STUDY ON EXTRACTION KINETICS AND FORMULATION OF NATURAL MOSQUITO REPELLENT SOLUTION FROM MARIGOLD FLOWER EXTRACTS

NOOR ASIKIN BINTI AHMAD SAFRI

Thesis submitted in fulfillment of the requirements
For the award of the degree of
Bachelor in Chemical Engineering

Faculty of Chemical Engineering and Natural Resources
UNIVERSITY MALAYSIA PAHANG

FEBRUARY 2012

Created with
nitro PDF professional
download the free trial online at nitropdf.com/professional

ABSTRACT

The uses of plant extract which is Marigold flower can reduce the uses of chemical in mosquito repellent. Thus a research will be conduct to study on extraction kinetics and formulation of natural mosquito repellent solution using ultrasonic extraction from Marigold flower. During the extraction process, the reaction kinetics is study. Lutein has been extracted from Marigold using ultrasonic extraction at various extraction conditions such as different temperature, extraction time and volume of solvent due to its pharmacological actions. Lutein content in the extract is also determined by high performance liquid chromatography (HPLC) method. Based on experimental results, the content of lutein extracted from Marigold flower was found to be 2.5354% and the maximum amount of lutein extracted was obtained at temperature 45^{o}C, volume of solvent at 150 ml and extraction time at 30 mins. The optimization results demonstrated that temperature was the influential variable on the extraction content of lutein. The extraction rate constant, k of lutein decreased with increasing temperature and volume of solvent, and the k values were (0.0405-0.2712) min^{-1}. The lutein with higher concentration is used as main ingredient in mosquito repellent which gives the positive effect.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

ABSTRAK

Penggunaan ekstrak tumbuhan iaitu bunga Marigold boleh mengurangkan penggunaan bahan kimia di dalam kandungan penghalau nyamuk. Oleh itu, kajian ini menjalankan kajian ke atas kinetik pengekstrakan dan kandungan penghalau nyamuk daripada tumbuh-tumbuhan semulajadi dimana mengunakan kaedah ultrasonik daripada bunga Marigold. Semasa proses pengekstrakan, tindak balas kinetik akan dikaji. Pengeluaran lutein daripada bunga Marigold menggunakan kaedah pengekstrakan ultrasonik pada faktor-faktor tertentu seperti perbezaan dari segi suhu, masa dan isipadu pelarut akibat daripada tindakan farmakologi. Kandungan lutein dalam ekstrak juga ditentukan oleh kaedah kromatografi cecair prestasi tinggi (HPLC). Berdasarkan keputusan ujikaji, jumlah maksimum kandungan lutein yang diekstrak daripada bunga Marigold ialah 2.5354% diperolehi pada suhu 45^{o}C, isipadu pelarut sebanyak 150 ml dan pada masa 30 minit. Keputusan optimum yang diperolehi menunjukkan bahawa suhu adalah pembolehubah yang mempengaruhi kandungan pengekstrakan lutein. Pemalar tindak balas, k dapat dilihat bahawa lutein menurun dengan pertambahan suhu dan isipadu pelarut, dan nilai-nilai k ialah (0405-0,2712) min^{-1}. Kandungan lutein yang berkepekatan tinggi digunakan sebagai bahan utama dalam penghalau nyamuk yang memberikan kesan positif.

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

TABLE OF CONTENTS

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

LIST OF TABLES

Created with nitroPDF® professional
download the free trial online at nitropdf.com/professional

LIST OF FIGURES

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

LIST OF SYMBOLS

g	gram
^{o}C	degree celcius
y	area under the graph
m	slope of the graph
x	concentration of lutein
b	intercept at y-axis
%	percentage
k	rate constant
t	extraction time
S_o	total content of extractible compounds
S_t	remained extractible compounds after extraction time
V	volume of solvent
T	temperature

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Over one million people worldwide die from mosquito diseases every year because of the diseases that the mosquitoes carried out such as malaria, yellow fever, dengue fever, and others (Buderer et al., 2004; Darling, 2007; Kwoen et al., 2008; Bowden et al., 2010; Singh & Ing, 2010; Specos et al., 2010). Due to the concern of the health, many repellent were produced to avoid the diseases which are repel the mosquitoes from the human, plant, and building structures.

At present, the mosquito repellent formulation usually contain the chemical compound to obtain the high efficiency of repellent because the excellent mosquito repellent effect. However, these types of chemical have a side effect and have potential to be harm to the human (Kwoen et al., 2008). Widely used compound in mosquito repellents formulation is N, N-diethyl-m-toluamide, also called N, N-diethyl-3-methylbenzamide and commonly known as DEET (Choochote et al., 2007; Gillij et al., 2008). Although DEET had a remarkable safety profile for the last 40 years of worldwide use, there are a number of reports on its toxicity against the skin, generally happening when the product is used incorrectly or in the long term (Blackwell et al., 2003; Choochote et al., 2007). Fradin and Day (2002) said that other undesirable effects of this substance are an unpleasant odor, uncomfortable oily or sticky feeling, and danger to plastics and synthetic rubber. Due to these disadvantages, many customers prefer to use alternatives such as repellents from natural origin (Choochote et al., 2007; Kwoen et al., 2008)

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

There are numerous plants and derived products have been investigated and described as potentially natural sources of mosquito repellents due to their eco-friendly and biodegradable nature. Most plant-based mosquito repellents currently on the market contain essential oils from one or more of the following plants: citronella, cedar, eucalyptus, geranium, lemon-grass, peppermint, neem and soybean (Prajapathi, 2005; Choochote et al., 2007; Gillij et al., 2008). Vasudevan, Kashyap, and Sharma (1997) found that Marigold flowers also have a potential to become repellent of mosquitoes.

An active compound in Marigold flower is use as repellent with addition of other chemical. Marigolds originated in Central America and now inhabitants of much of Asia, Europe and the Americas. They have been used in many applications such as perfumes, dyes, inks, paints, ornamental arrangements, in landscape design, and in religious ceremonies. These plants are sometimes confused with the European-origin calendula, but their properties are not same.

There are several methods to extract the plant based to obtain main component in the material. There are like steam distillation, hydro distillation, and solvent extraction but this study focus on extraction using ultrasonic extraction method. In this research, the flower petals are extracting to get the active compound in the Marigold flower using the ultrasonic extraction. Due to the extraction process, the kinetics occurs in different conditions which involve the movement between the solute and solid and also the solute and the solvent also studied.

1.2 Problem Statement

In particularly, the production of mosquito repellent usually using a lot of chemical compound mainly DEET where it has an unpleasant odor and strong penetration into the skin which can be harmful to the human. The uses of plant extract can reduce the uses of chemical in mosquito repellent. To extract the plant to be uses in repellent, there are many ways to produce lutein from Marigold flowers such as microwave extraction and supercritical carbon dioxide extraction. These types of conventional extraction method have their own disadvantages due to long extraction time and poor stability of free lutein as stated by Liu (2010).

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

1.3 Research Objectives

i. General objective:

- To develop formulation of mosquito repellent using Marigold flower extract

ii.Specific objective:

- To study the yield of lutein and kinetic of ultrasonic extraction from Marigold flower

1.4 Scope of Research

In order to achieve the objectives stated above, the following scopes of study have been drawn:

- To study the different parameters (effect of solvent volume, extraction time and temperature) which give optimum conditions to extract lutein
- To find value of rate constant, k with varied of extraction time in optimal condition
- Testing the mosquito repellent in the place with many mosquito

1.5 Rationale and Significance

The ultrasonic extraction method for lutein in the present invention utilizes intensive vibration, high acceleration, intensive cavitations effect, and stirring action induced by ultrasonic waves to accelerate the entrance of lutein into solvent, so as to increase the extraction rate of effectiveness components and shorten the extraction time. Compared with supercritical method, the disclosed method possessed low equipment investment and simple processing.

Other than that, the ultrasonic extraction technique is a low temperature physical extraction process, which is more beneficial for extraction of lutein with poor thermal stability. Compared with microwave extraction, ultrasounds extraction has the main advantage of working at ambient temperatures, thus avoiding the thermal overexposure,

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

a very important asset for industry. Like ultrasound extraction, microwave intensification needs special equipment to be functional, which means higher investments, and electricity to produce waves, which means higher operating costs than classic techniques.

The active component in Marigold flower can be used as repellent same effective as DEET and at the same time can lower the cost of mosquito repellent. This repellent is non-toxic and very safe for every age group and do not harm pets also. The smell of the oil relieves from new mosquito repellent can calms the body.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

CHAPTER 2

LITERATURE REVIEW

2.1 Mosquito Repellent

A mosquito is a very harmful insect which are carries disease causing viruses and parasites from person to person without catching the disease themselves. Mosquitoes are estimated to transmit disease to more than 700 million people annually in Africa, South America, Central America, Mexico, Russia and much of Asia with millions of resulting deaths which at least 2 million people annually die of these diseases (Crosby, 2005, p. 12). Due to this problem, many product of mosquito repellent are produced to use for personal protection and it is quite popular among citizens in this country.

2.1.1 Formulation of Mosquito Repellent

Mosquito repellent formulation is well known to use for personal protection from mosquitoes. Usually the repellent exists in the form of lotions, aerosol spray, or cream, which displays the warning labels especially for the children (Bowden, & Bowden, 2010). Such formulation are normally applied to the skin of humans or the coats of animals to provide repellency which last a few hours (Vlasblom, 1996). Most of the insecticides are harmless not only to the mosquitoes but can also be harmless towards human and other forms of life in the environment. The compositions in the repellent should not cause illness or death to the human and other communities.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

DEET is the active ingredient found in many insect repellent products. It is used to repel biting pests such as mosquitoes and ticks, including ticks that may carry Lyme disease. Products containing DEET currently are available to the public in a variety of liquids, lotions, sprays, and impregnated materials (e.g., wrist bands). The book of *Travelers' Health* (2009) state that DEET has higher effectiveness where 100% DEET was found to offer up to 12 hours of protection while several lower concentration DEET formulations (20%-34%) offered 3–6 hours of protection. Formulations registered for direct application to human skin contain from 4 to 100 percent DEET but in United States currently restricts using DEET more than 20% in mosquito repellent for the public use (Kwoen et al., 2008). The use of DEET has been restricted for children, pregnant women, and people with sensitive skin and so on to use it (Darling, 2007; Gillij et al., 2008). Therefore, efforts have been made to develop mosquito repellent using natural plant to replaced chemical composition in formulation (Baker, 2008).

Natural mosquito repellents consist of a combination of numerous ingredients that keep mosquitoes at bay. The plants whose essential oils have been reported to have repellent activity include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, basil, thyme, and peppermint. Most of these essential oils provided short-lasting protection usually lasting less than 2 hours. Many essential oils and their monoterpenic constituents are known for their mosquito repellent activity against *Culex* species (Choi et al., 2004; Traboulsi et al., 2002). The oils from Basil, Thyme, Fennel, Allspice, Lavender, Pine, Garlic, Soybean, Verbena, Pennyroyal, Cajeout, and Neem are less common, but also used in natural mosquito repellents (Gaborik, 2011).

The formulations of repellent should be long lasting product and lesser of toxicity than chemical repellent. The ingredient in natural repellent consist 100% of essential oil or addition of some chemical to increase the effectiveness. Researcher of repellent, Runkel (2003) stated that the addition of glycerol in repellent give advantage of this feature that it is possible to make available formulations that are pleasant to the skin, or particular galenical presentations can be offered, for example skin sprays, lotions, creams, or sticks. Other than that, the present of glycol as the adjuvant can extends the period of effectiveness in repellent thus resulting in an extension of the

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

period effectiveness with relatively small quantities of actual active ingredient (repellent), thus creating an effective and at the same time tolerable as mosquito repellent agent.

Although these oils are capable of repelling mosquitoes, certain factors can lower their effectiveness. Wind and high temperature cause them to evaporate, rain, perspiration and swimming dilute them, and various sunscreens lower their potency. Furthermore, they are quickly absorbed into the skin. Consequently, it is suggested that a natural mosquito repellent be reapplied every two hours.

2.2 Natural Plant

2.2.1 Plant Description

Marigolds are most useful in repelling or warning away insects when planted along with vegetables and fruits. It has pungent smell that repels insects including mosquitoes and usually the villagers planted it around their house and farm. This statement is supported by Vasudevan et al. (1997) and also by Sarin (2004). It also is a potential plant whose essential oil from flowers has been effective repellent against insects (Ray et al., 2000).

2.2.2 Active Compound in Marigold Flower

A number of papers are now available on the repellent activities from Marigold against different type of mosquito species. It has an active ingredient which is lutein. Lutein is one type of material that can be use in repellent to replace DEET. It obtained from the Marigold flower petals where it is one of the major constituent of yellow or orange fruits and vegetables such as mango, papaya, prunes and others (Cromble, 2004; Hojnik et al., 2008). Lutein is usually useful for preventing cataract and arteriosclerosis, enhances immunity and also has significant functions for preventing cancer formation where it can delay cancer development (Liu & Fan, 2010). Figure 2.1 shows the lutein structure with molecular formula is $C_{40}H_{56}O_2$ and molecular weight is 568.87 g/mol. It is insoluble in water, but soluble in fats and lipophilic solvents.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

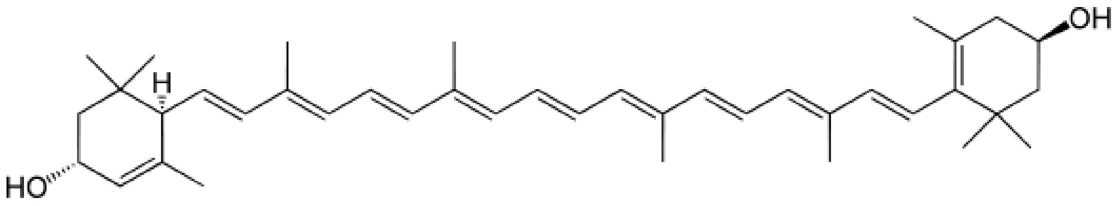

Figure 2.1: Lutein structure

2.3 Method of Extraction

There are many methods reported in literature for the extraction of lutein and esters of lutein from marigold flowers (Modad et al. 2000; Barzana et al. 2002; Navarrete- Bolanos et al. 2005). Generally, lutein is extracted from marigold flowers by solvent (hexane) extraction of dried flowers followed by the removal of solvent to obtain oleoresin, which is subjected to further purification steps to obtain a mixture of lutein and xanthophylls that is suitable for human consumption as a food additive or as nutritional supplement (Breithaupt and Schlatter 2005). The advantages of drying flowers are reduction in the bulk, lower water activity and ease of extraction of pigment.

There are several methods reported in literature for the extraction of lutein from Marigold flowers to increase the productivity such as ultrasonic extraction, microwave extraction and supercritical fluid extraction to improve the yield and quality of extracted products (Wang, 2006). The lutein content in Marigold petals is variable and can be as low as 0.03% (Piccaglia et al. 1998).

2.3.1 Ultrasonic Extraction

Ultrasonic were employed to extract active compounds such as saponins, steroids and triterpenoids from Chresta spp. about three times faster than the conventional extraction methods (Schinor et al., 2004). The source of energy for the ultrasonic techniques is the ultrasonic field. The mechanical waves formed by the ultrasound enable generation, locally, micro-cavitations in the liquid surrounding the plant material and therefore heating this material and furthering release the extract.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

There are two effects which are mechanical disruption of the cell's wall releasing its content and local heating of the liquid, increasing the extract diffusion. The kinetic energy is introduced in the whole volume following the collapse of cavitation bubbles at or near walls or interfaces thus improving the mass transfer across the solid-liquid interface. The mechanical effects of ultrasounds induce a greater penetration of solvent into cellular membrane walls, facilitating the release of contents of the cells and improve mass transfer (Alupului et al., 2009).

Many factors affect the course and efficiency of extraction using ultrasounds. They are such parameters associated with acoustic field as wave frequency, ultrasound intensity, acoustic energy density; raw material: structure, breaking up level, type and amount of extracted substance; solvent's physical properties; as well as the process itself: duration, temperature, pressure, etc.

Furthermore, the ultrasonic waves are mechanical pressure waves formed by actuating the ultrasonic transducers with high frequency, high voltage current generated by electronic oscillators. The generated ultrasonic waves propagate perpendicularly to the resonating surface. The waves interact with liquid media to generate cavitation implosions. High intensity ultrasonic waves create micro vapor/vacuum bubbles in the liquid medium, which grow to maximum sizes proportional to the applied ultrasonic frequency and then implode, releasing their energies. The cavitations size is smaller when the frequency is higher.

The high intensity ultrasonic can also grow cavities to a maximum in the course of a single cycle. At 20 kHz the bubble size is roughly 170 microns in diameter At a higher frequency of 68 kHz, the total time from nucleation to implosion is estimated to be about one third of that at 25 kHz. At different frequencies, the minimum amount of energy required to produce ultrasonic cavities must be above the cavitation threshold. In other words, the ultrasonic waves must have enough pressure amplitude to overcome the natural molecular bonding forces and the natural elasticity of the liquid medium in order to grow the cavities. For water, at ambient, the minimum amount of energy needed to be above the threshold was found to be about 0.3 and 0.5 W/cm^2 per the transducer radiating surface for 20 kHz and 40 kHz, respectively.

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

The energy released from an implosion in close vicinity to the surface collides with and fragments or disintegrates the contaminants, allowing the detergent or the cleaning solvent to displace it at a very fast rate. The implosion also produces dynamic pressure waves which carry the fragments away from the surface. The implosion is also accompanied by high speed micro streaming currents of the liquid molecules. The cumulative effect of millions of continuous tiny implosions in a liquid medium is what provides the necessary mechanical energy to break physically bonded contaminants, speed up the hydrolysis of chemically bonded ones and enhance the solubilization of ionic contaminants. The chemical composition of the medium is an important factor in speeding the removal rate of various contaminants.

The ultrasonic extraction system for biologically active compounds has many advantages over other conventional extraction methods. Ultrasonic extraction methods is more simple and required shorter time, less solvents, provide higher extraction rates, high safety and better products with lower costs (Bjorklund & Eskilsson, 2000; Belanger et al., 2003; Mandal, 2007). It's also using low temperature physical extraction process which is more beneficial for extraction of lutein with poor thermal stability (Liu & Fan, 2010).

2.3.2 Microwave Extraction

The application of microwave extraction to natural compounds such as glycosides, alkaloids, carotenoids, terpenes, and essential oils has been reviewed by Kaufmann (2002). The use of microwave energy for the extraction of active substances from plant materials results in more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start up and increased production rates (Alupului et. al., 2009).

In case of microwave irradiation on biological material, electromagnetic waves are indeed absorbed selectively by media possessing a high dielectric constant. During absorption, the microwaves energy is converted into kinetic energy, thus enabling the selective heating of the microwave absorbent parts of the plant material. The volume increased in this way makes cells explode, releasing their content into the liquid phase.

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

When the liquid phase absorbs the microwaves, the kinetic energy of its molecules increases and consequently, the diffusion rate increases too (Mandal, 2007). While in microwave extraction, the kinetic energy is introduced locally through heating and then it propagates in the whole mass of the liquid phase increasing the diffusion rate.

2.3.3 Supercritical Fluid Extraction

The high pressure of carbon dioxide is use in supercritical extraction method where carbon dioxide becomes a supercritical state above the critical point. This method uses two different pressures within the extraction chamber. The first extract which is at first pressure containing carotene is obtained. At second pressure, the lutein is obtained with free of beta-carotene. The amount of carbon dioxide needed for obtaining the lutein is higher approximately 80 kg/kg feed. The results show that the production of lutein was generally low at all temperatures investigated (Skerget et al., 2010). The disadvantage of this method is use a high equipment investment and high production cost where the uses of high pressure (Liu & Fan, 2010).

2.4 Factor Affecting Extraction Process

2.4.1 Temperature

Commonly, temperature has a positive effect on extraction efficiency and extraction rates when it is not too high where several of active components in plant may degrade with temperature. Hojnik et al. (2008) found that when temperature is increase from 20 to 40^{o}C, a small increase in the final extraction efficiency of lutein can be observed and remains constant with further rise of temperature to 60^{o}C.It is due to film resistance and intra element diffusion are controlling the rate of process where fast diffusion coefficient of lutein is decrease.

In ultrasonic extraction, a higher temperature means a higher efficiency in the extraction process due to the increase in the number of cavitation bubbles and in the surface contact area. However, this effect tends to disappear when the temperature is near the boiling point. It must be borne in mind that the effect of temperature depends

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional

on the analyte. For some other compounds, increasing the extraction temperature to 45-70°C will increases the recovery. In contrast, other analytes can be easily degraded with an increment of temperature. During ultrasonic extraction the solvent temperature increases with the extraction time and the sonic power applied, owing to the sonication process.

Guihua & Quancheng (2011) also state the same thing with Hojnik et al. (2008) where the amount of lutein extracted significantly increased with increasing temperature, but the content of lutein extracted slightly decreased with increasing temperature. Increasing the temperature will increase the solubility of the lutein which results in higher yields. Instead of that the increasing temperature can contributed to damage the particle cell wall, and as the result lutein availability for extraction was increased.

2.4.2 Extraction Time

Liu and Fan (2010) investigated the effect of extraction time on extraction efficiency. They said that when the extraction time is short, the dissolution balance of lutein and the extraction solvent is not yet reached, therefore the extraction rate is small, but when the extraction time is extended, as lutein is unstable to heat, heat generated by ultrasound has a certain damaging effect on lutein. Therefore, the extraction rate is lower if the extraction time is longer than a certain time.

2.4.3 Volume of Solvent

The adequate selection of the solvent plays very important role in increasing the efficiency of extraction of particular active ingredient due to the corresponding properties of its solubility and selectivity. The solubility properties of the solvent with different polarities used can increases the extraction efficiency. If the reaction is one in which the products are more polar than the reactants then a polar solvent accelerates the reaction. So the reaction is accelerated in the presence of polar solvents like benzyl alcohol.

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

On the other hand if the reactants are more polar than the products, a polar solvent decreases the reaction rate. In general a polar solvent hastens the reaction in the direction of increasing polarity. When both reactants and products are non polar, polarity of solvents will have no influence on the rate of the reaction and the rate is independent of the nature of the solvent. As discussed by Liu and Fan (2010), when the ratio of liquid to material (v/m) is larger than 40, the extraction efficiency is reduced along with the increase of solvent amount which shows that when ratio of liquid to material is 40, lutein and extraction solvent substantially reach dissolution balance.

2.5 Extraction Kinetics

There are different researches and studies have been conducted to describe the kinetics of the extraction processes (Kadi et al., 2006; Kadi, & Meziane, 2008). The main part of the solute gets extracted rapidly because of the scrubbing and dissolution caused by driving force of the fresh solvent and then comes the next stages where the extraction process gets much slower accomplished by external diffusion of the remains solute in the solution (Abidin et al., 2009). As the content of the solute in solid varies with time and distance, the diffusion coefficients of solute in solid can be determined by observing the adjusted in its concentration in the surrounding liquid by means of time. In this research, the process of lutein extraction depends on the rate of extraction from the composition in Marigold to the free lutein.

Furthermore, extraction kinetics of lutein from Marigold flowers also has been studied by Hojnik et al. (2008) with alkali treatment. Effect of enzyme pretreatment in comparison with acid and alkali pretreatment of fresh Marigold flowers on kinetics of extraction of pigment has been reported in the present study. A uniform and porous solid sphere, initially with a homogeneous concentration, is immersed in a well stirred liquid. The solute linked to the solid matrix by physical or chemical forces be required to transferred to the solvent phase by dissolution or desorption (Hojnik, et al., 2008). Next, the solute or solvent mixture diffuses to the solid surface and finally moves across the stagnant film around the particle to the bulk fluid phase (Campos et al., 2005).

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

Lavecchia and Zuorro (2006) have been investigated the kinetics of lutein stability in sunflower and rice bran oils using first order reaction. Their finding shown that thermal degradation of lutein followed the first-order kinetics, with apparent activation energies of 60.9 kJ mol^{-1} (in sunflower oil) and 44.9 kJ mol^{-1} (in rice bran oil).

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

CHAPTER 3

METHODOLOGY

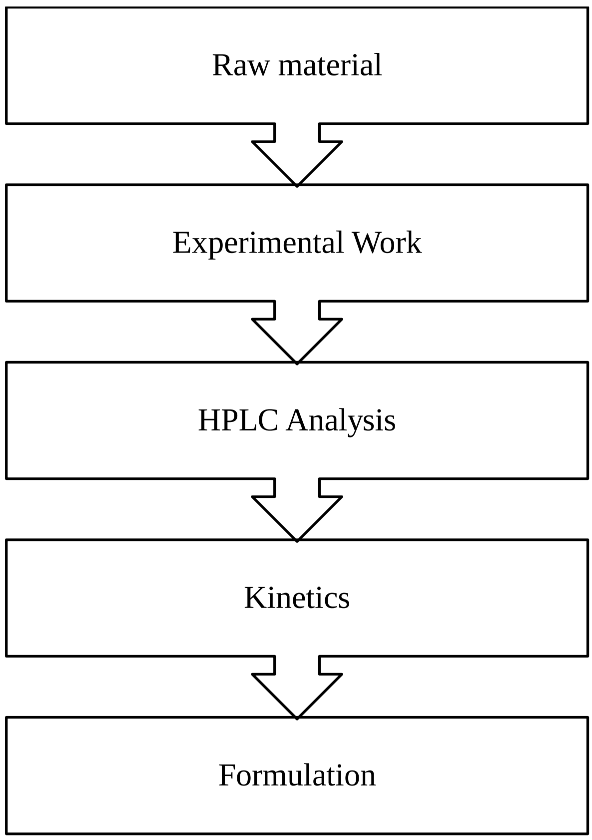

Figure 3.1: Flow pattern to produce mosquito repellent

Created with
nitroPDF professional
download the free trial online at nitropdf.com/professional

3.1 Raw material

Figure 3.2: Fresh Marigold flower petals

Figure 3.3: Marigold flower after dried

Fresh Marigold flowers (Figure 3.2) were collected from villagers of Felda Lepar Hilir. Then, the petals of flower were separated from the seed and this flower petals will dried from some period of time under the room temperature as shown in Figure 3.3. After that, the dry flower petals were grounded into powder using grinder before the extraction (Figure 3.4 & 3.5).

Figure 3.4: Grinder machine

Figure 3.5: Powder of Marigold flower petals

Created with nitroPDF professional
download the free trial online at nitropdf.com/professional