COMPARATIVE STUDY ON COMPOSITION AND GEL FORMULATION OF ALOE VERA EXTRACT

CHONLADA KOMARAKUL NA NAKORN

MASTER OF SCIENCE
IN
COSMETIC SCIENCE

SCHOOL OF COSMETIC SCIENCE
MAE FAH LUANG UNIVERSITY

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Chonlada Komarakul Na Nakorn

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ABSTRACT

Aloe Vera used in cosmetic formulation as anti-inflammatory, soothing and wound healing. Polysaccharide and Anthraquinone components are the indicators in Aloe Vera. This study was to determine the content of Mannose-6-Phosphate and Aloe-emodin in the various forms of Aloe vera extract which are fresh, liquid and powder forms. Total carbohydrate content was carried out to analyse the glucose and mannose contents, fresh aloe vera extract contains 0.2329 g glucose eq./g, 0.178 g mannose eq./g liquid commercial extract contain 0.0370 g glucose eq./g. , 0.029 g.mannose eq./g and for Aloe Vera powder 200x extract contains 1.2849 g glucose eq./g, 0.981 g mannose eq./g

The Ultra-high performance liquid chromatography (UPLC) method was described to investigate the content of Aloe-emodin in Aloe vera extract. The method involved Column C_{18} (1.7 μm, 2.1 x 100 mm) at 25˚C with the mobile phase of methanol and 2% acetic acid (70:30, v/v), absorption at 254 nm. The retention time of Aloe-emodin was 2.9 minutes. The assay was linear from 10 to 100 μg/ml ($r^2 \geq 0.9921$).

Aloe vera extract were using as an active ingredient in after sun gel for various types of aloe vera extracts into 4 formulations include blank formulation, fresh aloe vera extract formulation, aloe vera liquid extract formulation and aloe vera powder formulation, stability test was measured via physical appearance by kept under freeze thaw condition. After the freezing thaw condition. stability test the gel was measured pH and viscosity. The result showed that the viscosity of formulation with fresh aloe vera extract was decreased from 31,800 cps to 79,200 cps and for the formulation of liquid extract and powder extract had viscosity from 81,800 cps to 70,300 cps and 101,000 to 58480 cps respectively.

Keywords: Aloe-emodin/Aloe Vera/Total Carbohydrate content/UPLC

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Now a day, Aloe vera extract is widely using in cosmetic and skincare products. This is because its benefit and affordable price. Other than that, Aloe vera is also a home grown vegetable that can be used for wound healing from burning skin.

Aloe vera carries over 200 nutrients such as minerals enzyme salicylic vitamins anthraquinones and sugar. There are many types of Polysaccharides that can be found in Aloe vera. And these Polysaccharides reinforce anti-allergy effect for skin. It is healing a wound as well as moistening the skin. In term of pharmaceutical, Aloe vera extract is also used as laxative or purgative effect. As Aloe vera consists of Anthraquinone which has Emodin that stimulate the intestines to contract and increase the water content in intestines (Ishii, Tanizawa & Takino, 1991). Aloin is an active compound in Aloe vera that widely used due to its ability to absorb UV and inhibit tyrosinase activity and also reduce the formation of melanin.

Due to a various characteristics of Aloe vera, many users disregard the appropriation of formula. This study aims to investigate the active ingredient in Aloe vera extract in fresh Aloe vera extract, liquid commercial Aloe vera extract that using propylene glycol as solvent extraction and Aloe vera powder 200X extract. And the active ingredients that will be analysed are Mannose-6-phosphate and Aloe-emodin in order to examine the concentration of them. This research is also testing the effect of Aloe Vera extract toward After sun gel formulation.

1.2 Objectives of the Study

1.2.1 Determine the content of mannose-6-phosphate and Aloe-emodin from fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200x extract.

1.2.2 Develop after sun gel formulation to suitable for using Aloe Vera Extract as raw material.

1.3 Scope of the study

1.3.1 Review the biology of Aloe Vera to apply in cosmetic field.

1.3.2 Collect fresh Aloe Vera leaf and prepare Aloe vera extract.

1.3.3 Standardize fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200X extract by freeze dry method.

1.3.4 Determine the content of mannose-6-phosphate from fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200X extract by Spectrophotpmeter.

1.3.5 Determine the content of Aloe-emodin from fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200x extract by UPLC.

1.3.6 Develop after sun gel by divided into 3 formulations by added the ingredient as fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200x extract. And test the stability of the products.

1.4 Significances of the Study

1.4.1 Total content of mannose-6-phosphate and Aloe-emodin from fresh Aloe vera extract, liquid commercial Aloe vera extract and Aloe vera powder 200x extract.

1.4.2 After sun gel product that have good stability when using Aloe Vera Extract as active ingredient.

CHAPTER 2

LITERATURE REVIEWS

2.1 Aloe Vera

Aloe Vera has botanical name as *Aloe Barbadenis* it belongs to the family Aloaceae. Its plant height around 0.25-0.5 meters. It has triangular leaves with serrated edges. Aloe Vera is a shrubby or a pea – green color plant. The inner gel parts is almost transparent and have slightly yellow green color (Pachanon, 2005)

(a) (b)

Note. (a) Leaf and (b) root

Figure 2.1 The picture of *A. Barbadenis*

2.2 Chemical Active Components

Due to Aloe vera is well known as medicine properties for a long time in many countries around the world. This is because its beneficial biological and physiological properties. As a result, the active components of Aloe Vera reveal more than 200 substances, including sugar, enzymes and amino acid. The mainly components found are Anthraquinones and Saccharides (Vogler and Ernst, 1999). All components are derived from leaf; the water content of homogenized Aloe vera gel was determined by Cryodesiccation techniques. That the raw leaf mucilage contains water 99.5 percent and pH at 4 -5. For remaining active components are as followed (Pachanon C, 2005).

Table 2.1 Constituents of Aloe vera

Anthraquinones	Saccharides	Vitamins	Inorganic Compounds
Aloin / Barbaloin	Cellulose	B1	Calcium
Isobarbaloin	Glucose	B2	Sodium
Aloe-emodin	Mannose	B6	Chlorine
Emodin	L-	Choline	Manganese
Aloetic Acid	Rhamnose	Folic Acid	Zinc
Ester of Cinnamic	Aldopentose	Ascorbic Acid	Chromium
Acid	Galactose	α- Tocopherol	Copper
Anthranol		β - Carotene	Magnesium
Chrysophanic Acid			Iron
Resistannol			
Anthracene			
Ethereal Oil			

Table 2.1 (continued)

Enzymes	Nonessential Amino Acids	Essential Amino Acids	Miscellaneous
Cyclooxygenase	Histidine	Lysine	Cholesterol
Oxidase	Arginine	Threonine	Triglycerides
Amylase	Hydroxyproline	Valine	Steroids
Catalase	Aspartic Acid	Leucine	β – Sitosterol
Lipase	Glutamic Acid	Isoleucine	Lignins
Alkaline	Proline	Phenylalanine	Uric Acid
phospahatase	Glycine	Methionine	Gibberellin
Carboxypeptidase	Alanine		Lectin
			Salicylic Acid

Source Vogler & Ernst (1999)

2.2.1 Polysaccharides

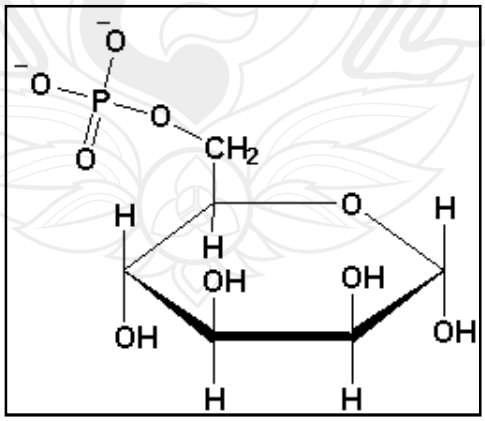


Figure 2.2 Structure of Mannose-6-Phosphate

Polysaccharides can be founded in latex and gel of Aloe vera (Pachanon, 2005). It is acemannan which is the derivatives of mannose including mannose-6-phosphate which stimulate collagen and increasing the rate of collagen produced. And also stimulates cell growth at the edge of the wounds and increasing in all attachment. As mannose-6-phosphate work as a connected to Growth factor receptor of the fibroblast (The World Health Organisation, 1999).

2.2.2 Anthraquinones

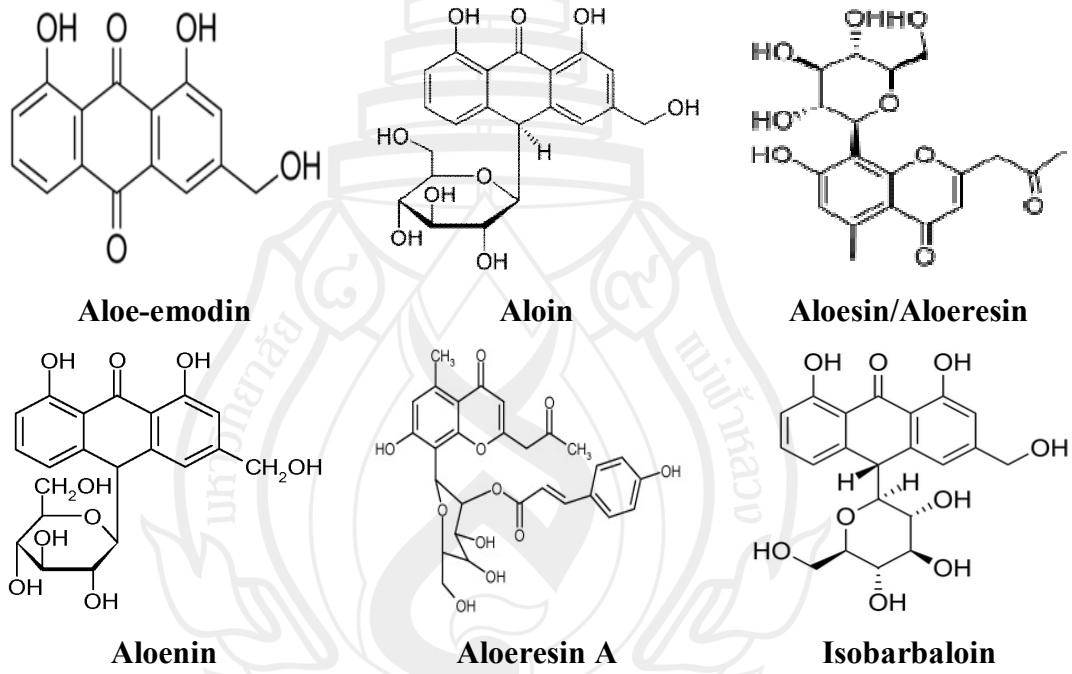


Figure 2.3 Chemical structure of main anthraquinone compounds that found in Aloe vera.

Anthraquinones is phenolic compound that consists of aloin and aloe-emodin these two compounds act as an anti-bacterial. Aloin can be found at 15-40% in aloe vera gel. Nevertheless, Aloin is a marker or key active ingredient in Aloe Vera for cosmetic industry. The content of Aloin analysed the by TLC HPLC and Column chromatography.

2.2.3 Aloin and Aloe-emodin

Aloin can be found in Aloe vera. It has a bitter taste and when hydrolysed it is called “Aloe-emodin”, slightly soluble in water and organic solvents (Ishii, Tanizawa & Takino, 1991). Aloe-emodin is part of anthraquinone, It acts as laxative and reduces inflammation. Aloe-emodin can also inhibit or slow down cancer cells. As Aloe-emodin is used in cosmetic to reduce inflammation, therefore, people have stereotype that Aloe vera can reduce inflammation as well.

In order to determine content of Aloe-emodin or other Anthraquinone can use the method of UPLC, TLC and HPLC. The method of UPLC to determine the Anthraquinones in plasma of patient after injuries and administration of Rhubarb and the determination of aloe emodin in Aloe vera extracts and commercial formulations by HPLC also have been reported.

2.2.4 Salicylic Acid

Salicylic acts as a keratinolytic agent, it causes epidermis cell to shed faster. This helps to open the clogged pores. Salicylic acid can be found in Aloe vera, this is one of the reason why using Aloe Vera can be used in anti acne formulation. And most of people are not aware about this role of Aloe vera as they only concern about Anthraquinone.

2.3 Dosage form of Aloe Vera in Cosmetic Formulations

Dosage form of Aloe vera that have been using in cosmetic formulation can be divided into 3 categories according to its physical appearance as follow

2.3.1 Fresh Aloe Vera Gel

Fresh aloe vera gel has been used for long time in cosmetic formulations. However, fresh aloe vera gel creates difficulties during the process of applying to the cosmetic products due to its quality standard cannot be control. Nevertheless, Soap manufacturers are still using fresh aloe vera gel for their formulation.

2.3.2 Aloe Vera Powder

Aloe vera powder extract is broadly used in cosmetic formulations because it is convenience to formulate. And it creates the marketing claim that it is 100% Aloe vera for example 100% aloe vera gel. As users believe that powder extract has the highest concentration and purification when compared to others form of Aloe vera. In the market, Aloe vera has many types categorize by its concentration and method of production. For example the concentration of aloe vera extract powder is called 100X and 200X which means 200 times concentrate than normal extraction. The processes of making Aloe Vera powder is either using freeze dry or spray dry method. Regarding the Spray dry method has to adding maltodextrin to act as carrier. And these caused the purity of Aloe Vera powder not to be as pure as people understand. According to figure 2.4 shown the process of Aloe Vera powder making.

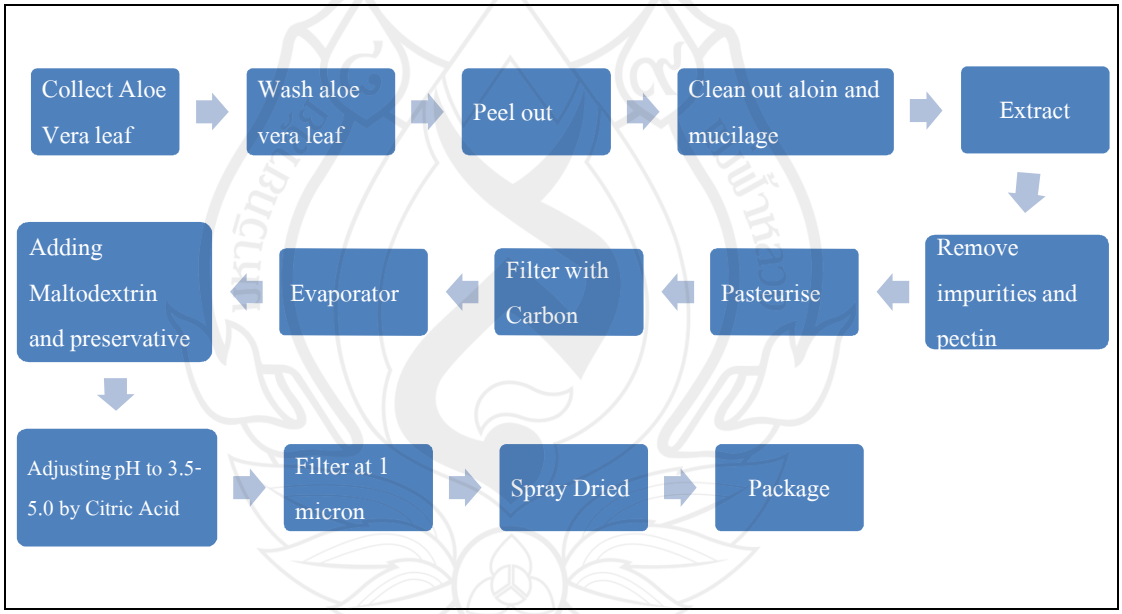


Figure 2.4 Manufacturer process of Aloe Vera powder 200X extract

2.3.2.1 Type of carrier in spray dry process

Carrier agents for spray drying are widely used among the food manufacturer. This is because without carrier agents the product such as aloe vera juice can easily stick on the chamber wall of the dryer and cause operation problem.

This carrier agents have to have high molecular weight. Examples of carrier agents are maltodextrins, gum Arabic, waxy starch and microcrystalline cellulose. In order to select the suitable carrier agents the formulator has to considers their particle size, shape, bulk density and their compatibility. According to cosmetic manufacturer maltodextrin is widely used.

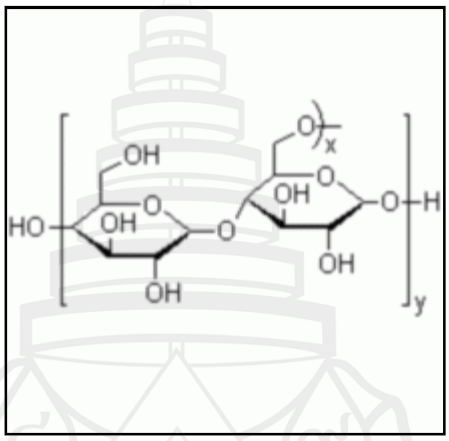


Figure 2.5 Maltodextrin chemical structure Maltodextrin consists of maltose and dextrin, which dextrin are mixtures if polymers of D-glucose.

2.3.3 Liquid Aloe Vera Extract

Liquid Aloe vera extract is popular type of extract apart from powder form. This dues to its quality and convenience to formulate as the formulator can easily add the extract without dissolve. However, its disadvantage is that it cannot be claim as 100% Aloe vera as its process requires solvent and preservative. In this research the type of liquid aloe vera extract is using Propylene glycol as solvent and using the process of maceration.

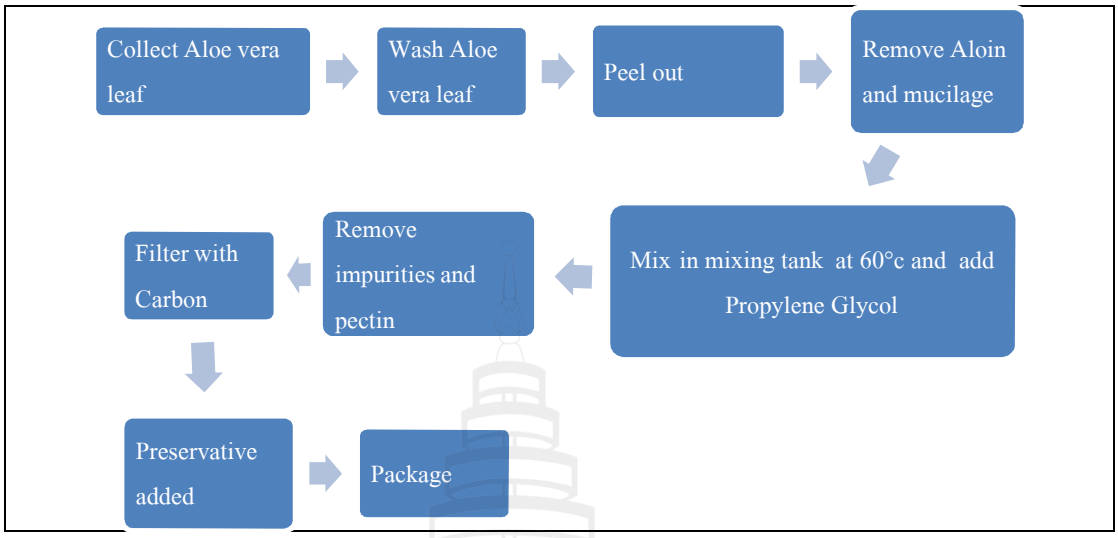


Figure 2.6 Manufacturer process of Liquid Aloe vera extract

2.4 Ultra High Performance Liquid Chromatography

Figure 2.7 UPLC Machine

Ultra-high performance liquid chromatography is a variant of HPLC (High performance liquid chromatography) using columns with particle size less than 2 µm. It provides better resolution, speed and sensitivity than HPLC. Chromatography is used to separate multi-component mixture of trace into individual fraction. It is separated through porous medium under the solvent.

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Equipments

3.1.1 Chemicals

3.1.1.1 Fresh aloe vera extract (Samutsakorn province)

3.1.1.2 Aloe Vera powder 200x extract (CAC, USA)

3.1.1.3 Commercial liquid extract (Kingvish Kompany, India)

3.1.1.4 Glucose (Merck, Germany)

3.1.1.5 Mannose (Merck, Germany)

3.1.1.6 Phenol (Merck, Germany)

3.1.1.7 Sulfuric acid (Merck, Germany)

3.1.1.8 Ethanol (Merck, Germany)

3.1.1.9 Acetic acid (Merck, Germany)

3.1.1.10 Carbomer (Zhongbao, China)

3.1.1.11 Propylene Glycol (Prolabo)

3.1.1.12 Sodium Hydroxide (Merck, Germany)

3.1.2 Equipments

3.1.2.1 Ultra- High Performance Liquid Chromatography (Waters, 600e)

3.1.2.2 Column 80 TM (5µm, 4.6 x 150 mm)

3.1.2.3 Spectrophotometer UV/VIS (GenTech)

3.1.2.4 pH meter (Satorius Docu-pH^{+} Meter PY-11, Germany)

3.1.2.5 Hot air oven (Binder BF 53, Germany)

3.1.2.6 Refrigerator

3.1.2.7 Stirrer (IKA, Germany)

3.1.2.8 Viscometer (DV-II + Pro, Brookfield, USA)

3.1.2.9 Analytical balance 0.1 mg. (Sartorius, Germany)

3.1.2.10 Vortex mixer (Vortex genic-Z)

3.2 Sample Preparation

3.2.1 Fresh Aloe Vera Extract Preparation and Extraction

Aloe vera leaf was collected from Samutsakorn province (22nd April 2014), cleaned and peeled. Aloe vera gel was rinsed with water to get rid of the aloin or yellow mucilage of aloe vera. Then grinded by using blender. Aloe vera juice was heated up to temperature of 60°C for 15 minutes to kill pathogen and to inactivate enzyme activity that can cause any change during storage. Aloe vera juice was filtrated by 16 cm mesh sieve before filled to container.

3.2.2 Standardize Aloe Vera Extract

Freeze dry was carried out to create the same condition of extract into powder form. For the fresh aloe vera extract and commercial liquid extract was under the freeze dry process due to its liquid form, this evaporated all the water from the extract.

3.3 Determination of Total Carbohydrate Content

By dissolved the sample of fresh Aloe vera extracts was used at the concentration of 0.5 mg/ml, the concentration of commercial liquid extract was 0.1 mg/ml and aloe vera powder 200X extract concentration was 0.5 mg/ml. All the extracts were prepared at the volume of 0.1 ml and 0.49 ml DI water was added. Mix with 0.5 ml of 5% Phenol solution. Lastly, added 2.5 ml of Sulfuric acid. Then incubated at 50˚C and avoid contacted with light for 20 minutes. The absorbance of sample was measured at 410 nm by Vis-Spectrophotometer. Standard curve of glucose and mannose were used. All the samples were done in triplicate.

3.3.1 Determination of Carbohydrate Content by Ethanol Precipitation.

3.3.1.1 Dissolved each aloe vera extract sample weight 0.1g in 10 ml of DI water.

3.3.1.2 Add 40 ml of 95% Ethanol and kept at 4°C for 4 hours

3.3.1.3 Centrifuged at 8000 rpm for 30minutes

3.3.1.4 Separated the precipitation

3.3.1.5 Dissolve the precipitation into DI water and repeat the determine carbohydrate content as mentioned in 3.3

3.4 Determination of Aloe-emodin by UPLC Method

3.4.1 Preparation of Standard Solution

3.4.1.1 Preparation of stock standard solution

Aloe-emodin solution was prepared to achieve the concentration of 0.1 mg/ml by weighing 1 mg of aloe-emodin in 10 ml volume metric flask and adjusted the volume by DI water.

3.4.1.2 Working Standard Solution

From the solution 3.5.1.1, diluted to get the concentration of 0.1, 0.5, 1, 5 and 10 mg/mL and adjust the volume by DI water.

3.4.1.3 Sample preparation

The sample of aloe vera extract was weighed for 5 mg into 10 ml volumetric flask and adjusted the volume with DI water.

3.4.2 UPLC Condition

UPLC analysis was carried out by Water 600e equipped with Aquity PDA eλ detector measured at 254 nm. Data analysis was performed by Empower III softwear. Separation was achieved isocratically at 25˚C on a 1.7 µm, 2.1 x 100 mm , Acquity UPLC Column. Used nylon syringe filter 0.2 µm. The mobile phase consisted of methanol and 2% acetic acid solution at 70:30 (v/v). Pumped at a flow rate of 1mL/min. The injection volume was 5 µL.

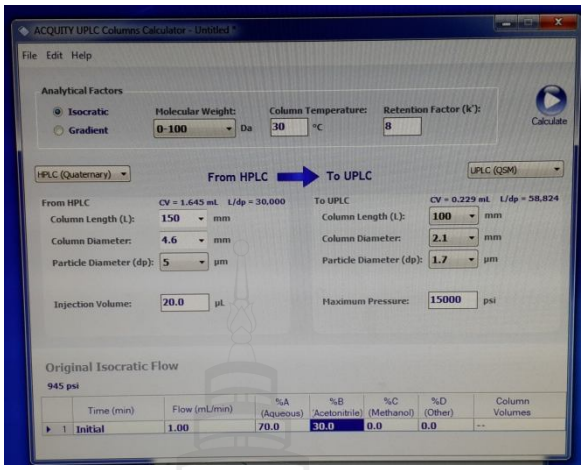


Figure 3.1 Empower III using for converted HPLC condition into UPLC condition

Empower III was using to convert the suitable condition of HPLC into UPLC condition as showed in figure 3.4.2. At the beginning the HPLC condition for determined aloe-emodin was 150 mm x 4.6 mm particle diameter was 5µm and injection volume was 20µL. Regarding the UPLC condition, it was converted as 100 mm x 2.1 mm,1.7µm and the maximum pressure was 15000 psi.

3.5 Stability Test and Factors Affecting of After Sun Gel from Aloe Vera Extract

Table 3.1 Formulation

Raw Material	Function	Control (%w/w)	FAV (%w/w)	CL (%w/w)	AVP (%w/w)
AloeVera Extract	Active	-	5	5	5
Propylene Glycol	Moisturizing	15	15	15	15
Carbopol 940	Thickener	0.5	0.5	0.5	0.5
Sodium Hydroxide	Neutralizer	0.25	0.25	0.25	0.25
Deionizer Water	Solvent	84	79	79	79
Germal Plus	Preservative	0.25	0.25	0.25	0.25

Note. FAV: Fresh Aloe Vera
CL: Commercial liquid
AVP: Aloe Vera powder 200X

3.5.1 Stability of After Sun Gel

3.5.1.1 Measured the pH value and viscosity from 4 formulations of after sun gel and recorded the results.

3.5.1.2 Kept at bottle volume 250 mL after sun gel in 45 ˚C for 24 hours then kept at 4˚C for 24 hours. Repeat this cycle for quadruplicate.

3.5.1.3 Measured the pH value and viscosity from 4 formulations of after sun gel and recorded the results.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Standardized Extracts by Freeze Dry Method

In order to create the same condition of the three extracts which are fresh aloe vera extract, commercial liquid extract and aloe vera powder 200x extract, Freeze dry method was used to make all the extracts into similar form and condition. However, the result after the freeze dry turned out as following; the beginning weight of fresh aloe vera extract was 5000 grams and weight after freeze dry was 19 grams. The commercial liquid extract the beginning weight was 1000 grams and weight after freeze dry was 458 grams.

Moreover, the liquid commercial extract did not turn out to be in powder form after the freeze dry process. It was still in liquid form which had little higher in term of viscosity. This was because propylene glycol was used as the solvent for the extraction. Propylene glycol could not turn into powder form via the freeze dry method.

Table 4.1 Weight of freeze dry method of fresh aloe vera extract and liquid

Extracts	Beginning Weight (g)	Yield (g)	Yield (%)
Fresh aloe vera juice	5000	19	0.38
Liquid commercial extract	1000	458	45.8

4.2 Determination of Total Carbohydrate Content

The total carbohydrate contents were determined by using coloring method for determination of sugars. The comparison of total carbohydrate from Aloe vera extract in gram glucose equivalent per gram of aloe vera extract. The glucose content founded in fresh Aloe vera extract, commercial liquid extract, and aloe vera powder 200x extract were 0.233, 0.037 and 1.285 mg GE/g, respectively. And for mannose contents there were 0.178, 0.029 and 0.981 mg ME/g respectively.

Table 4.2 Total Carbohydrate contents

Extracts	Total Carbohydrate contents	
	mg GE/g*	mg ME/g**
Fresh aloe vera extract	0.233	0.178
Liquid commercial	0.037	0.029
Aloe vera powder 200x	1.285	0.981

Note. * Glucose Content (mg Glucose Eq./g.)
** Mannose Content (mg Mannose Eq./g)

Regarding the result of total carbohydrate contents as showed in Table 4.2, the mannose content represented the purity of aloe vera, as mannose could be found as highest content when compared with others polysaccharide in aloe vera. Glucose content represented other polysaccharide that contained in aloe vera. However, aloe vera powder 200x showed the highest content of glucose which could be from its raw material during process such as maltodextrin. This maltodextrin used as carrier during spray dry process.

4.2.1 Determination of Carbohydrate Content by Ethanol Precipitation

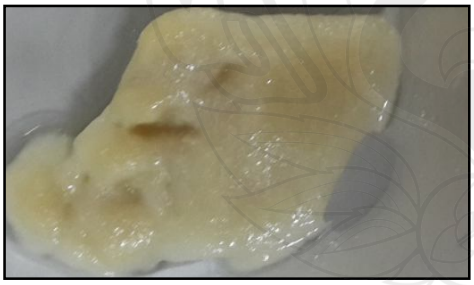
The total carbohydrate contents were calculated by using the method of ethanol precipitate this was done to make the polysaccharide to precipitate. The result showed that fresh aloe vera extract gave 53.51 g from 10 ml of extract. Aloe vera powder 200x showed 32.31 g.

Table 4.3 Weight before and after ethanol precipitation method of Aloe vera extract

Extracts	Beginning Weight (g)	Precipitate (g)
Fresh Aloe Vera	100	53.51
Aloe vera powder 200x	100	32.31

The precipitate of aloe vera extract has showed that the fresh Aloe vera extract had its precipitate in yellow brown color and the texture was similar to pectin. And for Aloe vera powder 200X had its precipitate as shinny slightly yellow and the outer layer was transparent. The precipitate of liquid commercial extract was not detected.

Figure 4.2.1 show the precipitate after centrifuged.



Precipitate of fresh Aloe vera extract

Precipitate of Aloe vera extract powder 200X

Figure 4.1 Precipitate of Aloe vera extract

After the precipitation process, total carbohydrate contents were measured by coloring method for determination of sugars. The results show as below.

Table 4.4 Total Carbohydrate contents by ethanol precipitation

Extracts	**Total Carbohydrate contents**	
	mg GE/g*	**mg ME/g****
Fresh aloe vera	0.015	0.011
Aloe vera powder 200x	0.202	0.154

Note. * Glucose Content (mg Glucose Eq./g.)

** Mannose Content (mg Mannose Eq./g)

From the determination of the Aloe vera precipitate it revealed that glucose content founded in fresh Aloe vera extract, and aloe vera powder 200x extract were 0.015 mg GE/g and 0.202 mg GE/g respectively. And for mannose contents it was 0.011 mg ME/g and 0.154 mg ME/g respectively.

4.3 UPLC analysis of Aloe-emodin in Fresh Aloe Vera Extract, Commercial Liquid Extract and Aloe Vera Powder 200x Extract

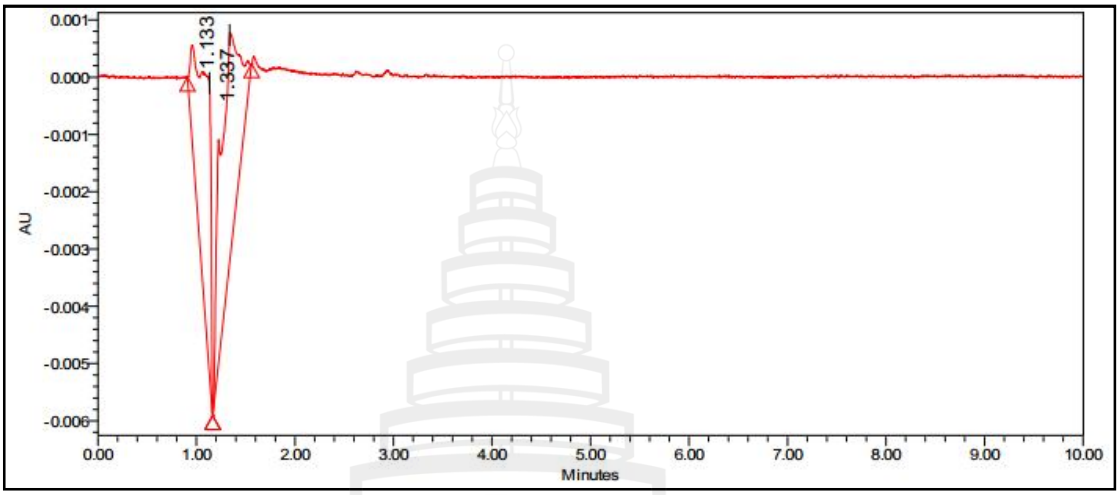
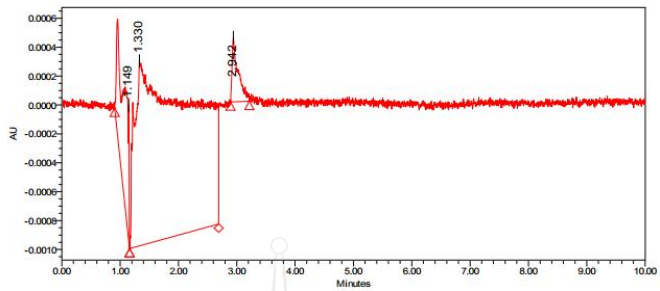


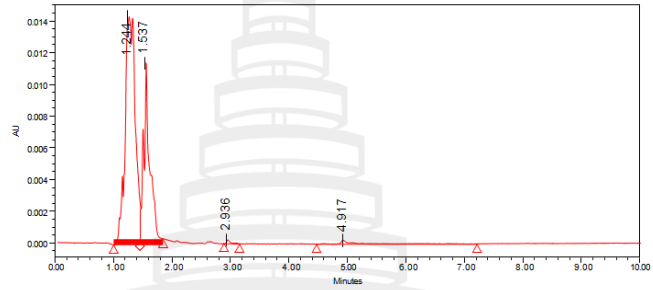
Figure 4.2 Chromatogram of Blank solution

The figure 4.2 showed the chromatograph of blank solution, which is DI water that have been filtered already. This blank solution was injected to examine its peak.

Aloe-emodin was not detected in Commercial liquid extract, and Aloe vera powder 200X. However, in fresh Aloe Vera extract the chromatogram shown that Aloe emodin was detected but the peak area was too small to use for calculate the Aloe-emodin content. The table 4.3 had revealed the peak area and retention time of aloe-emodin.



(a)



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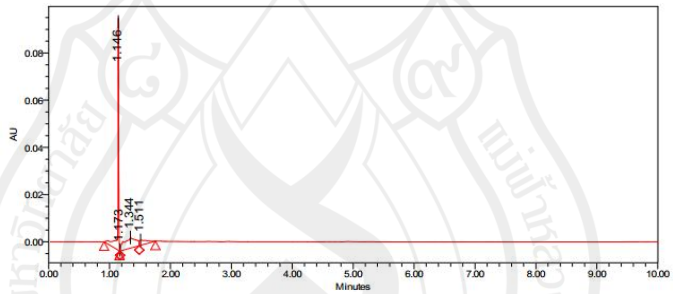

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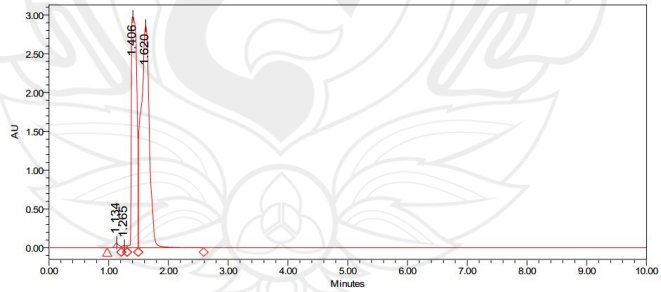

(d)

Note. Chromatogram of (a) standard solution (Aloe-emodin), (b) fresh aloe vera extract, (c) liquid commercial extract, (d) aloe vera powder 200x

Figure 4.3 UPLC-Chromatograms of the standards and samples.

Table 4.5 Peak area of each aloe vera extract and aloe emodin standard

Extracts	Retention time	Peak area	Concentration of aloe-emodin (mg / g of extract)
Aloe-emodin	2.942	3200	Standard
Fresh aloe vera extract	2.936	1191	0.12
Liquid commercial extract	n.d.	n.d.*	0
Aloe vera powder 200x	n.d.	n.d.	0

Note. *Not detected

4.4 Stability Test and Physical Effect on Aloe Vera Extracts in After Sun Gel Formulation

Regarding the stability test result, fresh aloe vera extract affected the viscosity of the gel to be increased from 31800 cps. to 79200 cps. and for liquid commercial extract the viscosity is slightly changed in term of decreasing which was 81800 cps. to 70300 cps. And Aloe Vera powder 200x affected the viscosity of the gel to decrease from 101000 cps.

However, the color of the gel had slightly changed as well, because for fresh aloe vera extract formulation, the gel lost its transparency. And in the beginning this fresh aloe vera extract after sun gel formulation was colorless but after freeze thaw process the gel became slightly yellow.

Figure 4.4 After sun gel with 4 formulations that kept at room temperature.

Figure 4.5 After sun gel that kept under freeze thaw condition for 4 cycles.

Table 4.6 Characteristics of After Sun Gel were recorded to check the stability and pH value.

Extracts	Viscosity*		pH Value	
	Before Freeze Thaw (cps)	**After Freeze Thaw (cps)**	**Before Freeze Thaw**	**After Freeze Thaw**
Formula 1	139000	129000	8.00	8.09
Formula 2	31800	79200	7.92	7.94
Formula 3	81800	70300	8.40	8.35
Formula 4	101000	58480	7.65	8.04

Note. * 5 rpm % torque ≥ 80%

Formula 1: Blank

Formula 2: Fresh aloe vera extract

Formula 3: Liquid Extract

Formula 4: Aloe vera powder 200x

CHAPTER 5

CONCLUSION

In conclusion, aloe vera powder 200x extract contains highest total mannose contents (0.981g. Mannose Eq./g.) followed by fresh aloe vera extract (0.0178 g. Mannose Eq./g.) and Liquid commercial extract (0.028 g. Mannose Eq./g.). Analysis Aloe-emodin contents by UPLC method, fresh aloe vera extract has Aloe-emodin 0.12 mg/g of crude extract. Liquid commercial aloe vera extract and Aloe vera powder 200x extract was not detected Aloe-emodin. Methanol and 2% acetic acid (70:30, v/v) is suitable for mobile phase solvent as to analyse Aloe-emodin, which is useful indicator to calculate the concentration of Aloe-emodin in crude extract.

Regarding the stability test of after sun gel, fresh aloe vera extracts is the only extract that increased the viscosity of After sun gel. And Aloe vera powder 200x extract has reduced the viscosity of the gel.

According the use of Aloe vera extract, formulator can used fresh aloe vera extract to give the highest result in anti inflammatory, as it contain highest result in aloe-emodin. Moreover, for the formulation that will give the highest moisturizing effects, Aloe vera powder 200x, fresh aloe vera extract and liquid commercial Aloe vera extract respectively.

For further study regarding this research, other anthraquinone such as Rhein and emodin can also be determined by the method in this research as well.

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APPENDICES

APPENDIX A

SPECIFICATIOM SHEET OF ALOE VERA EXTRACT

Kingvish Kompany

Email: Kingvish@kingvish.com| Website: www.kingvish.com

SPECIFICATION SHEET

PRODUCT : ALOE VERA EXTRACT (Clear) (Aloe Barbadenis)
FAMILY : ASPHODELACEAE

TEST PARAMETER	STANDARD
1. Appearance	Clear transparent
2. Solubility in water	Soluble
3. Odor	Odorless
4. Fruit acid	Present by TLC
5. pH	4.0 - 8.0
6. Specific Gravity @ 25 deg	1.0 - 1.1
7. Refractive Index @ 25 deg C	1.3 - 1.5
8. Total Microbial Count	100 cfu / gram Max.
9. E.Coli & Salmonella	Absent
10.Heavy Metals	10 PPM Max.

REMARKS: MATERIAL COMPLIES WITH THE SPECIFIED DETAIL.

PLEASE KEEP THE MATERIAL WITHIN 35 DEGREE CENTIGRATES AND AWAY FROM DIRECT SUN LIGHT.

APPENDIX B

STANDARD CURVE OF GLUCOSE AND MANNOSE

Table B1 Concentration of Glucose in aloe vera extract

Concentration of Glucose (mg/ml)	0	0.0014	0.0028	0.0054	0.0080	0.0104	0.0149
A490	0	0.079	0.169	0.359	0.505	0.643	0.840

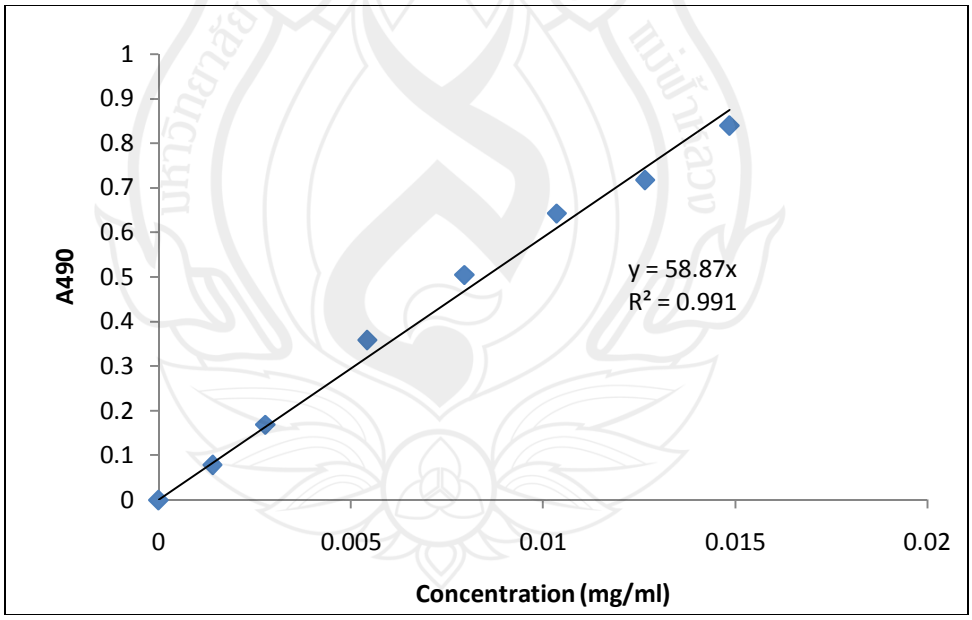


Figure B1 Standard curve of glucose content

Table B2 Concentration of mannose in aloe vera extract

Concentration of Glucose (mg/ml)	0	0.0014	0.0028	0.0054	0.0080	0.0104	0.0127
A490	0	0.155	0.263	0.437	0.622	0.765	0.975

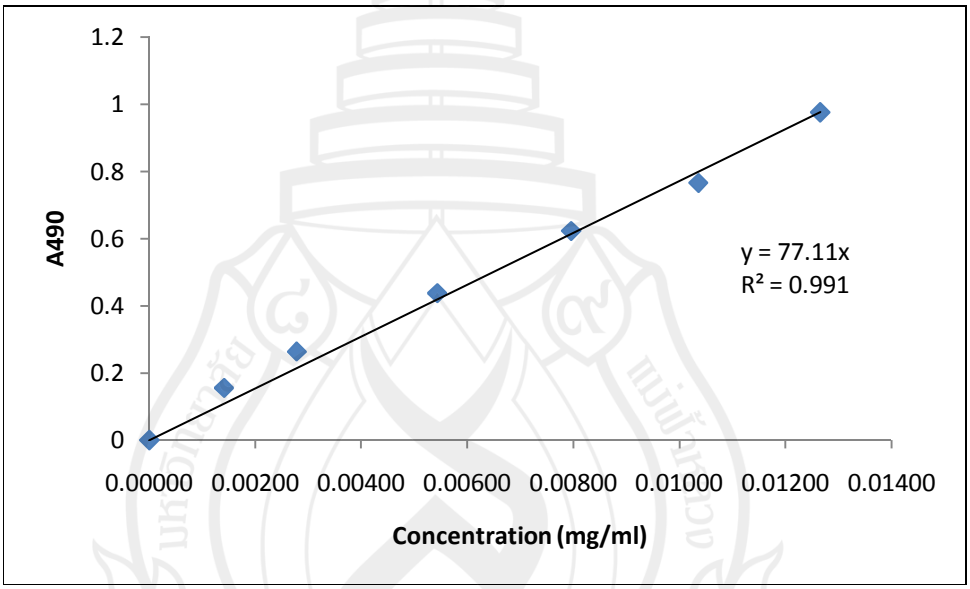
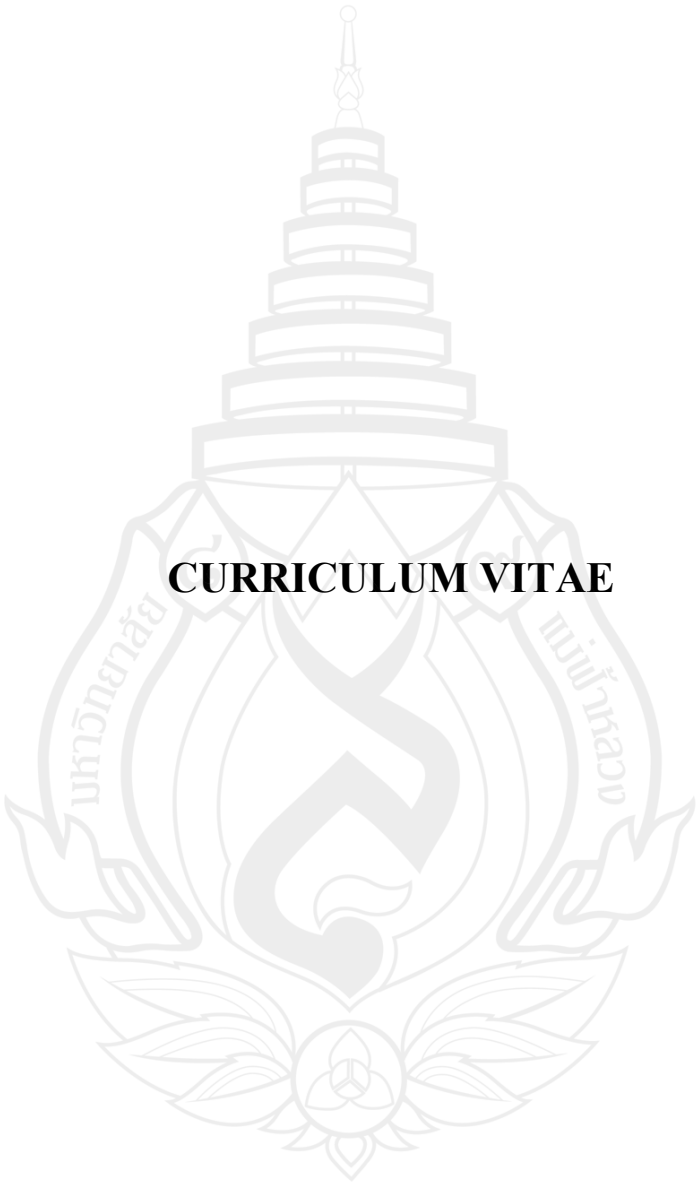


Figure B2 Standard curve of mannose content



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