EXTRACTION OF SAPONIN FROM GAC (*MOMORDICA COCHINCHINENSIS SPRENG*) SEED KERNELS USING SUBCRITICAL DIMETHYL ETHER COMPARED WITH CONVENTIONAL ORGANIC SOLVENTS.

KITTAPAT SOOKNUAN

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Engineering in Chemical Engineering - (Type A 1)
2023

มหาวิทยาลัยนเรศวร

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By Kittapat Sooknuan

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Engineering in Chemical Engineering - (Type A 1) of Naresuan University

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Title EXTRACTION OF SAPONIN FROM GAC (*MOMORDICA COCHINCHINENSIS SPRENG*) SEED KERNELS USING SUBCRITICAL DIMETHYL ETHER COMPARED WITH CONVENTIONAL ORGANIC SOLVENTS.

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ABSTRACT

The purpose of this study is to determine solvent system for enhance the saponins recovery from gac (*Momordica cochinchinesis* Spreng) seed kernels, the waste product from food industry. The preliminary results of analysis of gac seed sample and solubility approximation of target solutes in selected solvents were determined. Hansen solubility parameters (HSPs) and spheres were evaluated and used as a guideline for solvent selection. The prediction results revealed that single solvent system of methanol, ethanol, propanol, n-butanol hexane and dimethyl ether (DME) might recover saponins only one part of matrix or oil body of gac seed kernel which related to the experimental recovery results. The predictions suggested that co-solvent of DME with 10 to 15 %wt of methanol, 10 to 25% wt of ethanol, and 10 to 35% wt of n-butanol were potential systems for recovery saponins from both parts of matrix and oil body. The experimental results showed that ethanol and n-butanol at 25% wt gave 64.6% and 72.35% of saponins recovery, respectively and the highest saponin recovery was 87.27% by using 75% wt DME with 25% wt butanol as a co-solvent extraction to 120 minutes.

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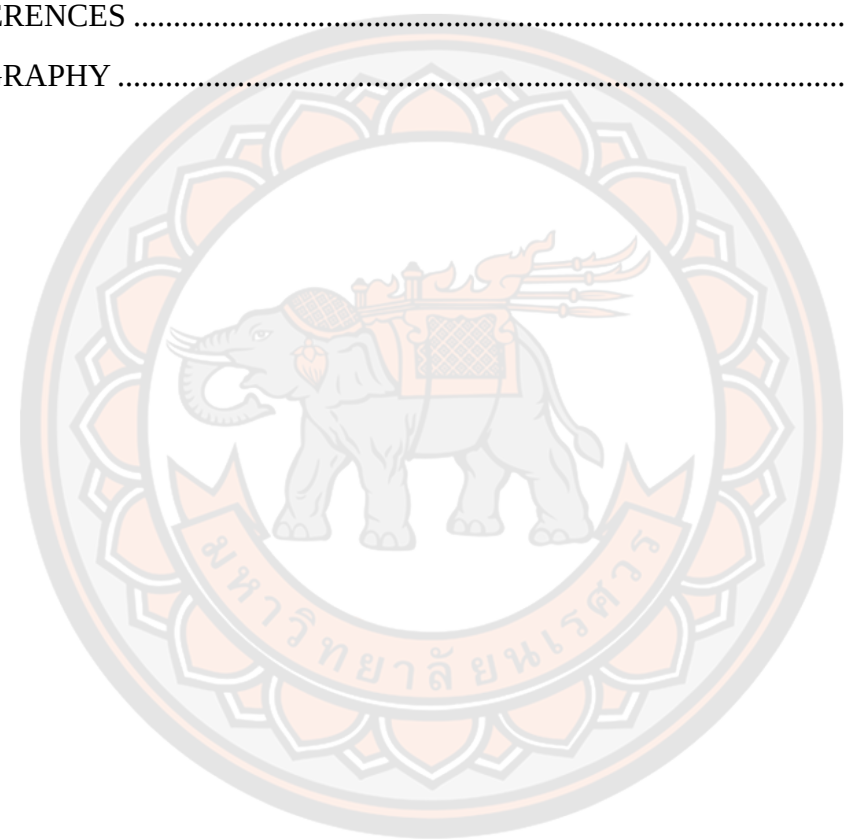

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CHAPTER I

INTRODUCTION

1.1 Introduction

Gac plant (Momordica cochinchinensis Spreng) belongs to the Cucurbitaceae family, and in nature its dioecious vines grow by climbing trees, reaching up to 20 meters in length. Gac originates from Vietnam and can be found widely in Northeastern Australia, South Asia, and Southeast Asia, including Thailand. The pulp, peel, and aril of the red-orange gac fruit are rich sources of carotenoids such as β-carotene, lutein and lycopene (Kha et al., 2013). Because of their antioxidant content, gac fruits are consumed fresh, or are made into juice and nutritional supplements (Do et al., 2019). This leaves the inedible seeds as waste (Le, Huynh, et al., 2018). However, it has been reported that kernel of the gac seeds contains high amount of saponins (38.8 - 100.3 mg/g of dried seed kernels) (Le, Huynh, et al., 2018; Le, Parks, et al., 2018), bio-active compounds known to have a variety of health benefits, including exhibiting anti-breast cancer, antioxidant, anti-gastritis, anti-inflammatory, and wound-healing abilities (Jung, Chin, Chung, et al., 2013; Zheng et al., 2015) . The most abundant saponin in gac seed kernels (GSKs) is a triterpene aglycone, called Momordica saponin (Yu, Kim, et al., 2017), whose chemical structure is shown in **Figure 1**. Moreover, GSK also contains high amounts of oil (50 to 60%wt in dried GSK) (V. Le et al., 2018b) which approximately 55 to 75% of total saponins are observed to partition in the oil body (V. Le et al., 2018a) (25 to 45% of total saponins partitioned in the matrices of GSK).

Saponins are polar compounds and are expected to dissolve in polar solvents (alcohols). But in presence of large amount of oil in GSK, alcohol could not easily access the saponins. To recover saponins, GSKs are normally first extracted with hexane to remove oil, prior to extraction with alcohol. However, since saponins in GSK resides in two parts: 55 to 75% in the oil body (V. Le et al., 2018a) and 25 to 45% in the matrix of the gac seed. Pre-extraction with hexane will cause considerable loss of saponins into the oil fraction. To achieve complete saponins recovery, it therefore generally requires two steps: extraction with hexane followed by alcohol such as ethanol, propanol, and n-butanol (V. Le et al., 2018a). The hexane saponin-oil extract and the alcohol extract of defatted GSK matrix are then combined. Extraction with hexane alone could not recover the fraction of saponin in GSK matrix, while alcohol could not recover saponin resided in the oil extract.

In term of economic, the one-step recovery of saponins is still more attractive since it requires less equipment. However, as mentioned, single solvent of hexane or alcohols leads to low performance of saponins recovery. To enhance the process, co-solvent system of hexane with possible alcohols such as ethanol or butanol might be possible but the toxicity of hexane is a major concern. The study of one-step recovery

of saponins using single solvent of ethanol, considering as a safe extraction solvent, was reported to be achieved. The significant higher of saponins recovery was observed compared with the pre-extracted with hexane sample (105.7±2.4 vs 25.8±2.3 mg/g of dried seed). However, the extraction process requires microwave-assisted to enhance the extraction performance that causes this system less practical for larger scale production because of its high equipment cost (Li et al., 2013).

Alternatively, liquified dimethyl ether (DME) is widely used as an organic solvent for extraction such as vegetable or seed oils and other bio-active components from natural sources such as microalgae, rubber seed, and marigold flower (Bauer et al., 2023; Boonnoun et al., 2019; Boonnoun et al., 2017) . DME is partially miscible in polar solvents including water and alcohols, making it possible to adjust the polarity of the solvent system to be suitable for this purpose. DME is also a low toxic gas at room temperature, with a low boiling point (-24.8℃), which allows for easy separation and removal from final extracts using pressure reduction. It has been approved as a safe solvent for the production of foodstuffs and food ingredients by the European Food Safety Authority (EFSA) and the Food Standards Australia New Zealand (FSANZ) (Sparg et al., 2004). Extraction using DME has been previously applied on a large scale, with reports of both pilot and industrial scale projects such as preparation of protein powder in the food industry as well as extraction of oils, polar lipids, and vitamins (Fang et al., 2018; Fang et al., 2020; Liu et al., 2019). Importantly, extraction with DME occurs at mild conditions (Goto et al., 2015; Noriyasu et al., 2015) , which makes the process practical and economical(Liu et al., 2019).

This current study therefore investigates the method of one-step recovery saponins from GSK without prior defatting process using DME as the main solvent with alcohols as co-solvent. The analysis of GSK sample was firstly performed to determine the composition of saponins and GSK oil in both parts of matrix and oil body. The composition was then used to estimate Hansen Solubility Parameters (HSPs), solubility prediction of specific solute in organic solvents (Detriche et al., 2008; Tirado et al., 2018), of the oil body solute (oil and Momordica saponin). The HSPs predictions of saponins in oil body defined as solute mixture and in the matrix defined as pure Momordica saponin. The predictions of two solutes were evaluated and used as a guideline for the suitable solvent system selection which firstly considered a single solvent system of DME and conventional solvents including methanol, ethanol, propanol, n-butanol, and hexane. The possibility of using DME with co-solvent to enhance saponins recovery was then investigated. The effects of co-solvent type and its composition were also reported in this work.

1.2 Objectives

1.2.1 Study of single step saponin recovery from GSK without defatting process.

1.2.2 Apply Hansen solubility parameters for predict and enhance the suitable solvent system for saponin recovery from GSK.

1.3 Scope of work

1.3.1 Study the comparison of different GSK sample preparation (defatted sample and full-fat sample) effect to saponins recovery.

1.3.2 Using HSPs to predict the possibility of solvent systems for saponins recovery therefore considered with extraction experiments.

1.3.3 Select the suitable solvent (methanol, ethanol, propanol, n-butanol, and sub-critical DME) for saponins recover.

1.3.4 Select the suitable solvents system for saponins recovery.

1.3.5 Adjust the suitable extraction condition (percentage of cosolvent and extraction temperature) for saponins recovery.

1.4 Expected outcome

The results from this work are expected to provide the important information of the suitable solvent system and extraction conditions to obtain the highest amount saponins recovery from gac seed kernels. This finding might be the guideline for the future work of extraction in larger scale such as pilot and industrial scale.

CHAPTER II

BACKGROUND & LITERATURE REVIEW

Articles used in this research are related to gac fruit, gac seed, saponins in gac seeds, conventional solvent extraction, supper/sub-critical solvent extraction, defatting process, and Hansen solubility parameters.

2.1 Gac fruit

Gac fruit (botanical name: Momordica *cochinchinensis* spreng) is in Curcurbitacece family and originally from Vietnam. Gac plant glows on vines twining up tree trunks or supported by trellises in commercial farms and home gardens and grown around South and Southeast Asia and Northeastern Australia (Kha et al., 2013).

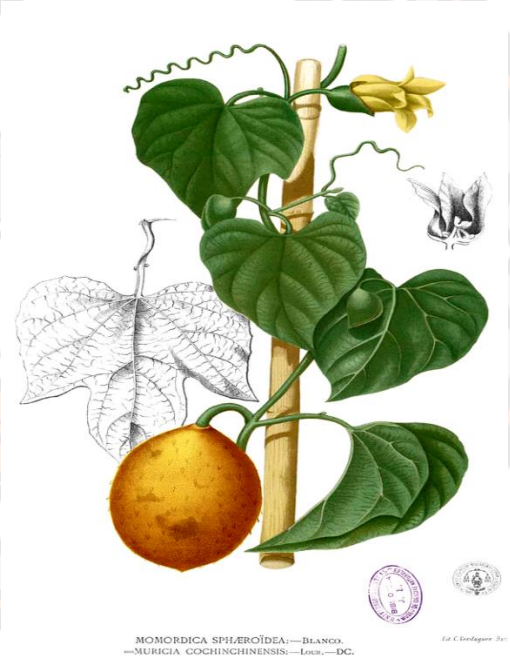

Figure 1 Gac fruit vines

Gac has been commonly used in its native countries, mainly as food and traditional medicine. Its use as medicine has been dated back to over 1200 years ago in China and Vietnam.

The names of this plant in any places will suggest its historical significance and widespread applications as food and medicine (Do et al., 2019) and various names as shown in **Table 1** (Huynh & Nguyen, 2020).

Table 1 Name of Gac fruit in many countries.

Language	**Name**
Latin	Momordica *cochinchinensis* Spreng, Muricia cochinchinensis Lour

English	Chinese bitter cucumber, spiny bitter gourd, Cochinchin gourd
Chinese	Mu Bie, Mu BieZi, Teng Tong, Tu Mu Bie
Vietnamese	Gac, MocMiet Tu
Thai	Bat-Khai-Du, Phak-Khao
Hindi	Bhat-Karela, Gangerua, Kakrol, Kantola
Laos	Khaawz
Malais	Teruah
Tagalog	Buyok-buyok, Sugod-sugod

Table 2 List of gac suppliers by Thailand and their products.

Manufacturers/Trading companies	**Gac products**
MagnaGac	Extract
Phusirath Company Limited	Powder
D2 Holding Company Limited	Juice
P.O.P. Siam Golden Fruit Limited	Blend
Partnership	Skin Balm
Lycopenelover Co,. Ltd.	Soap
Chaichada Co., Ltd.	

The ripe gac fruits will consist of 4 parts; there are seed, aril, pulp and spines. So, it has black seeds that covered by red aril (membrane), an orange pulp and dark orange peel with little spines on surface (**Figure 2**).

Figure 2 Anatomical components of gac fruit (1. Seed, 2. Pulp, 3. Aril, 4. Peel with spines) (Chuyen et al., 2015).

The gac seed membrane (red aril) contains very high concentration of β-carotene and lycopene. It was reported the β-carotene five time greater than amount measured in carrot and eight time greater than of lycopene content in tomatoes (Do et al., 2019; Kha et al., 2013).

Gac fruits is not only rich in β-carotene and lycopene but also contains high significant levels of other bioactive compounds such as α-tocopherol (vitamin E), phenolic compounds, saponins and flavonoids (Le, Huynh, et al., 2018) etc. In each

part of the gac fruits were found to be rich in any bioactive compound that shown in **Table 3**.

Table 3 Phytochemical compounds isolated from different parts of gac fruit and their functions.

Section	Function	Phytochemical compound
Fruit aril	Anticancer	35 kDa protein
	Antioxidant	β-Carotene, lycopene
	Antioxidant, anticancer	Lycopene, others
	Antioxidant, anticancer	Phenolics, flavonoids
	Antimicrobial	*
	Reproductive health, antioxidant	Phenolics, flavonoids
	Eye health	Carotenoids (β-carotene, lycopene, zeaxanthin, retinol)
	Anemia	Lycopene, β-carotene
Fruit peel	Antioxidant	Carotenoids, lutein
	Antimicrobial	*
	Antioxidant	Phenolics
Fruit pulp	Antioxidant	Apigenin
	Antioxidant	Carotenoids (β-carotene, lycopene, lutein), phenolics, flavonoids
	Antimutagenic	*
	Antimicrobial	*
Fruit seed (kernel)	Anticancer	Cochinchin B
	Trypsin inhibition	MCCTI-1 protein
	Trypsin inhibition	TI-1, TI-2, TI-3, TI-4, TI-5 proteins
	Intracellular targeting drug	MCoTI-I, MCoTI-II proteins
	Vaccine adjuvant	Saponins I and II
	Anticancer	MCoCC-1, MCoCC-2 proteins
	Anticancer	Momorcochin-S
	Anticancer	P-hydroxylcinnamaldehyde
	Anticancer	Saponins
	Anticancer	Trypsin inhibitors
	Glucose uptake activity	Saponins
	*	Triterpenoid ester
	Abortifacient	*
	Antiulcer, wound healing	*
	Anticancer	*
	Hypoglycemia	17 kDa protein
	Anti-inflammation	Saponin
	Antioxidation	Chymotrypsin inhibitor
	Antioxidation	Saponin

2.1.1 Gac seed

Gac seed is the part that covered by aril inside the fruit pulp. It is black or dark brown color and pretty hard skin when dried. Gac seeds consisted of 2 parts are gac seed kernel (GSK) and gac seed shell as shown in **Figure 3**. In each fruit contained about 15 – 20 seeds.

Figure 3 Gac seed kernels (GSK) and gac seed shell

The seed weight is important for the industrial processing of seeds (Le, Parks, et al., 2018) in general that not all seeds required the same grinding conditions and that variability in weight can affect the grinding time and the uniformity of the resulting powder. The average weight of fresh, dried seeds and dried kernels as shown in **Table 4**.

Table 4 Average seed weight (fresh and dried seeds)

Characteristics	**Component**	**Mean ± SD**
Average seed weight (g)	Fresh seeds	5.07 ± 0.19
Average seed weight (g)	Dried seeds	3.15 ± 0.12
Average kernel weight (g)	Dried kernels	2.09 ± 0.33
Moisture (%) Moisture Analyzer	Dried kernels	3.34 ± 0.10
Moisture (%) AOCS: Ab 2-49	Dried kernels	3.47 ± 0.11
Crude protein (%)	Dried kernels	17.33 ± 0.47
Oil content (%)	Dried kernels	53.02 ± 1.27

There are many studies reported about gac aril and gac pulp extraction studies unlike the gac seed, that because gac seeds was inedible and they are removed considered as waste (Do et al., 2019; Goto et al., 2015; Li et al., 2013). However, there are reported about bioactive compounds in GSK as shown in **Table 3** and the mainly is saponins (Le, Parks, et al., 2018; Yu, Kim, et al., 2017).

Table 5 Quantitative information of gac fruit

Title	**Range**	**References**
Fruit fresh weight (g/fruit)	316.78 - 704.06	(V. Le et al., 2018b)
Fruit number for year (fruits/tree/year)	18.00 – 69.00	

Number of seeds (seeds/fruit)	13.90 - 38.30	(Bhumsaidon & Chamchong, 2016)
Ratio fresh seeds in fruit (g seed/g fruit)	16.80	(Nhung et al., 2010)
Total Saponin content (g/kg dried kernels)	38.80 - 100.30	(Ishida et al., 2004; Le, Parks, et al., 2018)

So, the saponin content in GSK shown the potential of value. Moreover, utilization of gac seeds will help in the industrial waste reducing and there were lots of seeds supplies into that utilization process as shown in **Table 5**.

2.1.2 Saponins and saponins in GSK

Saponins are chemical compounds that occur in a wide range of herbs, seeds and vegetables. It's can also be found in starfish and sea cucumbers. In medicine, it's used in vaccine formulations to regulate immune function. Due to their antibacterial and foaming properties, these compounds are added to shampoos, soap, household cleaners and makeup products.

Several studies operated over the years confirm the health benefits of saponins. These chemicals may help reduce cholesterol levels, kill disease-causing bacteria, scavenge oxidative stress and inhibit tumor growth. According to the latest research, they improve lipid metabolism and may help prevent and treat obesity (V. Le et al., 2018a). The steroidal saponins are mostly found in monocotyledons, and triterpene saponins are greatly found in dicotyledons. While the main dietary sources of saponins are legumes (Airaodion et al., 2019). Saponins have been reported it had a wide range of biological properties. Which are summarized and listed alphabetically (Sparg et al., 2004) in **Table 6**.

Table 6 Reported biological activities of saponins.

Biological Activity
Adaptogenic
Adjuvant

Analgesic activity
Antiallergic
Antiedematous
Antiexudative
Antifeedant
Antifungal
Antigenotoxic
Antihepatotoxic inhibitory effect on ethanol absorption
Anti-inflammatory
Antimicrobial
Antimutagenic
Antiobesity
Antioxidant
Antiparasitic
Antiphlogistic
Antiprotozoal
Antipsoriatic
Antipyretic
Antispasmodic
Antithrombotic (effect on blood coagulability)
Antitussive (relieving or preventing cough)
Antiulcer
Antiviral
Chemopreventive
Cytotoxic
Diuretic
Effect on absorption of minerals and vitamins
Effect on animal growth (growth impairment), reproduction
Effect on cognitive behavior
Effect on ethanol induced amnesia
Effect on morphine/nicotine induced hyperactivity
Effects on ruminal fermentation
Expectorant
Haemolytic
Hepaprotective
Hypocholesterolemic
Hypoglemic
Immunostimulatory effects
Increase permeability of intestinal mucosa cells
Inhibit active nutrient transport
Molluscicidal
Neuroprotective
Reduction in fat absorption
Reduction in ruminal ammonia concentrations
Reductions in stillbirths in swine
Ruminant bloat
Sedative

Saponins can be classified into two group that are triterpene aglycones and steroid aglycone (sapogenin) and were shown in **Figure 4**

a) b)

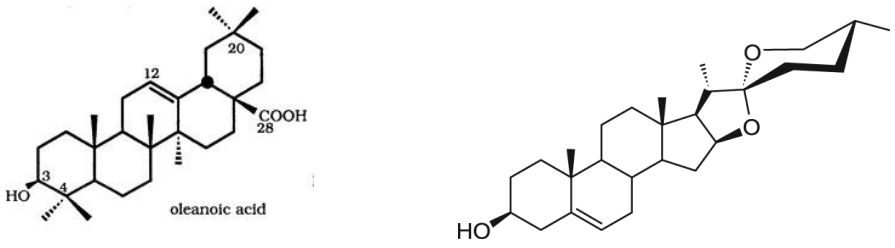

Figure 4 Structure of a) Triterpene aglycones and b) Steroid aglycone (Diosgenin)

The saponins in GSK was triterpene aglycones group. There are three of main saponins compounds as shown in **Figure 5** (Huynh & Nguyen, 2020; Le, Parks, et al., 2018).

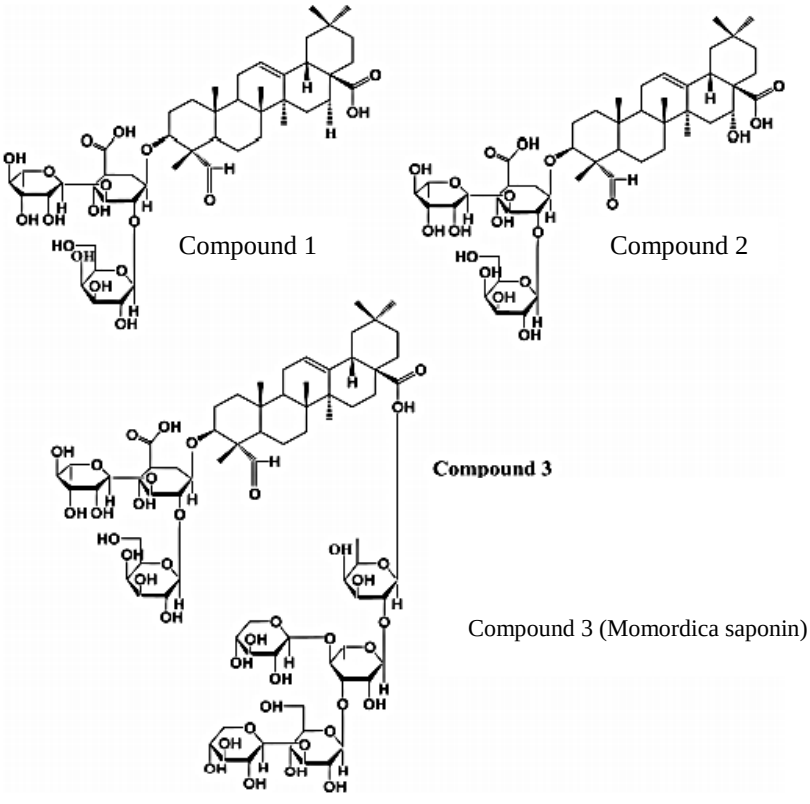

Figure 5 Molecular structure of saponins in GSK

The mainly compound is Momordica saponin. Previous studies have demonstrated this saponin exhibit anti-breast cancer activity, antioxidant, anti-gastritis and wound-healing effects, and anti-inflammatory properties. Biological activities of Momordica saponin was shown in **Table 7**.

Table 7 Biological activities of Momordica saponin

Activities	**References**
Anticancer	(Francis et al., 2002; Zheng

	et al., 2015)
Anti-inflammatory	(Fan et al., 2016; Yu, Roh, et al., 2017)
Glucose uptake activity	(Zheng et al., 2015)
Antioxidation	(Jung, Chin, Yoon, et al., 2013)

2.2 Solvent extraction

2.2.1 Conventional solvent extraction

Maceration is a generally extraction technique, that was used in wine making and has been certified to use for medicinal plant or herb research. Maceration was proceeded by soaking coarse grain or powdered of plant materials with a solvent in a sealed container and agitated frequently at room temperature for a while (Le, Huynh, et al., 2018).

Figure 6 Maceration extraction

The main processes have three parts are extraction part, filtration part and purification part. There are

- Extraction: solvent will be diffused through plant matrix caused swelling and soften, which increase a surface area of plant matrix to contact with solvent and break the cell wall to easy for elute the soluble phytochemicals.
- Filtration: The solution is strained and pressed by filtration to separate a supernatant liquid from moist solid material
- Purification: The liquid residue needed a step to separate a solvent used evaporation to obtain high concentration of extract.

2.2.2 Super/sub-critical solvent extraction

The supercritical extraction and fractionation are an alternative effective technology to separate heavy hydrocarbons into series of fractions with obviously different polarity, molecular weight and solubility to enhance their properties. Zhang et

al. (2019) using supercritical fluid extraction fractionation (SFEF) technology to separate coal tar pitch into a fractions series to enhance meso-carbon microbeads preparation. SFEF has ability to separating heavy hydrocarbons by aromaticity and polarity by adjusting the supercritical fluid (SCF) solubility. Each fraction showed different chemical and physical properties and performance of production which depended on solubility, pressure, temperature or polarity. The results confirmed that SFEF technology successfully separated component into a series of fractions and concentrating with various properties. The effectiveness of SFEF technology has thus been proved and the reason for this effectiveness has been analyzed (Liu et al., 1993). Schematic diagram of critical fluid extraction & fractionation (SFEF) apparatus shown in **Figure 7**.

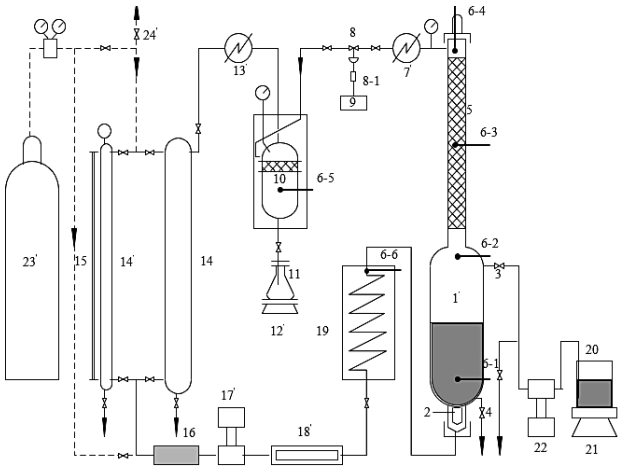

Figure 7 Schematic diagrams of supercritical fluid extraction & fractionation (SFEF) apparatus (Zhang et al., 2019)

Composition of SFEF are 1) Supercritical fluid (SCF) extraction column, 2) SCF solvent distributor, 3) Inlet of feed, 4) Outlet of residue, 5) Pack bed, 6) Thermocouple, 7) SCF cooler, 8) Pressure regulating valve, 9) Controlling center, 10) Solvent separator, 11) Fractions outlet, 12) Electronic balance, 13) Solvent condenser, 14) Solvent tank, 15) Solvent level gauge, 16) Solvent filter, 17) Solvent pump, 18) Flow rate gauge, 19) Solvent heater, 20) Feed tank, 21) Electronic balance, 22) Feed pump, 23) Nitrogen cylinder, 24) Emptying valve

2.2.3 Defatting process

The defatting is the preparation before saponin extraction, this process commonly uses the light petroleum as a solvent for extract the oil in sample then the crude will be used in saponin extraction, that because in saponin extraction normally used the medium polarity solvent such as alcohols and these solvents poor dissolve with oil.

Defatting is often carried out before the saponins are extracted. It helps to simpler for the saponin extraction in terms of technique. However, in some type of plant this method might decrease the total saponin content especially GSK that reported by Le, A.V et al. (2018), there was the comparison between full-fat GSK and defatted GSK saponin extraction. The result shown the 75% of GSK saponin had loss

in oil part, that because of the defatting process. So, when focusing on the amount of saponins recovery from GSK, the full-fat GSK extraction (without defatting process) was applied to use in this study.

2.3 Extraction solvents

2.3.1 Alcohol solvents

Several researches reported the used alcohol solvents shown the good potential in saponin extraction such as Fan R et al. (2016) used methanol and V. Le et al. (2018) used ethanol and methanol in saponin extraction. This is due to the polarity of saponin was similar to alcohols.

2.3.2 Super/subcritical extraction

Supercritical fluid is the substances at temperature and pressure condition above the critical point. In the supercritical area, the fluid exhibits particular properties and has an intermediate behavior between liquid and gas. In particular, supercritical fluids (SCFs) possess liquid-like densities, gas-like viscosities and diffusivities intermediate to that of a liquid and a gas. Thermophysical properties of these fluids are high diffusivity and density, low viscosity, and they can be easily changed by change of operating pressure and/or temperature (Zhang et al., 2019).

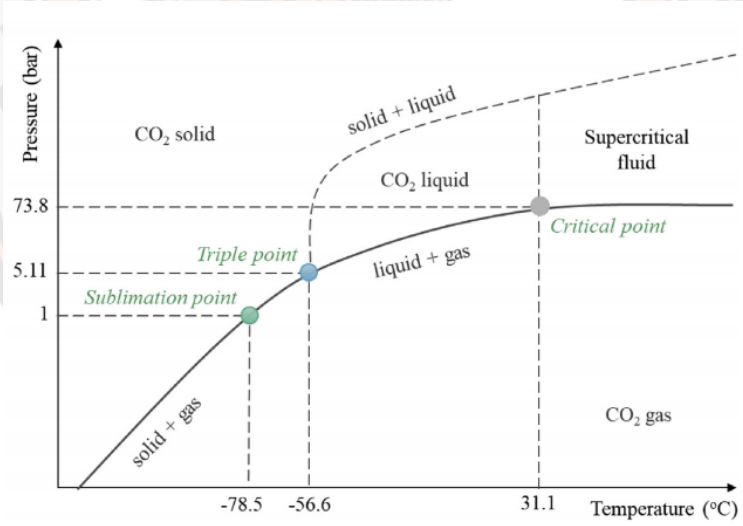

Figure 8 CO_2 Phase diagram

In literature reviews, the popularly is supercritical carbon dioxide. Phase diagram of carbon dioxide as shown **Figure 8**. The advantage of supercritical carbon dioxide extraction greater than conventional extraction techniques for isolation of thermo sensitive such as carotenoids is the processing on the mild and low temperature conditions. However, the supercritical condition has a big disadvantage is cost of equipment for support the high-pressure process. Therefore, another interesting condition is subcritical fluid.

Subcritical fluid extraction (SFE), also called pressurized low-polarity fluid extraction, is one of the most popular techniques which can overcome the defects of the conventional organic solvent extraction and expeller pressing methods. It is an excellent extraction that has numerous advantages such as lower operating

temperature and pressures, shorter extraction time, environmental compatibility, good selectivity, one step from the extraction to the separation and avoidance of residual solvent (Zhang et al., 2019).

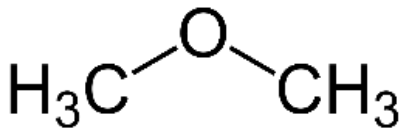

Figure 9 Structure of dimethyl ether

Sub-critical DME extraction is an interesting and effectiveness method to extract a bioactive compound. Previous study shown the concept of this method is low consuming of energy, dewatering and deoiling technology using liquefied DME. Liquefied DME is well known that have high dissolving ability of non-polar and polar substances in wide range and it acts as a good solvent to dissolve many hydrogens bonded substances. Liquefied DME could create a matrix with less viscosity and therefore enhance diffusion of the substances from the solid phase to the solvent (Vidović et al., 2021). Several protocols also have been presented to use liquefied DME as a solvent for extraction at low temperature. It has been proven that has ability to dissolved pigments and proteins derived from essential oils from peels of citrus and vegetable tissues (Obeid et al., 2018). The advantage of DME extraction is the simultaneous dewatering from the extracts due to water has low solubility in DME approximately 7–8 weight% at room temperature (Subratti et al., 2018).

Table 8 Chemical and physical properties of dimethyl ether

Properties	
Chemical formula	C_2H_6O
Molecular weight	46.069 g/mol
Boiling point	-24.82 °C
Melting Point	-141.5 °C
Flash point	-41°C
Autoignition temperatur	350 °C
Density of liquid	0.61 g/cm^3 at 25°C (liquefied)
Density of gas	1.91855 g/L at 1 atm and 25 °C
Vapour pressure	4450 mm Hg at 25°C
Solubility in water	7–8 weight% at room temperature

The boiling point of DME is about −24.8 ◦C. The critical temperature and pressure of Liquefied (sub-critical) DME are 126.85 ◦C and 5.37 MPa, respectively (Vidović et al., 2021). Liquefied DME has high correlated for oily substances and partial miscibility with water. It is non-toxic, decomposition rapidly in the atmosphere, environmentally friendly, easily available and cost effective. DME has

been approved as a safe solvent for extraction of the foodstuffs and food ingredients production by the European Food Safety Authority (EFSA), by the Food Standards Australia New Zealand, and by the United States (Rittner, 1992).

2.4 Hansen solubility parameters

The Hansen solubility parameters (HSPs) (Hansen, 2007) consist of the dispersion (δ_D^2), polar (δ_P^2), and hydrogen-bonding (δ_H^2) parameter, as well as their sum, the total solubility parameter (δ_T^2). HSPs are based on the assumption that the total cohesive energy (E) must equal the summation of nonpolar or dispersion interactions (E_D), polar or dipole-dipole and dipole-induced-dipole interactions (E_P), and hydrogen-bonding or other specific association interactions (E_H) as shown in Equation (2.4.1).

$$E = E_D + E_P + E_H \tag{2.4.1}$$

Dividing the individual cohesive energy terms by the molar volume (V_m) gives Equation (2.4.2).

$$\frac{E}{V_m} = \frac{E_d}{V_m} + \frac{E_p}{V_m} + \frac{E_h}{V_m} \tag{2.4.2}$$

The square of the total solubility parameter (δ_t^2) is the sum of the squares of the Hansen dispersion (δ_D^2), polar (δ_P^2) and hydrogen-bonding (δ_H^2) contributions.

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \tag{2.4.3}$$

The distance between two molecules, a solute *i* and a solvent *j*, in Hansen three-dimensional space defined as "distance", R_a (Equation (2.4.4)), depends on their respective partial solubility parameter components.

$$R_a = \sqrt{4\left(\delta_{Di} - \delta_{Dj}\right)^2 + \left(\delta_{Pi} - \delta_{Pj}\right)^2 + \left(\delta_{Hi} - \delta_{Hj}\right)^2} \tag{2.4.4}$$

By a trial-and-error system, solvents tested are plotted in Hansen three-dimensional space creating the "solubility sphere". Thus, this solubility sphere is defined as the region where solvent-solute combinations occur as a solution and the radius of the sphere is known as "interaction radius" (R_0). Thereby, the Relative Energy Difference (*RED*) is defined as follows:

$$RED = \frac{R_a}{R_0} \tag{2.4.5}$$

So, good solvents are comprised into the interior of the sphere or are at least on its surface ($RED \leq 1$), while a *RED* value higher than 1 indicates low affinity. Therefore, solubility requires that Ra has a smaller value than R_0. Nevertheless, R_0 can only be used when solubility experiments can be performed since it is based only on

experimental data of the observation of the interaction between studied solutes and well-known solvents.

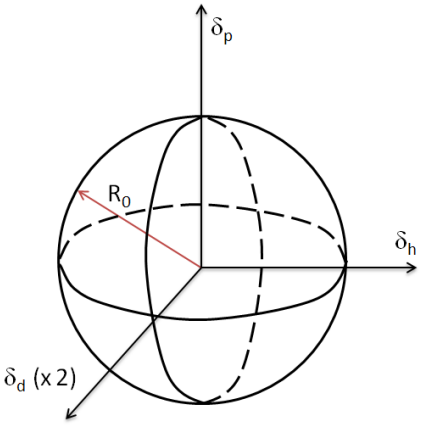

Figure 10 Hansen solubility sphere

2.4.1 HSPs of mixed.

The HSPs of the oil shown in **Table 14** were estimated based on the symmetrical triglycerides (TAGs) assumption which the fatty acid mass fractions were obtained from literature (Ishida et al., 2004). The HSPs of pure dimethyl ether (DME) and the conventional solvents including ethanol, methanol, propanol, n-butanol, and hexane were obtained from Hansen solubility parameters: a user's handbook (Hansen, 2007). The HSPs of solute and solvent mixture then can be calculated (shown in **Table 15**) by the following equation.

$$\delta_{mixture} = \sum x_i \delta_i \tag{2.4.6}$$

where x_i refer to mass fraction of each component, δ_i refer to each Hansen solubility parameter of each component.

2.4.2 HSPs of any condition

Temperature and pressure influence both δ_T and the individual HSP of the solvents. The impact of the operating conditions on δ_T and HSP could be calculated by Equations. (2.4.7) - (2.4.9) as a function of the molar volume. In these equations, the subscript ‘‘ref” indicates the HSP and molar volumes of the fluid at room temperature (298.2 K).

$$\frac{\delta_{D\,ref}}{\delta_D} = \left(\frac{V_{ref}}{V}\right)^{-1.25} \tag{2.4.7}$$

$$\frac{\delta_{P\,ref}}{\delta_P} = \left(\frac{V_{ref}}{V}\right)^{-0.5} \tag{2.4.8}$$

$$\frac{\delta_{H\,ref}}{\delta_H} = exp\left[-1.3210^{-3}(T_{ref} - T) - ln\left(\frac{V_{ref}}{V}\right)^{-0.5}\right] \quad (2.4.9)$$

2.4.3 HSPs calculation by group contribution

As solubility parameters are available for only a limited number of solvents, a method to predict these quantities from the chemical structure is valuable. The useful prediction method proposed by Van Krevelen was applied to calculate the dispersion, polar and H-bonding components (Van Krevelen & Te Nijenhuis, 2009):

$$\delta_D = \frac{\sum F_D}{V_m} \quad (2.4.10)$$

$$\delta_P = \frac{\sqrt{\sum F_P^2}}{V_m} \quad (2.4.11)$$

$$\delta_H = \sqrt{\left(\frac{\sum E_H}{V_m}\right)} \quad (2.4.12)$$

Group contribution technique was used for the calculation of F_d(dispersion group molar attraction), F_P (polar group molar attraction) and E_h (H-bonding) contribution for major saponins in gac seeds extraction.

CHAPTER III

RESEARCH METHODOLOGY

This chapter is a research methodology, explaining conventional extraction method, batch extraction using sub-critical dimethyl ether (DME) and analysis method of the obtaining extracts to determine the saponins recovery from gac seed kernel.

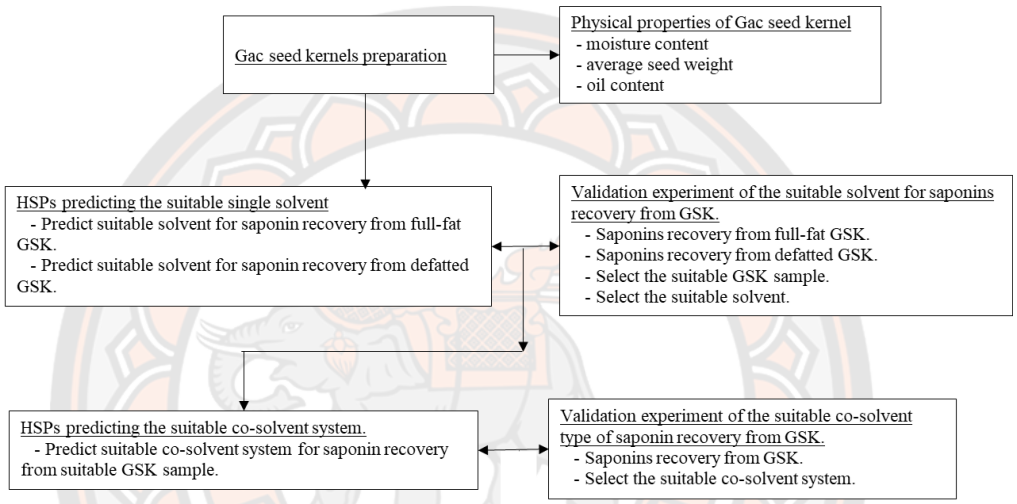

Figure 11 Schematic of methodology.

3.1 Chemicals

Solvents used for extraction including methanol (99.8%), ethanol (99.8%), propanol (99.8%), n-butanol (99.8%) and hexane (99.8%) were purchase from Merck, Singapore. Dimethyl ether (DME) spray can brand 420D was purchased from Siam Tamiya, Thailand. Aescin (99.5%), vanillin (99%), and sulfuric acid (97%) were purchased from Merck, Singapore.

3.2 Materials preparation

3.2.1 Sample preparation

The gac seeds were first separated from the fresh gac fruits (obtained from an orchard in Uttaradit Province, Thailand), and then the shells were removed to obtain the kernels. The kernels were then dried at 60°C for 72 hours and grounded into a fine powder (40 mesh). The dried GSK powder was stored in the desiccator until extraction.

3.2.2 Preparation of defatted GSK Powder

To prepare defatted GSK powder, the dried kernel powder was extracted three times for thirty minutes with hexane (1:5 w/v) on a magnetic stirrer at room temperature. Each time, the resulting slurry was suction-filtered and the final residue

was air-dried for 12 h and stored in a desiccator at ambient temperature until used. This GSK powder was referred to as defatted powder. The residues will be evaporated and bring to TSC analysis.

3.3 GSK properties

The GSK was measured the physical properties including moisture content using the AOCS Ab 2-49 method and using a MOC63u moisture analyzer (Shimadzu Corp) at 160 ℃, average seed kernel weight by gravimetric method.

The obtained GSK powder was analyzed to determine the oil content and saponins content. For the determination of oil content, the method was carried out as following. 10 grams of dried kernel powder was extracted with hexane (1:5 w/v) for 30 min. at room temperature for three times. Each time, the resulting slurry was suction-filtered. All filtrated liquids were summed and then evaporated using a vacuum rotary evaporator to remove the hexane. The solid residue was air-dried for 12 hours and collected as the defatted sample. The total amount of extracts was determined by gravimetric method. The oil content in GSK was then estimated by following equation.

Oil content = weight of extract - saponins content in the extract (3.3.1)

which the saponins content in the extract was determined by UV-visible spectrophotometer (the detail of analysis was described in section 3.7). The oil content therefore can be estimated.

For the determination of saponins content, the saponins in the extract was represented as saponins in oil body which the detail of analysis was mentioned above. The saponins content in the matrices of GSK was determined by re-extraction of defatted sample was re-extracted twice with propanol at a sample to solvent ratio of 1:5 w/v at room temperature for 30 mins. The extracts were summed and then evaporated using a vacuum rotary evaporator to remove the propanol. The resulting final extract was analyzed to determine the saponins content by UV-visible spectrophotometer.

3.4 Hansen solubility parameters

Computational methodology using Hansen solubility parameters (HSPs) to investigate the solubility between solvents and GSK’s solutes. List of solvents were consisted of sub-critical dimethyl ether (DME), methanol, ethanol, propanol, and butanol. Moreover, by the reason of possibility to obtain higher saponin recovery, the cosolvent systems will be applied in this study.

3.4.1 HSPs of GSK.

HSPs of GSK were considered to 2 sample conditions consisted of Momordica saponin only (as defatted) and solute mixture (as full-fat).

3.4.1.1 HSPs for Momordica saponin.

The HSPs for defatted part used the main saponin structure was Momordica saponin in GSK that calculated from molecular structure (**Figure 5**) by group contribution method (Van Krevelen & Te Nijenhuis, 2009) consist

of 3 equations (2.4.10 – 2.4.12). List of functional groups in any major structure of Momordica saponin as shown in **Table 9**.

Table 9 List of functional groups from Momordica saponin structure for applied the group contribution technique method of Hoftyzer–Van Krevelen (Van Krevelen & Te Nijenhuis, 2009).

Group	Frequency (Ni)	F_{di} $(MJ/m^3)^{1/2}/mol$	F_{pi}^2 $(MJ/m^3)^{1/2}/mol$	E_{hi} (J/mol)	V_m (cm^3/mol)
- CH3	9	3,780	0	0	193.95
- CH2-	14	3,780	0	0	217.7
-CH<	42	3,360	0	0	401.52
-CH=	1	200	0	0	13.18
COOH	2	1,060	352,800	20,000	52.2
OH	20	4,200	5,000,000	400,000	249
>C<	6	-420.00	0	0	21.36
>C=	1	70	0	0	7.18
-O-	16	1,600	2560000	48,000	103.2
Ring	13	2,470	0	0	208
COH	1	470	640000	4,500	0
Sum	-	20,570	8552800	472,500	1,467.29

Table 10 shown the result from the calculated, the HSPs of *Momordica* saponin shown low δ_P refer to low polarity that mean its dissolve well in low polarity solvents such as alcohols.

Table 10 HSPs of Momordica saponin.

Bioactive compound	$\boldsymbol{\delta_D}$	$\boldsymbol{\delta_P}$	$\boldsymbol{\delta_H}$
Momordica Saponin	14.02	1.99	17.94

3.3.1.2 HSPs solute mixture in GSK.

The full fat gac seed kernel there was 53% the oil part and this part had about 75% of whole saponin. However, the oily effected to the solubility parameter and main component of oil part in gac seed kernel was a fatty acid were reported (Ishida et al., 2004).

Table 11 HSPs of TAGs in GSK.

Substance	Composition	δ_D	δ_P	δ_H
PPP	0.056	16.2	2.4	2.2
SSS	0.605	16.9	1.9	2.1
LLL	0.203	16.5	2.0	2.9
OOO	0.09	16.6	2.1	2.3
AAA	0.016	16.6	2.2	2.0
EEE	0.03	17.3	1.7	2.2
Mixed TAGs	1.00	16.76	1.97	2.29

PPP=Tripalmitin, SSS=Tristearin, LLL=Trilinolein, OOO=Triolein,

AAA=Triarachidin, EEE=Trieicosenoin

Table 10 and **Table 11** were used to estimate the new HSPs by used equation 2.4.6.

3.4.2 HSPs for single solvent system.

The HSPs of Momordica saponin will compared with HSPs of any solvents and reported in *Ra* or *RED* value (equation 2.4.4 and 2.4.5) to tell which one is suitable for this study system and the HSPs of each solvent were shown in **Table 12**.

Table 12 HSPs of 5 solvents (ethanol, methanol, n-butanol, propanol, and DME)

Solvent	δ_D	δ_P	δ_H
Ethanol	15.80	8.80	19.40
Methanol	14.70	12.30	22.30
Butanol	16.00	5.70	15.80
Propanol	15.80	6.10	16.40
DME	15.20	6.10	5.70

HSPs of all solvent obtained from Hansen's handbook (Hansen, 2007).

3.4.2.1 Hansen solubility parameter for DME with cosolvent system.

Similar 3.2.3.2 but have to calculated HSPs of cosolvent sub-critical DME with any solvent (equation 2.4.6) before used to compared with HSPs of Momordica saponin.

3.5 Approximate solubility test

For the solubility of solute mixture, the hexane-extracted oil obtained from the previous described was weighed to be 0.1 gram then mixed with 2 ml of various composition of solvent mixture of Acetone and ethyl acetate for 1 hr and then observed the appearance. For the solubility of Momordica saponin, 0.01 gram of Aescin standard was used as solute and also mixed with 2 ml of various composition of solvent mixture of Acetone and ethyl acetate for 1 hr. The mixed solution was

filtrated by syringe filter (nylon 0.2-micron pore diameter) and the clear liquid was evaporated to remove solvent then the weight of solid residue was analyzed by gravimetric method.

3.6 Determination of suitable solvent system

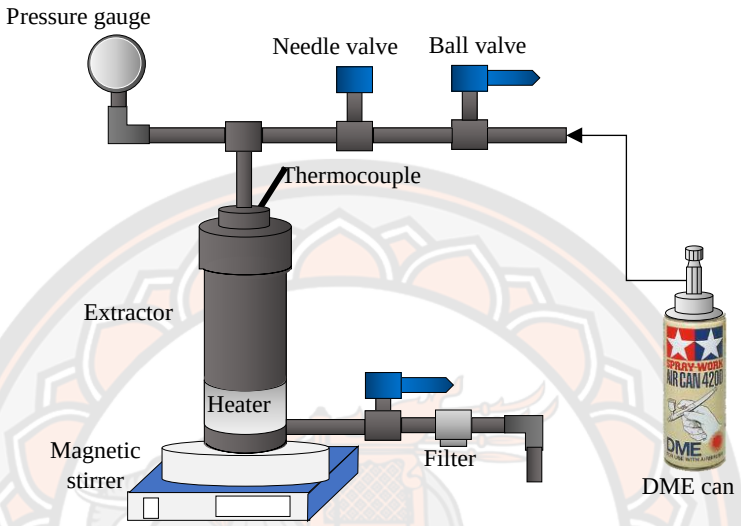

Figure 12 Sub-critical DME extraction column.

For DME extraction, 10 grams of GSK powder was placed in a cellulose thimble (30 mm x 100 mm) along with magnetic bar (8 mm diameter) and then loaded into an extractor (100 ml stainless steel) then the extractor was sat on a scale. DME from a pressurized can was filled into the pre-weighed extractor to reach a solvent to sample ratio (g/g) of 5:1. The mixture was then agitated at 500 rpm at desired temperature for 30 mins. After extraction, a separation unit was connected to the bottom of the extractor, and the solution was allowed to flow through a stainless-steel filter with a pore diameter of 7 microns. Each extract thus obtained was stored at -20°C until analysis.

For conventional solvent extraction, 10 grams of GSK powder was placed in a 250 ml beaker. Methanol, ethanol, propanol, n-butanol and hexane were each tested using a same solvent to sample weight ratio with DME extraction (5:1 g/g). The extraction was performed at 25°C for 30 mins. After extraction, each solution was filtered and a rotary vacuum evaporator was used to evaporate the solution down a viscous residue. Each concentrated extract was collected and stored at -20°C before use in the total saponin content analysis.

In case of co-solvent study, the extract amount of co-solvent was added into the extractor before loading DME and the co-solvent was removed from the extracts by a rotary vacuum evaporator to obtain the final products which were analyzed to determine saponins content.

The recovery of saponins was reported as %recovery which can be calculated by the following equation.

$$\%Recovery = \left(\frac{saponins\ content\ in\ each\ experiment}{total\ saponins\ in\ GSK}\right) \times 100\% \qquad (3.6.1)$$

3.7 Saponin content analysis

The saponins content was analyzed according to the method of [28] using a 0.3 ml sample mixed with 0.3 ml of 8% (w/v) vanillin solution and 3 ml of 72% (v/v) sulfuric acid. The mixture was stirred, incubated at 60°C for 15 mins, and then cooled on ice for 10 mins. The absorption of the mixture was measured at 560 nm using a UV-visible spectrophotometer (Helios Omega). Aescin was used as a standard.

3.8 Statistical analysis

All experiments were performed in triplicate and the results reported as the mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) and the post hoc Turkey's test on IBM SPSS statistical software. Significance was set at $p < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 GSK sample

4.1.1 GSK sample analysis

To estimate the mass fraction of solute mixture, the dried GSK was extracted with hexane triplicate and then removed the solvent residue which the weight of extract was determined by gravimetric method to be 0.57 g/g dry weight. The hexane-extract was analyzed saponins content which analyzed to be 0.033 g/g dry weight then the oil content can be calculated to be 0.54 g/g dry weight. The results in **Table 13** were then used to calculate the mass fraction of Momordica saponin and oil which are 0.06 and 0.94, respectively. In addition, from these results, 40% saponins were found to partitioned in matrices of GSK and 60% in oil body. The mass fraction of solute mixture was then used to estimate the HSPs as the results shown in **Table 14**. The dispersion term, polar term, and hydrogen term of the solute mixture between saponins and GSK oil could be estimated as 16.51, 1.97, and 3.23, respectively.

Table 13 Content and mass fraction of Momordica saponin and oil content in GSK

Part	Momordica saponin Content (g/g dry weight)	Oil Content (g/g dry weight)
In matrices of GSK	0.022	-
In oil body	0.033	0.54*
Total	0.055	0.54
Mass Fraction in oil body (x_i)	0.06	0.94

*The oil content was calculated by equation 1, which is the weight of extract was determined as 0.57 g/g dry weight.

Table 14 Hansen solubility parameters of Momordica saponin, oil (mixed TAGs), and their mixture.

Substance	composition	δ_D	δ_P	δ_H
Momordica saponin	0.06	14.02	1.99	17.94
Oil (Mixed TAGs)	0.94	16.76	1.97	2.29
Solute Mixture	1.00	16.51	1.97	3.23

4.1.2 Comparison of GSK sample preparation

The comparison between GSK preparation with defatting process and without defatting process (full-fat) was purposed to investigate the main assumption that the GSK oil part was rich in saponins and the defatting process was the reason of saponins loss and possibility to single step extraction for the cost advantage in reduce processing. Therefore, HSPs were used to predict the trend of extraction results then and it was used to discuss with extraction experiment results. The HSPs and experiment results were showed as **Table 15** and **Figure 13** respectively.

Table 15 HSPs prediction and extraction results

Sample	Sorted	Hansen's prediction		Experiment	
		Solvents	Ra	Solvents	TSC (mg/g)
Defatted (Momordica saponin)	1	Methanol	11.28	Methanol	23.16
	2	Ethanol	7.82	Ethanol	24.52
	3	Propanol	5.65	Propanol	41.19
	4	n-Butanol	5.83	Butanol	37.72
	5	DME	13.13	DME	18.61
Full-fat (Solute mixture)	1	Methanol	21.99	Methanol	2.40
	2	Ethanol	17.61	Ethanol	8.99
	3	Propanol	13.88	Propanol	26.91
	4	n-Butanol	13.15	n-Butanol	30.86
	5	DME	5.48	DME	44.97

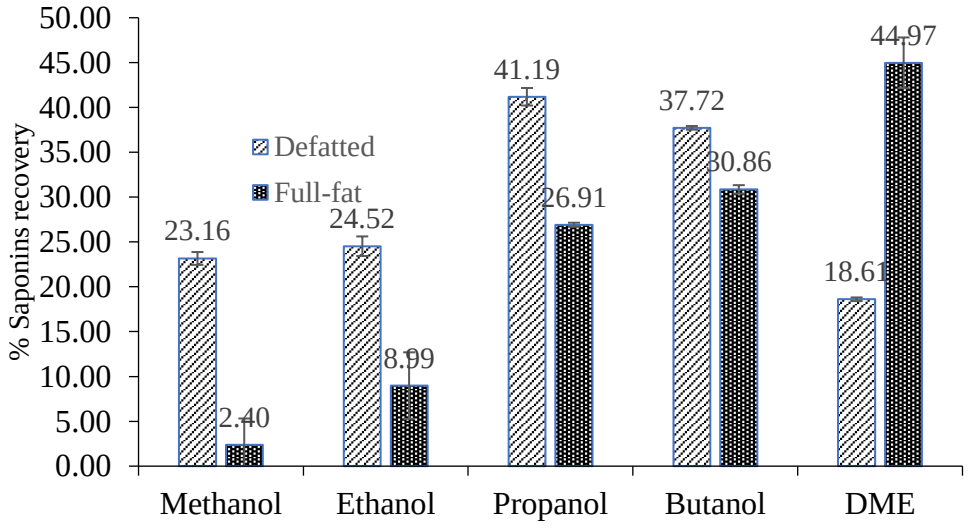

Figure 13 Comparison of Saponin recovery between defatted GSK and full-fat GSK

From the extraction results as shown in **Figure 13**, for conventional saponin recovery with defatting process the suitable solvent obtained high recovery rate was propanol as 41.19 % following by butanol 37.72 %, ethanol 23.52 %, methanol 23.16 %, and DME 18.61 % and considered with HSPs prediction were showed agreement with the experimental results that less R_a value was mean to higher solubility cause to high extractability in solvent maceration extraction. In contrasting, saponin recovery rate at full-fat showed higher than recovery rate from defatted GSK moreover the suitable one was the DME that poor extractability with defatted GSK. The saponin recovery with DME was 44.97 % following by butanol 30.86 %, propanol 26.91 %, ethnol 8.99 %, and methanol 2.4 % and it have agreement with HSPs results that showed the less R_a was DME.

Accordingly, the study of this part was not only showed that possibility of single step saponin recovery from GSK without defatting process, it showed that the saponin extraction from full-fat GSK will be obtained higher saponin recovery than defatted GSK extraction. Therefore, for obtained more saponin recovery full-fat GSK will be used.

4.2 Determination of suitable solvent system

4.2.1 Solubility results

The experimental of solubility testing was purposed to estimate the R_0 for sphere surface determined. The result of both solute including to Momordica saponin and solute mixture were showed as **Table 16** and **Table 17** respectively.

Table 16 Determination of R_0 for Momordica saponin.

Solvent	δ_D	δ_P	δ_H	R_a	Approximate Solubility (mg/ml)
Ethyl acetate/Acetone (90/10 v/v)	15.77	5.81	7.18	12.03	51.5
Ethyl acetate/Acetone (80/20 v/v)	15.74	6.32	7.16	12.26	37.95*
Ethyl acetate/Acetone (70/30 v/v)	15.71	6.83	7.14	12.49	0.7
Ethyl acetate/Acetone (60/40 v/v)	15.68	7.34	7.12	12.72	2.0
Ethyl acetate/Acetone (50/50 v/v)	15.65	7.85	7.1	12.95	2.4
Ethyl acetate/Acetone (40/60 v/v)	15.62	8.36	7.08	13.19	1.75
Ethyl acetate/Acetone (30/70 v/v)	15.59	8.87	7.06	13.42	1.25
Ethyl acetate/Acetone (10/90 v/v)	15.53	9.89	7.02	13.88	2.2
Acetone (100%)	15.50	10.40	7.00	14.12	0.9

Table 17 Determination of R_0 for solute mixture.

Solvent	δ_D	δ_P	δ_H	R_a	Approximate Solubility (mg/ml)
Ethyl acetate/Acetone (90/10 v/v)	15.53	9.89	7.02	8.99	<500
Ethyl acetate/Acetone (80/20 v/v)	15.56	9.38	7.04	8.55	<500
Ethyl acetate/Acetone (70/30 v/v)	15.59	8.87	7.06	8.10	<500
Ethyl acetate/Acetone (60/40 v/v)	15.62	8.36	7.08	7.67	>500*

Ethyl acetate/Acetone (50/50 v/v)	15.65	7.85	7.1	7.25	>500
Ethyl acetate/Acetone (40/60 v/v)	15.68	7.34	7.12	6.84	>500
Ethyl acetate/Acetone (30/70 v/v)	15.71	6.83	7.14	6.44	>500
Ethyl acetate/Acetone (20/80 v/v)	15.74	6.32	7.16	6.06	>500
Ethyl acetate/Acetone (10/90 v/v)	15.77	5.81	7.18	5.70	>500

From **Table 16** and **Table 17**, R_0 was the first R_a value (or minimum *Ra*) that found the rapid increasing of approximate solubility due to its properties was over range of solubility sphere according to Hansen solubility theory as explained in section 2.4. Therefore, R_0 of Momordica saponin and solute mixture were 12.26 and 7.67 respectively. Moreover, the both of R_0 were used to generate their Hansen solubility sphere as shown in **Figure 13**.

4.2.1 Single solvent system

The HSPs predictions were considered two parts of saponins which are the pure Momordica saponin in matrix of GSK together with Momordica saponin in oil body defined as solute mixture. The HSPs prediction results of pure Momordica saponins and solute mixture are shown in **Table 18**. For clearly see the effect of single solvent on HSPs predictions, the Hansen solubility sphere of solute mixture and pure Momordica saponin were established by GeoGebra online software (free version). The boundary of Hansen solubility spheres (R0) were estimated by using experimental data of the approximate solubility of Momordica saponin and the solute mixture which the results are shown in **Table 12**, respectively. From the results, it can be defined R0 for Momordica saponin and the solute mixture as 12.49 and 7.67, respectively.

Table 18 HSPs and R_a of methanol, ethanol, propanol, n-butanol, DME, and hexane for solute mixture and pure Momordica saponin.

Solvent	**HSPs**			R_a	
	δ_D	δ_P	δ_H	**Solute mixture**	**Momordica saponin**
Methanol	14.70	12.30	22.30	21.87	11.27
Ethanol	15.80	8.80	19.40	17.61	7.82
Propanol	15.80	6.10	16.40	13.88	5.65
n-Butanol	16.00	5.70	15.80	13.15	5.83
DME	15.20	6.10	5.70	5.48	13.13
Hexane	14.90	0	0	4.97	18.14

As seen in the **Figure 13**, single system of polar solvents including methanol, ethanol, propanol and n-butanol lied in the Hansen solubility sphere of pure Momordica saponin. It might be implied from the results that alcohols could be dissolved or recovered saponins from only in matrices of GSK. On the other hands, it might be implied from the sphere that hexane as non-polar solvent could recover saponins from only the oil body. DME also lied on the area of solute mixture but it located closer to the intersection area that means DME has more potential to recover

saponins from both matrices of GSK and oil body when combined with the suitable co-solvent at suitable composition.

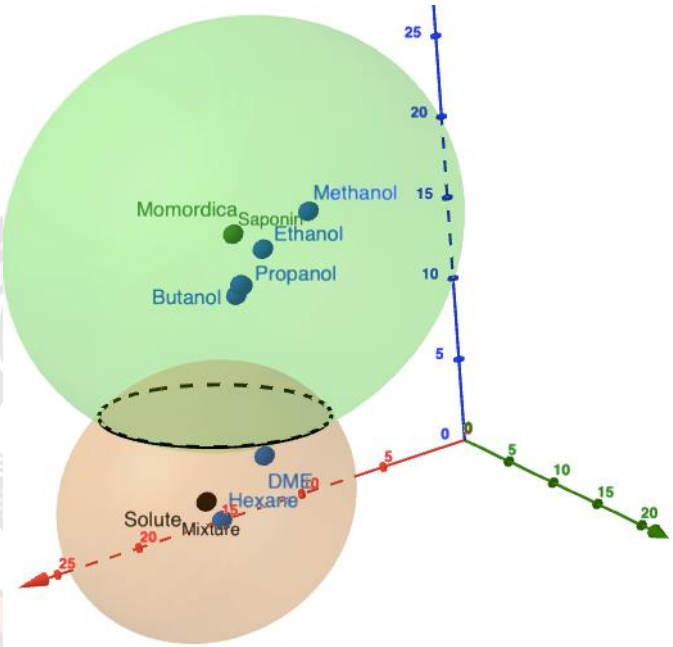

Figure 14 Hansen parameter sphere of solute mixture and pure Momordica saponin for single solvent system.

The saponins recovery experiments were performed and the results are shown in **Figure 15**. The highest %saponins recovery from GSK was obtained using DME (44.98%), followed by hexane (40.45%), n-butanol (30.86%), propanol (26.91%), ethanol (8.99%), and methanol (2.40%), respectively. The experimental results of using alcohols as solvents for recover saponins and HSPs predictions were supported that the alcohols might recover saponins only from the matrices of GSK. As mentioned in previous results, the saponins partitioning in the matrices of GSK was 40%. The alcohols can recover saponins 2 to 31%, combining with the Hansen sphere results in **Figure 14**, implying that the lower %saponins recovery caused by they could not dissolve the saponins in oil body. The non-polar hexane gave higher %saponins recover compared with alcohols but it still lower comparing with DME at the same extraction conditions. It might be because DME as the compressed gas, has high diffusivity and higher extraction performance than conventional extraction using hexane. These trends have also been observed in the previous studies in which DME had high extraction performance for extraction of substances that existing together with oils such as carotenoids (β-carotene) in C. *humicula* (Eghbali Babadi et al., 2020), xanthophylls (lutein) from marigold flowers (Boonnoun et al., 2017), and oil from spent bleaching clay (Zhang et al., 2021).

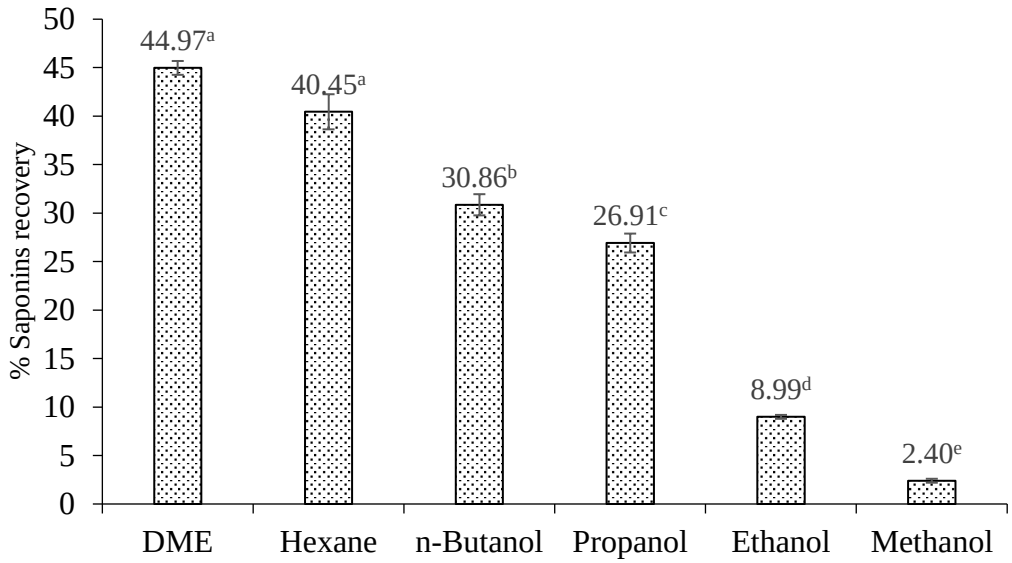

Figure 15 Saponins recovery from GSK using various solvents: extraction conditions of 25°C for 30 minutes and 5:1 (ml/g) solvent to sample ratio.

4.2.2 Co-solvent system

From the previous experiment, DME was the most suitable single solvent for saponins recovery but %saponins recovery still quite low (45%). In this section, the possibility of using 10% (w/w) alcohols as co-solvents with 90% (w/w) DME was study to enhance the %recovery. The HSPs predictions of solute mixture and pure Momordica saponin for DME with different co-solvents of methanol, ethanol, propanol, and n-butanol at various compositions are shown in **Table 19** to **Table 22** and the Hansen spheres are shown in **Figure 16** to **Figure 19**. The HSPs predictions and spheres revealed that adding methanol at 10% and 15% wt into DME resulted in the possibility to enhance saponins recovery since HSPs lied on the intersection area of solute mixture and pure Momordica saponin spheres, implying that saponins from both matrices of GSK and oil body might be recovered by using these solvent systems. However, at higher methanol composition (20 to 35% wt), HSPs predictions lied on the Momordica saponin sphere, suggesting that too high amount of methanol led to lower recovery of saponins since it might recover saponins only in matrices of GSK. These trends of results also observed for using ethanol, propanol and, butanol as co-solvent. However, the HSPs predictions suggested the different compositions for the different alcohols used. For ethanol as co-solvent, 10 to 25% wt were the potential compositions for recovery saponins but 10 to 35% wt were suggested for propanol and n-butanol as co-solvent. However, HSPs predictions of propanol as co-solvent were very close to n-butanol. Altought the appeal of propanol is its lower boiling point compared n-butanol (97 °C versus 117 °C, respectively), meaning lower energy consumption for removing the solvent residue, but n-butanol has the lower toxicity (Henderson et al., 2011) and it can be obtained from agricultural waste (Wechgama et al., 2017). From the results, it could be used as a guideline for saponins recovery experiments that 10 to 15 %wt of methanol, 10 to 25% wt of ethanol, and 10 to 35% wt of n-butanol were the potential co-solvent systems.

Table 19 Hansen solubility parameters of solute mixture and pure Momordica saponin for mixture solvent of DME and methanol at various composition

Solvent System	HSPs			R_a	
	δ_D	δ_P	δ_H	Solute mixture	Momordica saponin
DME/methanol: 90/10	**15.25**	**6.32**	**6.84**	**6.19**	**12.16**
DME/methanol: 85/15	**15.14**	**6.88**	**7.79**	**7.24**	**11.49**
DME/methanol: 80/20	15.12	7.15	8.51	7.90	10.97
DME/methanol: 75/25	15.09	7.42	9.25	8.60	10.47
DME/methanol: 70/30	15.07	7.71	10.00	9.33	10.01
DME/methanol: 65/35	15.05	7.99	10.76	10.08	9.58

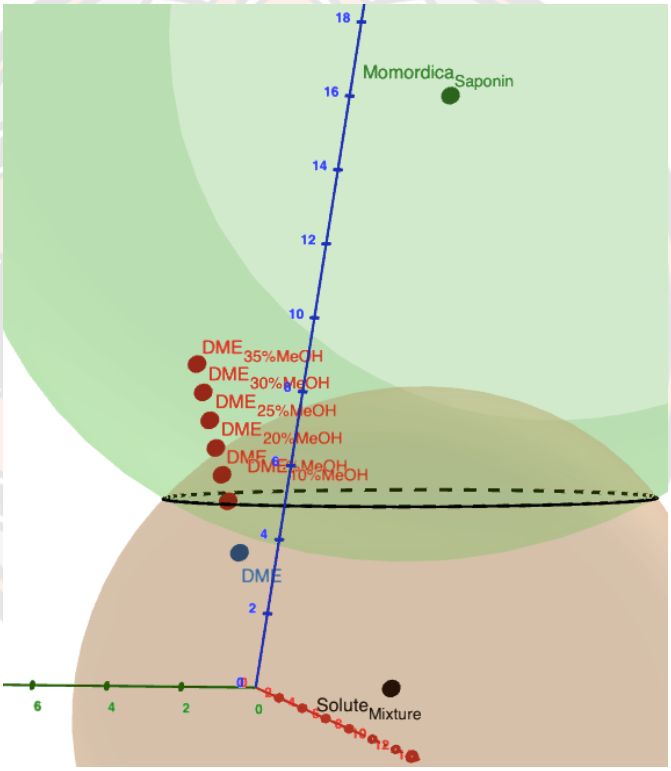

Figure 16 Hansen parameter sphere of solute mixture and pure Momordica saponin for various composition of methanol as co-solvent with DME.

Table 20 Hansen solubility parameters of solute mixture and pure Momordica saponin for mixture solvent of DME and ethanol at various composition

Solvent System	HSPs			R_a	
	δ_D	δ_P	δ_H	Solute mixture	Momordica saponin
DME/ethanol: 90/10	**15.25**	**6.32**	**6.84**	**6.19**	**12.16**
DME/ethanol: 85/15	**15.28**	**6.44**	**7.43**	**6.61**	**11.63**
DME/ethanol: 80/20	**15.30**	**6.65**	**8.03**	**7.06**	**11.21**

DME/ethanol: 75/25	**15.33**	**6.68**	**8.64**	**7.55**	**10.74**
DME/ethanol: 70/30	15.36	6.80	9.26	8.06	10.28
DME/ethanol: 65/35	15.38	6.93	9.89	8.60	9.83

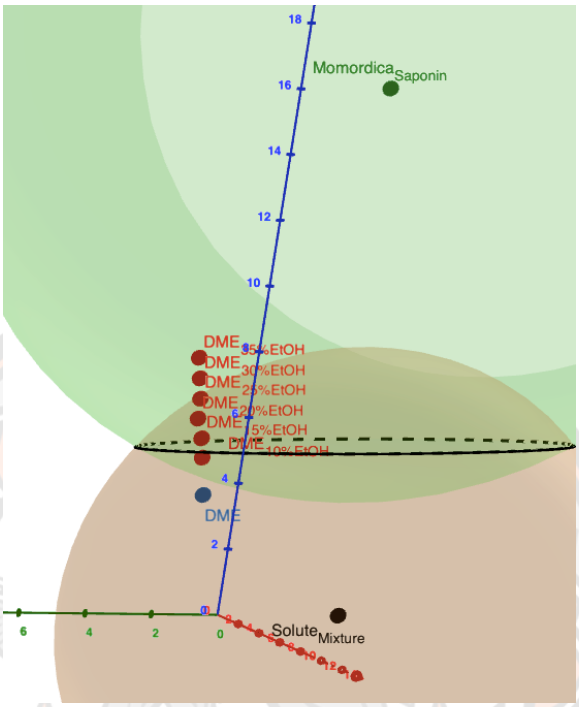

Figure 17 Hansen parameter sphere of solute mixture and pure Momordica saponin for various composition of ethanol as co-solvent with DME.

Table 21 Hansen solubility parameters of solute mixture and pure Momordica saponin for mixture solvent of DME and propanol at various composition

Solvent System	**HSPs**			**R_a**	
	δ_D	**δ_P**	**δ_H**	**Solute mixture**	**Momordica saponin**
DME/propanol: 90/10	**15.25**	**6.10**	**6.58**	**5.88**	**12.32**
DME/propanol: 85/15	**15.27**	**6.10**	**7.03**	**6.13**	**11.93**
DME/propanol: 80/20	**15.30**	**6.10**	**7.49**	**6.41**	**11.52**
DME/propanol: 75/25	**15.33**	**6.10**	**7.96**	**6.71**	**11.10**
DME/propanol: 70/30	**15.35**	**6.10**	**8.44**	**7.04**	**10.69**
DME/propanol: 65/35	**15.38**	**6.10**	**8.93**	**7.39**	**10.27**

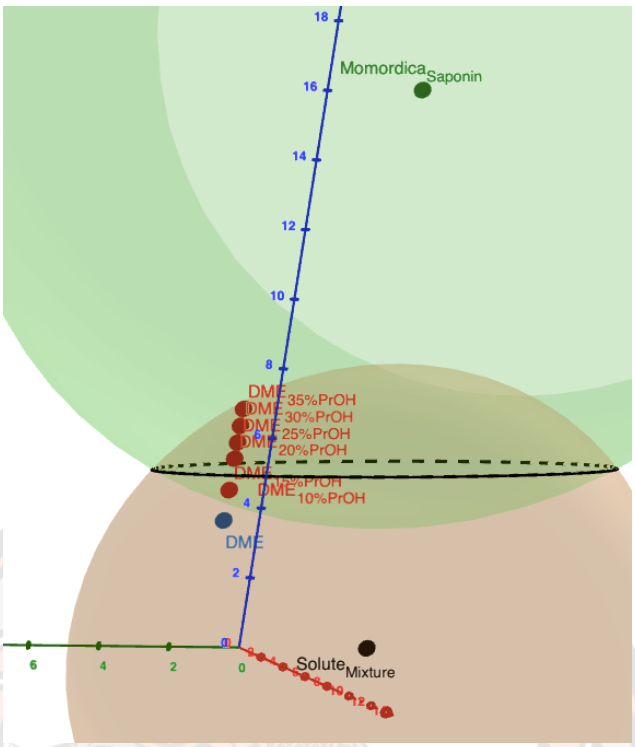

Figure 18 Hansen parameter sphere of solute mixture and pure Momordica saponin for various composition of propanol as co-solvent with DME.

Table 22 HSPs and R_a of DME with various percentage (w/w) of n-butanol as co-solvents for solute mixture and pure Momordica saponin.

Solvent System	HSPs			R_a	
	δ_D	δ_P	δ_H	Solute mixture	Momordica saponin
DME/n-Butanol: 90/10	15.27	6.07	6.56	5.83	12.34
DME/n-Butanol: 85/15	15.30	6.05	7.00	6.06	11.94
DME/n-Butanol: 80/20	15.34	6.03	7.45	6.31	11.55
DME/n-Butanol: 75/25	15.37	6.01	7.91	6.59	11.14
DME/n-Butanol: 70/30	15.41	5.99	8.37	6.89	10.74
DME/n-Butanol: 65/35	15.45	5.98	8.84	7.22	10.33

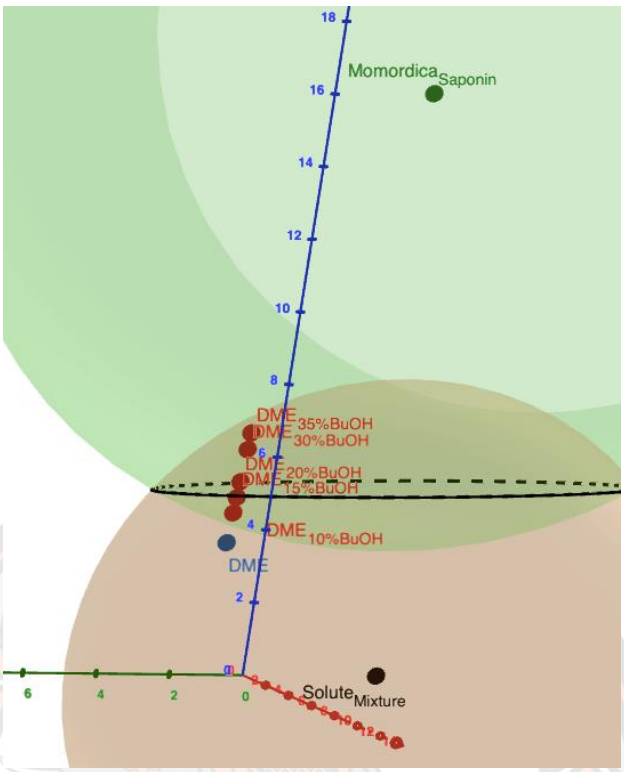

Figure 19 Hansen parameter sphere of solute mixture and pure Momordica saponin for various composition of butanol as co-solvent with DME.

The results of saponins recovery experiment are shown in **Figure 19** and they revealed that adding methanol at 10 and 15 % wt to DME resulted in an improvement of saponins recovery compared with the use of methanol as single solvent (39.0% and 43.5% versus 2.4%). However, the % saponins recovery was slightly lower compared with DME as single solvent (39.0% and 43.5% versus 44.9%). In addition, there was no significantly different of %saponins recovery between methanol composition at 10 and 15 % wt. It might be indicated from the results that methanol might be not suitable co-solvent in this case. These results could be explained by the HSPs prediction and spheres which adding methanol caused the bended out from the both solutes even they lied in the intersection area. In case of ethanol, as seen in **Figure 19**, %saponin recovery was improved compared with both ethanol and DME as single solvent. The highest %saponin recovery for ethanol as co-solvent (64.6%) was observed at the composition of 25%wt. In case of n-butanol, when the amount of n-butanol increased from 10 to 25% wt, saponins recovery also increased from 56.03% to 72.35%, respectively. However, when the n-butanol percentage was over 25% wt, saponins recovery decreased sharply. One possible explanation is that the higher amount of n-butanol might reduce the gas-like properties of sub-critical DME, lowering its diffusibility (Anas et al., 2020) and extraction efficiency as a result.

From the results, it was clear that methanol has less potential to be used as co-solvent for this purpose. However, it should be considered the possibility of using ethanol and n-butanol as co-solvent. The most suitable composition of using ethanol and n-butanol was the same composition at 25% wt which gave 64.6% and 72.35% of saponins recovery, respectively. The results were clearly indicated that n-butanol as

co-solvent had most saponins recovery performance. However, ethanol, in term of energy consumption for removing the solvent residue and toxicity, was also the potential co-solvent for saponins recovery form GSK.

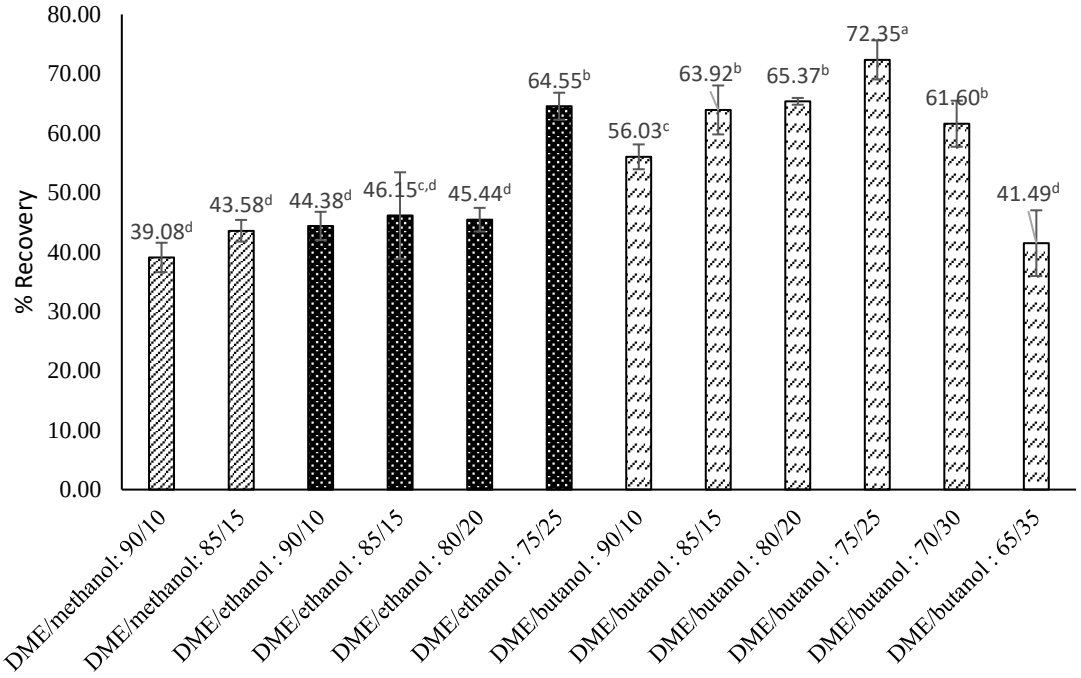

Figure 20 Saponins recovery from GSK using DME with various co-solvents and compositions: extraction conditions of 25°C for 30 minutes and 5:1 (ml/g) solvent to sample ratio.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings from this work revealed that pure solvent system of polar solvents including methanol, ethanol, propanol, and n-butanol or dimethyl ether (DME) as non-polar solvent cannot achieve the saponins recovery from oil body and matrices of gac seed kernel, leading to the low %recover observed. The experimental results were related to HSPs predictions which suggested to use co-solvent system of DME and n-butanol to enhance %recover of saponins. Moreover, the saponins %recover was increased by optimized extraction conditions which gave 75.61% recovery using dimethyl ether with n-butanol as co-solvent at 75 to 25 w/w ratio at extraction temperature of 35°C. This one-step extraction method has advantages over two-step method of de-fatting prior to extraction because it requires lower step and energy consumption, resulting to more economical and practical process.

5.2 Recommendations

The solubilities study for other solvents type should be studied in future work to obtain more information for more comparison result.

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