Research, 2019; 5(2): 29-4.
7. Sailaja A and Supraja R. An overall review on topical preparation gel. Innovat International Journal Of Medical & Pharmaceutical Sciences, 2016; 1(1): 17-2.
8. Barry BW: Novel mechanism and devices to enable successful transdermal drug delivery, European journal of pharmaceutical sciences, 2001; 14: 101-14.
9. Hortense P, Pauline B, Stephane A, Anne L et al. Development of a Natural Anti-Age Ingredient Based on *Quercus pubescens* Willd. Leaves Extract—A Case Study. Cosmetics, 2018; 15(5): 1-21.
10. Bhagyashree V, Rohit K and Shriniwas P. Formulation and Evaluation of Cream containing curcumin and lycopene for the antioxidant and antibacterial activity. European journal of Pharmaceutical and Medical research, 2017; 4(5): 394-9.
11. Rungruang R, Modsuwan J and Srisukong A. Antioxidant and Anti-wrinkle Activities of Tubtim Chumphae Rice Extract for Application in Facial Cosmetics. KMUTT Research & Development Journal, 2019; 42(1): 95-7.
12. Das S, Haldar P and Pramanik G. Formulation and Evaluation of Herbal Gel Containning *Clerodendron infortunatum* Leaves Extract. International Journal of Pharm Tech Research, 2011; 3(1): 140-3.
13. Ministry of Industry. Thai SMEs; Herbal body cream. 15-2562.
14. Malesev D and Kuntic V. Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. J. Serb. Chem. Soc, 2007; 72(10): 921-9.