A STUDY ON ULTRASONIC ASSISTED EXTRACTION AND FORMULATE NATURAL HAIR SHAMPOO FROM SAPINDUS EMARGINATUS

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We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for award of the Degree of Chemical Engineering.

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I hereby declare that the work in this thesis is my own expect for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Special Dedication to my family members,

My fellow lecturers,

my friends,

and all faculty members

Thanks for all your Care, Love, Encouragement and Best Wishes

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ABSTRACT

Hair shampoo is a personal care product that can removes dirt, skin particle, and sebum from hair skin. Mostly of hair shampoo consist of dangerous ingredient such as Sodium Laureth Sulfate which it produces skin and hair damages, including cracking and severe inflammation of tissue. Thus, *Sapindus emarginatus* will be used as main ingredient for a new formulation of hair shampoo. This herb material contains an active compound known as saponin which can replace the dangerous compound in hair shampoo. The objective of this research is to extract saponin from Sapindus emarginatus using ultrasound assisted extraction in order to formulate natural hair shampoo. The yields of saponin was measured by HPLC by study the effect of different size particle, sonication time and temperature. Based on result, the maximum amount of saponin extracted obtained at size particle 315 µm, sonication time at 60 minutes and temperature at 60°C .The herbal extract was mix with other ingredients such as Methyl paraben and EDTA to form of a new formulation. The shampoo was tested using pH test. The extraction rate constant, k of saponin decreased with increasing temperature and sonication time, and the k values between range 0.1091 to 0.430. Thus, the saponin with higher concentration is used as main ingredient in order to formulate natural hair shampoo.

ABSTRAK

Syampu rambut merupakan produk penjagaan diri yang boleh menghilangkan kotoran, kotoran pada zarah kulit dan sebum daripada kulit rambut. Kebanyakannya syampu rambut terdiri daripada bahan berbahaya seperti Sodium Laureth Sulfate yang menghasilkan kerosakan kulit dan rambut termasuk keradangan retak dan tisu yang teruk. Oleh itu, sapindus emarginatus akan digunakan sebagai bahan utama untuk formulasi syampu rambut yang baru. Bahan herba ini mengandungi sejenis bahan aktif yang dikenali sebagai saponin yang boleh menggantikan komponen berbahaya di dalam syampu rambut. Objective bagi kajian ini adalah untuk mengeluarkan saponin daripada Sapindus emarginatus menggunakan ultrasonic dalam membuat formula shampoo asli Untuk kaedah, dalam usaha untuk mendapatkan komponen bioaktif ini, sapindus emarginatus perlu dalam bentuk serbu. Apabila masa semakin meningkat, terdapat perubahan pada suhu. Semakin tinggi suhu, semakin maksimum hasil yang diperolehi bagi masa yang tertentu.. Seterusnya, untuk merumuskan satu shampoo yang jelas, ekstrak herba telah bercampur dengan bahan lain seperti methyl paraben dan EDTA untuk membentuk formulasi baru. Shampoo tersebut telah diuji dengan menggunakan ujikaji pH. Pemalar tindakbalas k dapat dilihat dengan bahawa saponin menurun apabila pertambahan suhu dan juga sonikasi masa dan nilai k diantara kadar 0.1091 sehingga 0.430. Maka, kandungan saponin yang berkepekatan tinggi digunakan sebagai bahan utama dalam melakukan formula bagi shampoo yang asli.

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LIST OF SYMBOLS

g	gram
°C	degree celcius
y	area under the graph
m	slope of the graph
x	concentration of saponin
k	rate constant
t	sonication time
T	temperature
S_o	total content of extractible compounds
S_t	remained extractible compounds after extraction time

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Sapindus Emarginatus commonly known as soapnut is a medium sized tree found in India. It is a genus of about five to twelve species of shrubs and small trees in the Sapindaceae, native warm temperature to tropical regions in both the Eastern and Western Hemispheres. In addition, it is distributed in Indian Peninsula, chiefly in South India. Members of these genus also called "soapberries" because of its pulp have special properties which can produce soap. In addition, it is also an economically important tree which has been brought under silvicultural practices (Troup R, 1921). Based on its special properties, inside from their pulp contain important bioactive compound like saponin to produce shampoo. It also reported that high content of saponin inside pericarp (The Wealth of India, 1972; Gupta and Ahmed, 1990). From its pericarp, two Pisicidal triterpenoid saponins, acetylated triterpene saponins, hederagenin, sweer acyclic sequieterpene glycoside and Mukurozioside IIb15 have been isolated (Wilawan,Pittaya et al. 1990). The soapnut extracts contained 11.58-19.58 % of total saponin which increase importance of soapnut (Battal, 2002)

Sapindus emarginatus contain saponin, the natural washing ingredient which are likely to soap. When the shells of the soapnut have some contact with water, the saponin will be released. The major commercial sources of saponin are *Yucca schidigera* and also *Quillaja saponaria*. Saponin has a large family which containing a steroidal or triterpenoid aglycone that linked to one or more oligosaccharide moieties. *Sapogenin* or *genin* is the aglycone or non saccharide portion that contain in saponin molecule. This saponin can be divided into three major classes which are Triterpene glycosides (C_{30}), Steroid glycosides (C_{27}) and also Steriod alkaloid glycosides (Hostettmann and Marston, 1995). Steroid glycosides saponins are shown in Figure 1.1.

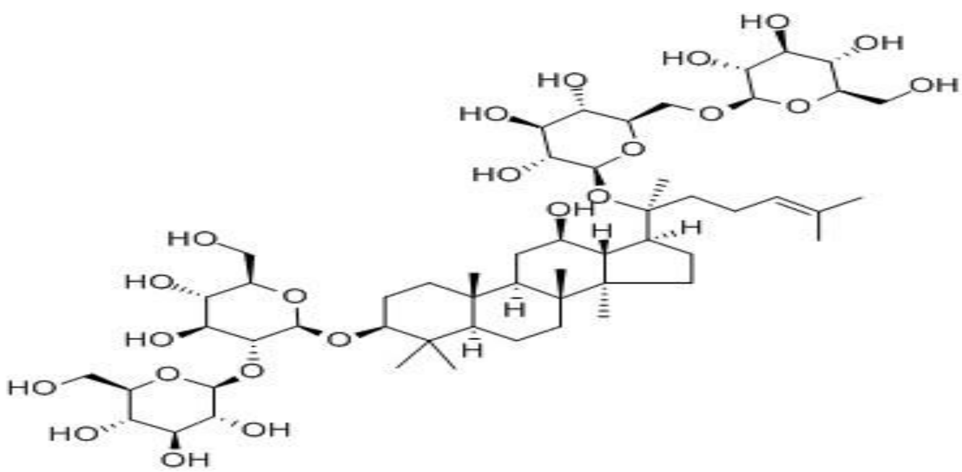

Figure 1.1: Molecular structure of steroid glycosides saponin

The definition of saponin is through their surface activity which has detergent properties. This is because they contain both water-soluble and fat soluble components. They consist of a fat-soluble core, either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates. They are also strongly surface active and can form stable foams which act as emulsifying agents and form detergent (Hostettmann and Marston 1995). Saponin can be found in many plants especially certain desert plants. It is present in the small amount in some type of food and peas. It is advisable not to consume a large quantity of food that contains saponin. It is more toxic for some other creatures such as fish (Chevallier, 1996). Phytochemicals is chemical that presents naturally in plants. The phytochemicals saponin have spectrum which can act as antifungal and antibacterial agents and also inhibition of cancer cell growth. Recently, there has been numerous reported on the application of high power ultrasound in the extraction of various phytochemicals such as alkaloids, flavonoids, polysaccharides, proteins and essential oils from various parts of plant and plant seeds. (Hamburger, 1992)

Ultrasonic is sound that ranging from 20kHz to 1 GHz which generated by transducer that can converts electrical energy into high frequency vibrations (Sun and Wands,2008). Ultrasound assisted extraction is a technique for extract an important compounds from vegetal materials (Vilkhu,et al, 2008). It is also can be used either small or large scales (Vinatoru, 2001). If this technique is compared with other extraction technique such as microwave assisted extraction, it is more cheaper and also the operation is much easier (Chen et al.,2008;Wang & Weller, 2006). The ultrasonic cleaning bath and ultrasonic probe system is the general ultrasonic devices (Vinatoru, 2001; Luque-Garciá & Luque de Castro, 2003). The effect of mechanism by using ultrasound will give greater penetration towards membrane walls, the contents of cell easy to release and also will improve mass transfer for the compound (Kiel,2007). In addition, it can be carried out at lower temperature to avoid extracts become damage in higher thermal and also loss of volatile components.

1.2 Problem Statement

Nowadays, chemical shampoo is hair care product that consists of dangerous chemical compound such as Sodium Laureth Sulfate that will give long term effect towards our body. Thus, the problem for this research is to replace the dangerous compound from existing hair shampoo with a bioactive compound in herbal material such as saponin. The bioactive compound usually extract by soxhlet method with ultrasonic assisted to reduce time consuming of extraction process.

1.3 Objectives of Study

The objective of this research is to extract saponin from Sapindus emarginatus using ultrasound assisted extraction in order to formulate natural hair shampoo.

1.4 Scope of Study

There are some scopes which need to be focused in order to meet the objectives.

1.4.1 To study the optimum amount of extraction yield from saponin by using different operating conditions such as size of particles, sonication time and temperature.

1.4.2 Test the formulate hair shampoo in term of Ph

1.4.3 To study the kinetic of extraction in term of k (rate constant) value.

1.5 Rationale and Significant of Research

The new formulation of natural hair shampoo from *Sapindus emarginatus* can give big impact in pharmaceutical and cosmetic industries. Most of shampoos contain hazardous compounds such as Sodium Laureth Sulfate. By using plant material, it will produce natural shampoo which will replace the dangerous ingredient. Other than that, this type of shampoos is non toxic and good for health. Thus, the shampoo will be safer to be used regularly towards ourselves.

The extraction of bioactive compound from natural plant such as soapberries will give source of saponin which is can be found in every plant material. The extraction by using ultrasound assisted extraction, it will produce higher amount of yield. Besides that, this type of extraction will have lower operating cost. Thus, it will reduce the cost for industrial.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant Material

2.1.1 Plant Description

Sapindus Emarginatus is fairly large, deciduous tree which are usually up to 12 m in height, with a globose crown and rather fine leathery foliage. Its leaves in average 30 until 50 cm long, alternate, commonly its narrowly bordered and often slightly falcate. These trees have flower that’s inflorescence a compound terminal panicle in length 30cm with pubescent branches. For its fruit, it is a globose that has 1 seeded drupe or sometimes 2 drupels together. It is in diameter 0.8- 1.3cm that smooth, black color and also loose in dry fruit. This fruits have so many names depends which countries it has been planted such as sapindus rarak, sapindus mukorrasi and etc. It also has bioactive compound in these fruits which is saponin that used to formulate natural hair shampoo in this research. This tree also is multifunction as all part can produce variety of products as shown in Table 2.1.

Table 2.1: Product from parts component in soapnut trees

Products	Part of plant	Explanation
Food	Seed	Prepare industrial protein from globulin fraction.
Fodder	Leaves	Fodder is used for cattle
Essential oil	Seed	Essential oil produce
Medicine	Fruit and seed	As cure for epilepsy. From seed, it is used to stop the dental caries and also considered haemolytic.
Others	Main part Pulp	Use as a substitute for shampoo.

2.1.2 Benefit of Sapindus Emarginatus

Traditionally, *Sapindus emarginatus* is used as an anti-inflammatory, antipruitic and also have been used to purify the blood. Its seed are crushed to make an effectives and environmentally friendly natural soaps. In other words, their seed are intoxicant and its fruit has an oxytropic action. Its powder also used as nasal insufflations. Their powdered are said to posses insecticide properties. It cleanses the skin of oil and even use as cleanser washing hair and hair tonic, and forms a rich, natural lather. *Sapindus emarginatus* also has showed that it is a strong anti bacterial activity against the tested bacterial strains (Nair R, 2005). In addition, their pericalp has been reported that contain high content of saponins (Venkatesh V & Sharmal JD et al,2002).

2.2 Saponin

Saponin is a bioactive compound that can be found in plant herbs. It has been reported that saponins presents in fruits which have higher chances including these fruits.

For any type of sapindus, it has four acylated saponins with the main a glycone, hederagenin which is triterpene-type sapogenin have been identified by Hamburger et al in 1992. Triterpene-type saponins series need to be isolated and characterized in other species of sapindus such as S.saponaria (Lemos et al.1994) and S.emarginatus (Kachanapoom et al., 2001). Actually, saponin are easily to find in plant herbs which are group among anti-nutritionally which may caused photosensitization (Flaoyen and Wilkins, 1997: Meagher et al., 2001: Pirez et al,.2002). In others findings, it has shown that saponin has beneficial effect towards animal and also environment by reducing amount of methane produced by the animals (Wallace et al,.2002; Hess et al.,2003 a.b). It also can decrease or eliminate protozoan in the rumen without inhibiting bacterial growth by interact with cholesterol in membranes of eukaryotic cells. In addition, saponins are phytochemical compound that linked towards each chain on sugar chains. In recent years, these type of phytochemical compound will be increase their yielding by using ultrasonic (Z.Hromadkova, 2003).

2.3 Extraction Process

2.3.1 Ultrasonic assisted Extraction (UAE)

Ultrasonic assisted extraction is one of the possible methods to extract the *Sapindus Emarginatus.* This method is using ultrasound device of ultrasonic which are offers great potential in the processing liquids and slurries. This is regarded as a new way of technology in food, chemical and also pharmaceuticals industries (M.Vinatoru, 2001). It is represent a clean way to accelerate and improve mass transfer process. The function of high power of ultrasound for bioactive compound has been reported by many researchers (Bruni et al, 2002;Albu et al,2004; Wang and Weller,2008). For the enhancement of extraction efficiency of organic compound using this technology is attributed to a phenomenon called cavitation. Cavitation is formation of bubbles of a liquid in a region where the pressure of a liquid falls below its vapor pressure. When ultrasound is applied, a large amount of bubbles formed in liquid medium. When agitated by intense ultrasound, they oscillate and undergo explosive growth and subsequently collapse (Leighton, 1994). The reason collapse because of limited “space” for them to expand which are bubbles can reach thousands of bar in pressure and Kelvin in temperature. Extraction can be significantly improved with the aid of an ultrasound wave.

Even though the effects of ultrasonic have been studied in over hundred years of herbal species such as ginseng, tobacco but it effect on the UAE on this sapindus emarginatus has not been discovered.

2.3.2 Traditional Extraction

Extraction system is a separation process either solid or liquid. It has several examples of traditional extraction. Soxhlet extraction is one of example of traditional extraction that has been used long time ago and until now. It is required where the desired compound has limited solubility. This kind of extraction is very time consuming and also required a large amount of solvent.

If the desired compound has a significant solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. Normally, a solid material contain some of the desired compound placed inside a thimble that make from thick filter paper that loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor placed onto a flask containing the extraction solvent.

The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the thimble of solid. Slowly, the chamber containing the solid material that fills with warm solvent. When the Soxhlet extractor almost full, the chamber automatically emptied by siphon side arm, with the solvent running back down to the distillation flask. The Soxhlet extractor apparatus are shown in Figure 2.1.

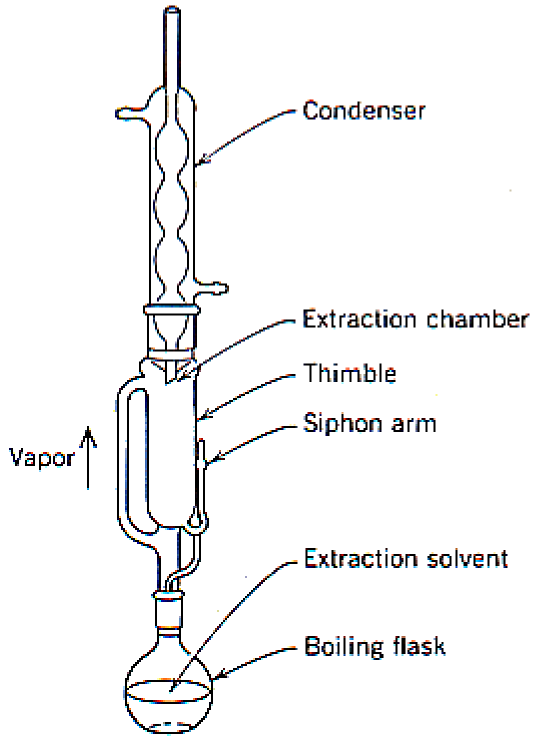

Figure 2.1: Soxhlet Extractor Apparatus

For the past 126 years, Soxhlet extraction has been the most respected among all other conventional techniques (M. Itelliar et al,1999) . It serves a dual purpose of extraction step for the isolation of phyto-constituents and as a well established model for the comparison of new extraction alternatives. One of the major significant shortcomings of Soxhlet extraction is the lengthy extraction time that can be 8, 16, 24 hours or more (Pastot,1997) , which results in consumption of considerable time and heat energy. The lengthy time requirement makes it more labor-intensive and limits the number of samples that can be processed which may not be entertained from commercial aspects. Use of large amount of organic solvents requires an additional recovery step and subsequent evaporation to environment.

2.3.3 Differentiate between UAE and Soxhlet Extraction

The differences between UAE and Soxhlet extraction are given in Table 2.2.

Table 2.2: Difference between UAE and Soxhlet extractor

Difference	Ultrasonic assisted extraction (UAE)	Soxhlet extraction
Process	The sound ranging from 20 kHz to 1GHz which generated by transducer that converts mechanical or electrical enegy into high vibrations. (Sun & Wands, 2008)	Soxhlet extraction used to extract soluble compounds from plant material.
Power used	20kHz	None
Equipment	Ultrasonic cleaner	Combination condenser and soxhlet
Time consuming	Less time	Longer time
Amount of solvent	Less solvent	Many solvent
Amount of yield	Higher yield	Less yield
Difficulties level	Lower	Higher
Cost	Lower	Higher
Environmental	Friendly user	Friendly user

From Table 2.2, it shows that UAE has more advantages than soxhlet extraction. This is because of time consuming for UAE is faster than soxhlet which required longer time to extract the bioactive plant. Other than that, UAE only need less solvent otherwhile soxhlet need many amount of solvent. Both of techniques are friendly users which not harm to the environment. Therefore, UAE was used to extract bioactive compound from plant material.

2.4 Mechanism in Ultrasonic assisted extraction

Ultrasonic has been used for years in research and also diagnostics which are turning this laboratory based prototype technology into fully operational processes such as food and cosmetics industries. From higher sound, it can convert mechanical or electrical energy into high vibrations (Sun & Wands, 2008). Thus, it will provide some unique condition to derive chemical reactions. These unique conditions will derive from acoustic cavitation which are have sound induced growth and collapse of micrometer sized cavities in a liquid. Through these condition, it will result such as emission of light and dissociation of chemical bonds. Thus, it will refer as sonochemistry.

Sonochemistry is about the effect of sonic waves and wave properties on chemical systems. The chemical effect is indirect interaction with molecular species. Some studies shows that no direct coupling with chemical species on molecular level. It is arises from acoustic cavitation which the formation, growth and implosive collapse of bubbles irradiated with sound. Cavitation is a process where mechanical activation destroys the attractive forces of molecules in the liquid phase. When sonicating liquid at high intensities the sound wave will propagate into the liquid media result. By applying the ultrasound, compression of the liquid is followed by the rarefaction which a sudden small pressure drops form, oscillating bubbles of gaseous substance.

From Figure 2.2 shows that the bubble collapse in liquid phases will produces enormous amounts of energy from the conversion of kinetic energy of the liquid motion into heating the contents of the bubbles. The compression of the bubbles during cavitation is more rapid than thermal transport which generates a short-lived localized hot spot. During the implosion very high temperature and pressure are reached locally. The implosion of the cavitation bubbles also results in liquid jets of up to 280 m/s velocity. The resulting shear forces break the cell envelope mechanically and improve material transfer. The ultrasound can have either destructive or constructive effects to cells depending on the sonication parameters employed.

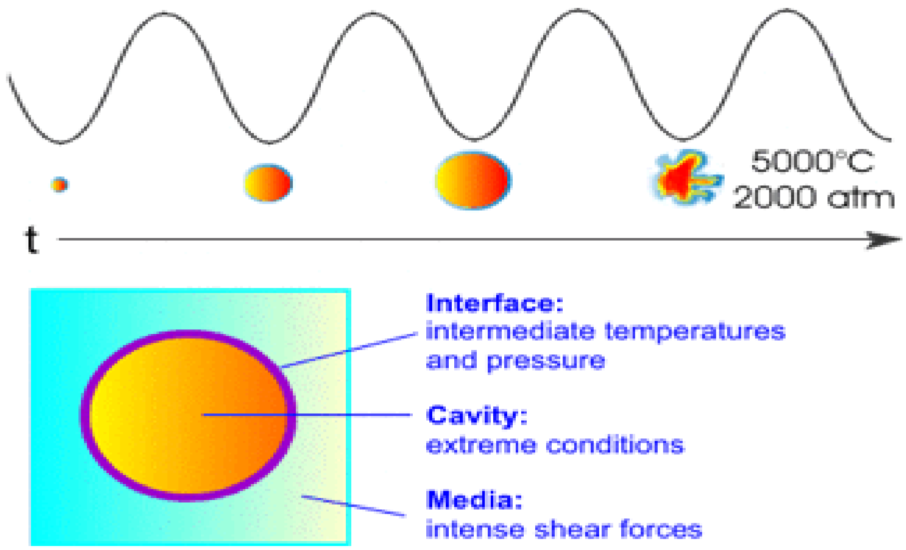

Figure 2.2: Implosion of bubbles

When liquids contain solids, similar phenomena may occur with exposure to ultrasound. Once cavitation occurs near an extended solid surface, cavity collapse is nonspherical and drives high speed jets of liquid to the surface. These jets and associated shock waves can damage the now highly heated surface. The liquid powder suspensions produce high velocity inter particle collisions.

The extraction of bioactive compounds from seed classically based upon combination of solvent, heat and agitation. This significantly improved by using the high power of ultrasound which are energy that has been generated from collapsing bubbles. This collapsing bubble provides greater penetration of the solvent into the cellular material and also will improve mass transfer (Vinatoru,2001; Vilkhu,Mawson & Bates,2006). At higher ultrasonic intensities, this extraction process can be improved by the disruption of cell wals and thus it will release the bioactive components. From previous journals, it has studied about the effect of ultrasonic on supercritical extraction of ginger. Balachandran (2006) states that both rate and their final yield have been improve significantly. The cavitational events in a supercritical fluid seem impossible due to absence of gas and liquid boundaries. Otherwhile, Carcel and co-workers showed that the intensity of brine solution into meat proportional to the applied intensity. The chemical effect of ultrasound irradiation does not depend on the molecules present to the collapsing cavity, it also depend on the magnitude of the temperature rise.

Temperature will affect the vapor pressure, viscosity of the liquid and also surface tension (Muthukumaran et al, 2006). When temperature keep increasing, the number of cavitation bubles also will increasing, thus the higher vapor pressure will dampened. At the higher viscous, the cavitation bubbles will be less. So when the temperature increased, the viscosity will decrease and it will allow more violent collapse. Thus, there will be an optimum temperature at which to form enough violent cavitation bubbles to avoid the dampening of vapor pressure.

CHAPTER 3

METHODOLOGHY

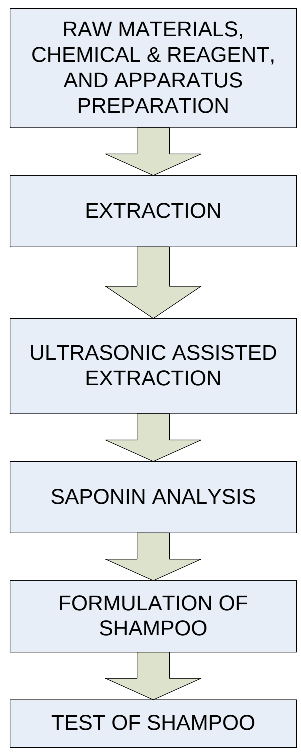

Figure 3.1: Procedures of experiment

3.1 Experimental

The flow chart for describing overall experiment is shown in Figure 3.1

3.1.1 Raw Material, chemical & reagent and apparatus preparation

Dry *Sapindus Emarginatus* used in this study were collected from India. All samples have been washed and dried under the shades for 14 day (Mali et al, 2010). The dried of samples were sliced into smallest pieces. Then, it need dry until no moisture in their shells. Next, these drying shells were ground into fine powder before extraction. The picture of soapnut is illustrated as in Figure 3.2.

Figure 3.2: Sapindus Emarginatus seed

Table 3.1: List and purpose of chemical used

Purpose	Chemical
Isolation of saponin	Methanol, Butanol, and Diethylether
HPLC analysis	Methanol (HPLC grade), Acetic acid (HPLC grade), Saponin standard
Formulation of shampoo	Xantham gum, EDTA, Methyl paraben, Orange oil

Table 3.2: List and function of apparatus used

Apparatus	Purpose
Ultrasonic water shaker	To disintegrate the cells of cell cultures
Rotary evaporator	To separate solvent from solution
High performance liquid chromatography (HPLC)	To identify the bioactive compound in the samples
Mechanical shaker	To mix and shake the solution
Magnetic stirrer	To stir the solution

3.1.2 Extraction

The extraction of total saponin conducted in several stages. First, the air dried plant powder was extracted with methanol which is set up at 60°C for 10 hour, yielding reddish crude extract. This methanolic extract was used in rotary evaporator to separate between solvent and concentrate saponin. Then, this concentrate was dissolved in a minimum amount of distilled water and was decanted with butanol. For the final stage, the butanolic extract was precipitated using diethylether and filtered (Hanquet B, 1993). This method was refer to Mali et al, 2010 and Shuna Zhao et al, 2006.

3.1.3 Ultrasonic-assisted extraction

Five grams of fine powder were extracted in a sealed flask (250 ml) containing volume of extraction solvent according to the experimental design. The ultrasonic has been setup with constant frequency. Thermometer was used to determine the temperature for the solvent. The extraction temperature was controlled by immersing the flask into an automatically adjustable temperature water-bath (Wu et al, 2001). After extraction, the crude extract were centrifuged, filtered and then analyze by HPLC.

Figure 3.3: Ultrasonic assisted extraction

3.1.4 Saponin Analysis

3.1.4.1 Optimization of ultrasonic assisted extraction condition

The size of particle (315µm to 800µm) from Sapindus Emarginatus was determined for the maximum yield of saponin Then, the ultrasonication time (15 to 60 min) and extraction temperature (30 to 70 °C) on the extraction yield of saponin from *Sapindus Emarginatus* were investigated. Each of these parameters were use to determine the maximum yield.

3.1.4.2 Extraction yield

The percentage yield of saponin was calculated using the following equation:

$$Yield\ (\%) = \frac{\mathrm{Wi}}{\mathrm{Wt}} \times 100\% \qquad (3.1)$$

where W_i is the weight of saponin obtained from extraction and W_t is obtained from the initial amount of saponin used.

3.1.4.3 Isolation of saponin

The methanolic extract after concentration was dissolved in a minimum amount of distilled water and decant severa times with n-butanol. In the final stage, the total saponin present in butanolic extract was precipitated using diethyether and then filtered. The extract was then tested for the presence of saponins (Lacaille M, 1993)

3.1.4.4 Test for saponin

The samples-solvent mixture was centrifuged for few minutes. Then, the supernatants were discarded and the solvents in samples removed. The concentrated samples were tested to determine presence of saponin by dilute 1 ml of alcoholic extract with distilled water to 20 ml and solution was shaked for several minutes. Then, a one centimeter layer of foam were appear indicates saponin.

3.1.4.5 HPLC method

Five milligrams of saponin were weighed into 100ml volumetric flask and diluted to volume with ultrapure water for final concentration of 0.05 mg/ml for saponin standard.

Chromatographic analyses were carried out and the HPLC system that consisted of photodiode array detector, DGU-14A degasser and SCL-10A system controller. Separation of saponins was carried out using column C18 (250×4.6 mm ID, particle size 5 µm at 1.5ml min^{-1}. The detection was made at 254nm and 25°C.

The HPLC mobile phase consisted of methanol, water and acetic acid in the ratio of 60/34/6 (v/v). The mobile was filtered and degrased prior to use has shown in Figure 3.4. From Table 3.5 shown that the standard and samples of saponin were analyze using high performance liquid chromatography (HPLC). Then, the compounds appearing in chromatograms were identified on retention times and spectral data by comparison with standard is shown in Figgure 3.6.

Figure 3.4: Mobile phase of HPLC

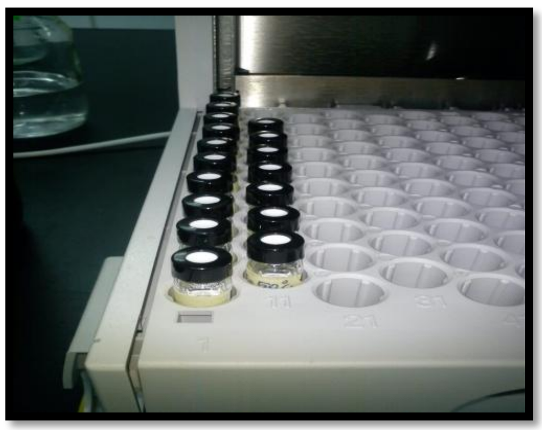

Figure 3.5: Standard and samples of HPLC

Figure 3.6: High Performance Liquid Chromatography

Table 3.3: Standard calibration of saponin

Concentration standard (ppm)	Area under curve (mAU)	Concentration (mg/ml)
1	267.2451	0.95
2	721.5918	2.027
3	1103.930	2.927
4	1659.082	4.235
5	1922.358	4.855

3.1.5 Formulation of Shampoo

Table 3.4 shows the components and percentage of ingredients used within the final formulation.

Table 3.4: Formulation of herbal shampoo

Compounds	Formulation (w/w)
Sapindus emarginatus (extract)	20
Xanthan gum	2
Glycerin	1
EDTA	0. 15
Methyl paraben	0.05
Orange oil	q.s

To formulate a clear base shampoo, definite amounts of saponin and salt were added to an aqueous solution containing extracts, and juices along glycerin, methyl paraben, and EDTA etc. Formulation was prepared by slightly heating and adding the weighed quantity of herbal ingredients.

Herbal extracts were diluted with distilled water then glycerin, EDTA, xanthan gum and methyl paraben were added with steering. Juices were mix on mechanical shaker for 25 minutes. The extracts are mix with slow steering on a magnetic stirrer. Then, orange oil was added and mixed with slow steering on a magnetic stirrer. Finally, volumes are made up to 100 ml with distilled water.

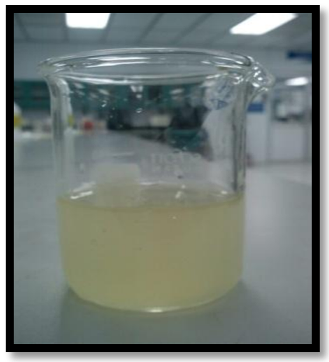

Figure 3.7: Formulated shampoo

3.1.6 Test of Shampoo

To evaluate the prepared formulations, the quality control test including visual assessment and physicochemical controls such as pH were performed.

3.1.6.1 Determination of pH

For the pH tested, the solution was tested by using the pH tester. First, the tester needs to do some calibration. Next, 10% of shampoo solution was tested by put the pH test into that solution. Firstly, the solution without addition of water was tested. If the pH shows below than 5.5, it means that the solution need to added 20ml of water between 5 minutes. This step was repeated until the pH of solution achieved range between 5.5 until 5.9 (Mainkar and Jolly,2001)

From previous research (Mankar, 2000) the pH was tested by added 10% shampoo solution in distilled water was added at room temperature 25 ° C.

Figure 3.8: pH test for shampoo

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Experimental Studies

In order to know the amount of extracting yield, three parameters such as size of particle, temperature and sonication time of raw materials were varied. The best operating condition for saponin production were determined based on the amount of maximum of saponin obtained.

4.1.1 Effect of size particle

During the study of the effect of size of particle, the temperature of saponin solution was fixed at 60° C and the time is about 60 minutes**.** The saponin amount also was fixed at 5g. As illustrated in Figure 4.1, the percentage of yield is decreased with increases in size of particle. The particle size that used in these three ranges is 315, 630 and 800 µm. As expected that when the size particle is smaller, the percentage of yield become higher than bigger particle. This is because when the size particle is smaller, the surface area is increasing. Surface area of particles is important to determine the extraction rate. For a fixed weight of material, the surface area are increased as the particle size decreased. Thus, for size particle range 630µm and 800 µm, the diffusion may play a important role so that the yield at a given time would be expected to increase with the reduction of size. However, for the smallest particle size which lower than 315 µm, most of cells will affected by ultrasonic which higher amplitude given when process is run. The higher amplitude can rupture wall cells and diffusion would not be a significant step in the extraction process.

Thus, for extraction of such small particles, the further decrease in the size would not result in increase in extraction rate. This hypothesis also has been supported by where the extraction rate increased when the size particle decreased. Other than that, Shuna Zhao (2007) have studied same parameter which in three ranges less than 0.3, 0.3-0.45 and 0.45-0.9mm. Consequently, the particle size range of 0.3-0.45 mm was chosen.

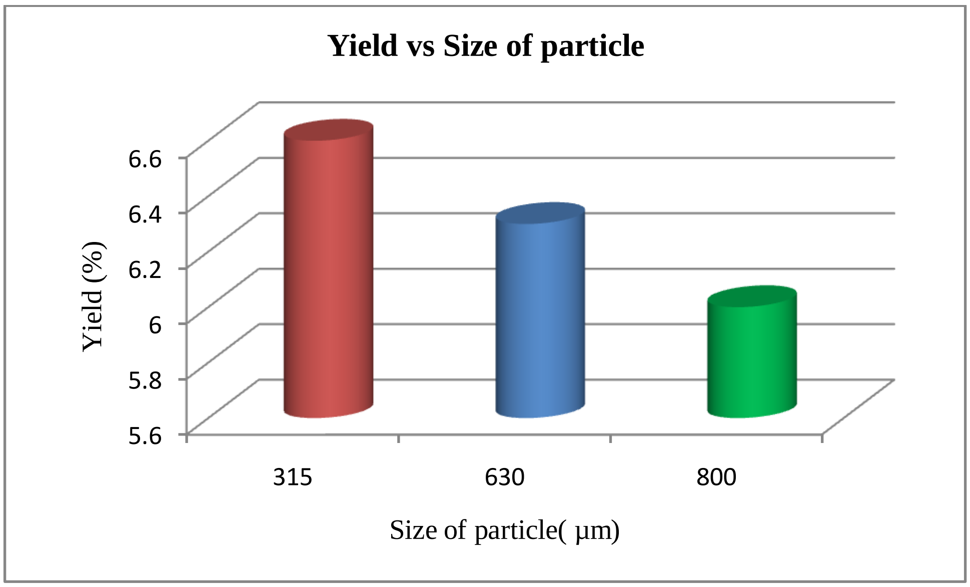

Figure 4.1: Effect of particle size on extraction yield. Other conditions were fixed at T=60°C and t = 60 min.

4.1.2 Effect of temperature

The effect of the temperature of ultrasonic assisted extraction towards saponin was investigated. The amount of methanol is 100 ml. Methanol is used as extraction solvent for extraction of saponin. The time taken for extraction will be constant for 60 minutes. The temperature was varied for 30, 40, 50, 60 and 70 °C respectively. The temperature influenced the reaction rate and percentages yield of saponin significantly. Increasing the temperature of reaction increases the kinetic energy of particles which increases the number of collisions so the reaction rate increases. Increasing kinetic energy of particles will have the minimum amount of energy required to form yield. Figure 4.2 shows that the extraction yield of saponin raises as extraction temperature increases. The result also shows that at 60 °C, it has approaches peak value. The results revealed that the highest yield of saponin was obtained at the value of 6.6 % when the sample was extracted with methanol at 60°C. This is due to the increases of mass transfer that resulted from increasing temperature of saponin. However, when the extraction temperature reaches 60°C, the extraction yield became decrease. This is probably cause by decrease of number acoustic cavitation bubbles that had been created by ultrasound and thermal degradation of saponin. Other than that, previous researcher (Shuna Zhao 2006) also had shown that as temperature approached the boiling point of solution, ineffective of sonication occurred as result of decrease in surface tension and increase in vapour pressure with microbubbles, in which turn caused the damping of ultrasonic wave. The higher vapor pressure especially near boiling point of the liquid can cause cavitation reduced to nearly zero. It has been explained by Hemwimol.S (2006) that higher vapor pressure would tend to had more bubbles but collapsed with lower intensity of pressure difference. Thus, the diffusion of active components was intensified when temperature increased, so was the extraction yield at a fixed time period. Thus, 60 °C was chosen as the optimum temperature.

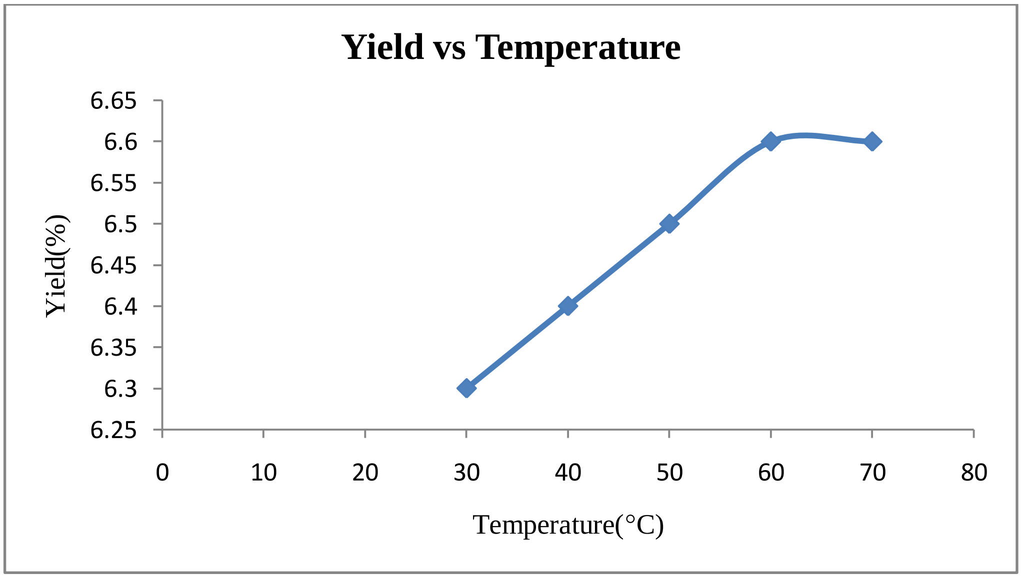

Figure 4.2: Effect of temperature on extraction yield. Other conditions were fixed at t = 60 min, particle size=315µm

4.1.3 Effect of sonication time

The effect of ultrasonic and its extraction time on the extraction yield of saponin were shown in Figure 4.3. The duration of ultrasonic irradiation was 15, 30, 45 and 60 min, respectively. The results shown in Figure 4.3 clearly stated that as ultrasonication time is extended from 30min to 45 min, and then increase is diminished when ultrasonication time is longer than 45 min. Previous researchers that choose this parameter on saponin, it resulted that the extraction yield increased rapidly in the first 30 min of sonication instead of 45 min. Since swelling and hydration could accelerate by ultrasonic, thus it can cause an enlargement in the pores of cell walls (Sun & Tomkinson, 2002). It would result as an improvement mass transfer of solute constituents from plant material to solvent. The disruption of plant cells after the cavitation bubbles could increase the rate of penetration into plant tissue (Paniwnyk et al, 2001). Therefore, this technique would allow target components to dissolve in the solvent, thus boosting yield with a relevant shorter time. If the ultrasonic time was more than several minutes, the extraction yield of saponin could decreased with the increased of ultrasonic time. This is because of saponin is easily decomposed when they kept at higher temperature. This was also observed in the ultrasonic assisted extraction of epidemin C from fresh leaves of *Epimedium* and extraction mechanism, where recovery of some epidemin C decreases with increasing extraction time (Hua et al, 2009).

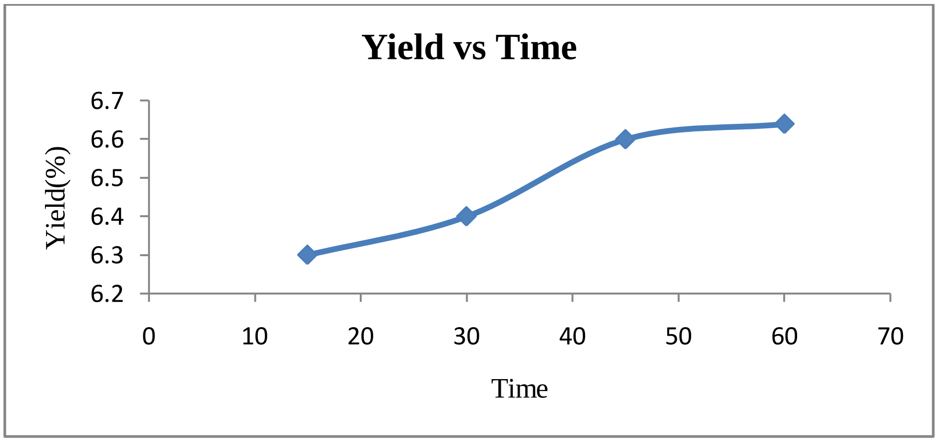

Figure 4.3: Effect of sonication time on extraction yield. Other conditions were fixed at T=60°C and particle size=315µm.

4.2 Test of Herbal Shampoo

4.2.1 Physical Appearance

The result for physical appearance for this formulation had the good characteristics with respect to foaming.

4.2.2 pH

The pH of shampoo has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. The current trend is to promote shampoos of lower pH is one of the ways to minimize damage to the hair. Mild acidity prevents swelling and promotes tightening of the scales, there including shine. As seen in Table 4.1, the shampoo were acid balanced and were ranged to 5.5 to 5.9, which is near to the skin pH (Mainkar and Jolly, 2001).

Table 4.1: Evaluation of pH

Amount of Water (ml)	pH
0	3.13
20	4.25
40	4.47
60	4.36
80	4.38
100	4.40
120	4.41
160	4.45
180	4.79
200	4.90
220	5.00
240	5.34
260	5.40
280	5.49
300	5.57

4.3 Kinetics of Ultrasonic Assisted Extraction

The extraction is carried out in aqueous solution. By using the Michaelis Menten equation, the value of extraction rate constant, k can be calculated. The equation of Michaelis Menten takes the familiar form

$$-r = \frac{Vmax\ (S)}{Km + (S)} \tag{4.1}$$

where V_{max} represent the maximum rate of reaction, K_M is Michaelis Menten equation and S is the concentration. At constant volume, assuming that the mass transfer process could be formally treated as an irreversible first order reaction. This is because the low concentration in solution. This equation above show the relation between V_{max}, K_M and extraction rate constant, k

$$k = \frac{Vmax\ (S)}{Km} \tag{4.2}$$

The equation of extraction rate constant is:

$$k = \frac{1}{t} ln \frac{So}{St} \tag{4.3}$$

where k is the extraction rate constant (min^{-1}), t is the extraction time (min), S_0 is the total content of extractible compounds (w/w) and S_t is the remained extractible compounds after extraction time t (w/w). The extraction rate was calculated on the basis of obtained result of total saponin extraction yield. In this study, the total content of extractible compound, S_0 was 50g/kg. Extraction time was conducted for 60 minutes because of extraction rate was almost constant..

Table 4.2: Rate constant value

Extraction time, min	St (w/w)	Rate constant, k (min^{-1})
15 min	32	0.430
30 min	33.5	0.217
45 min	34.5	0. 145
60 min	34.7	0. 1091

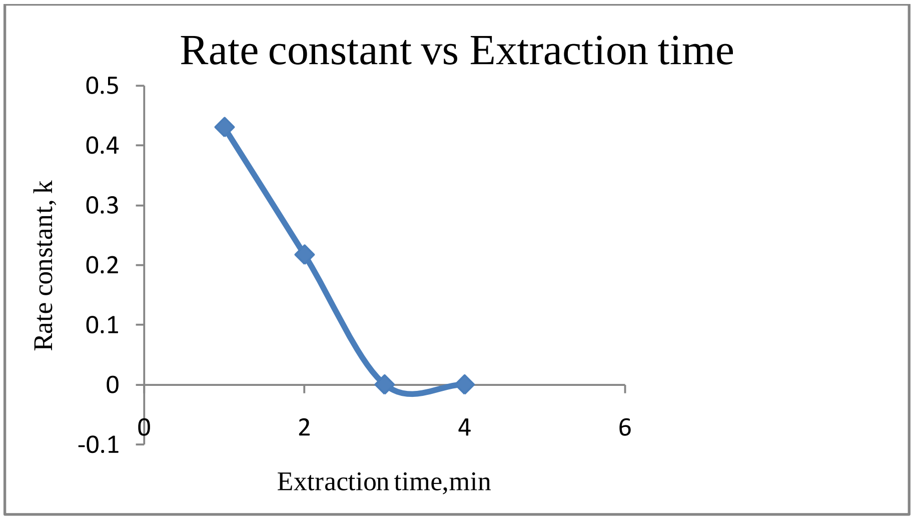

Figure 4.4: Graph of rate constant

The results shown in Figure 4.4 clearly stated that the rate constant decreased when the extraction time increased. This graph shows that the rate constant is inversely proportional with time. Thus, it can influence the extraction rate of saponin.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The most important variables that influence the yield of extraction are:

i. Size of particle
ii. Temperature
iii. Sonication time

It was observed that yield of extraction is increase when the temperature is increases. However, the test was conducted up to 60 °C. Further increase in temperature will maintain the amount of yield. When the reaction was carried out in 315 µm, 60 °C and 60 min, the amount of highest yield is 0.33 g. The extraction rate constant of saponin extraction has been calculated based on the experimental results.

Thus, it is as expected where the extraction yield increased when the size of particle decrease. Other than that, the higher temperature can give higher extraction rate. Last but not least.

- The extraction yield will be increase when size of particle reduce
- Higher temperature will give higher extraction rate.
- For sonication time, it will boosting yield at shorter time

5.2 Recommendation

This natural herbal shampoo need to further test to ensure that it is suitable with human skin and safe to use. The test is use to evaluate the formulation prepared, quality control tests including organoleptic assessment and physicochemical control such as pH, density and viscosity. It is to assure that the quality of product is guaranted.

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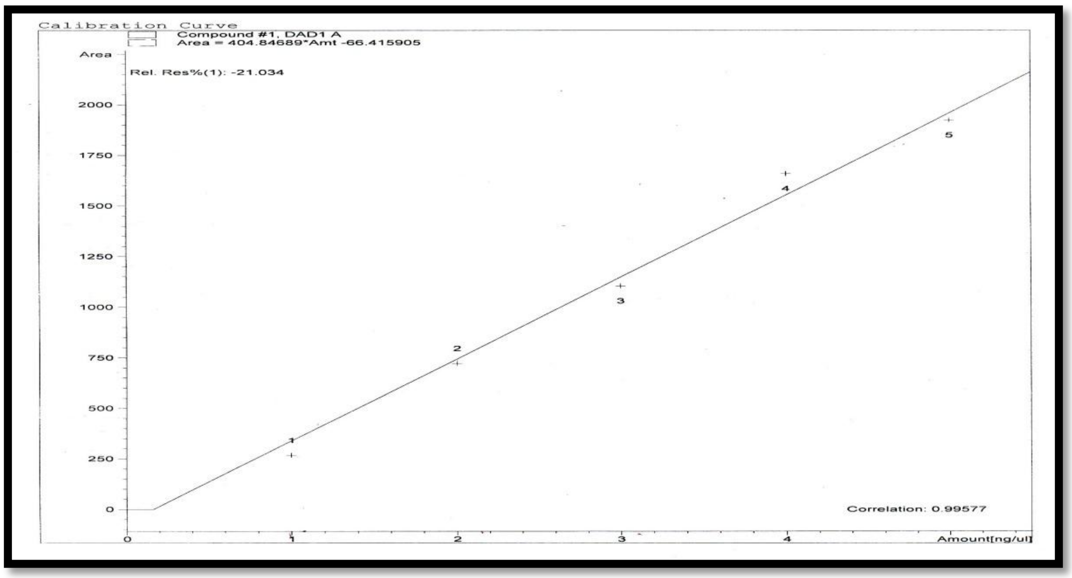
APPENDIX

HPLC RESULTS

APPENDIX 1

Calibration curve



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
1.522	1	1	1.00000	267.24515	3.74188e-3			
		2	2.00000	721.59180	2.77165e-3			
		3	3.00000	1103.93042	2.71756e-3			
		4	4.00000	1659.08240	2.41097e-3			
		5	5.00000	1922.35815	2.60097e-3			

APPENDIX 2

HPLC result at S=315µm, T=60°C, t=60 min

```
Injection Date   : 12/19/2011 11:12:57 AM        Seq. Line :    1
Sample Name      : 315micro                       Location : Vial 1
Acq. Operator    : AIN                                 Inj :    1
Acq. Instrument  : Instrument 1                 Inj Volume : 20 µl
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Method           : C:\CHEM32\1\METHODS\AIN.M
Last changed     : 12/19/2011 11:11:39 AM by AIN
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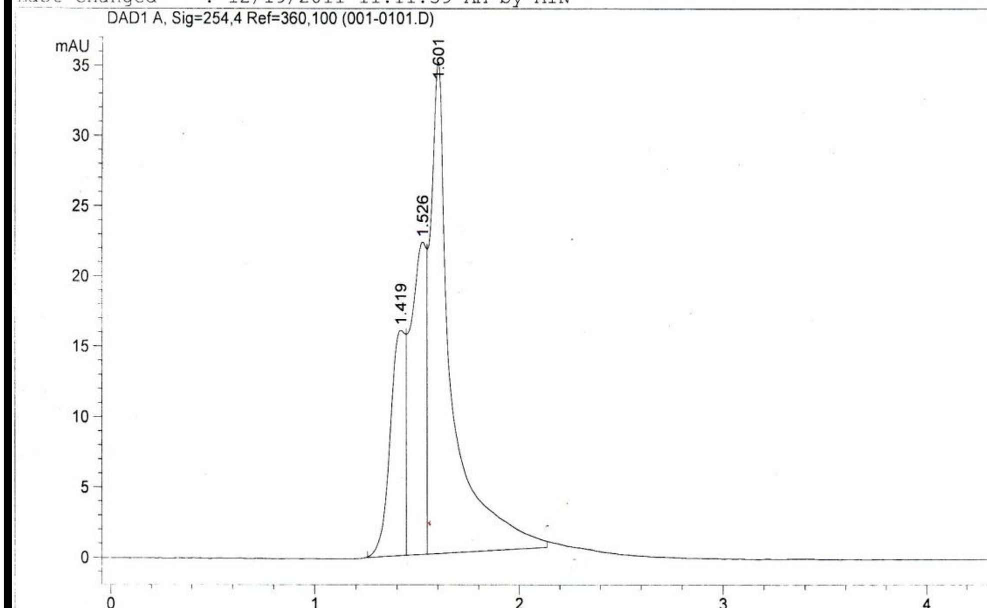

Area Percent Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.419	BV	0.0778	82.15139	16.02314	17.9116
2	1.526	VV	0.0761	118.37605	22.22953	25.8097
3	1.601	VB	0.0997	258.12250	35.13109	56.2788
Totals :				458.64993	73.38375	

APPENDIX 3

HPLC result at S=630µm, T=60°C, t=60 min

```
Injection Date   : 12/19/2011 11:19:57 AM          Seq. Line :    2
Sample Name      : 630micro                         Location : Vial 2
Acq. Operator    : AIN                                   Inj :    1
Acq. Instrument  : Instrument 1                   Inj Volume : 20 µl
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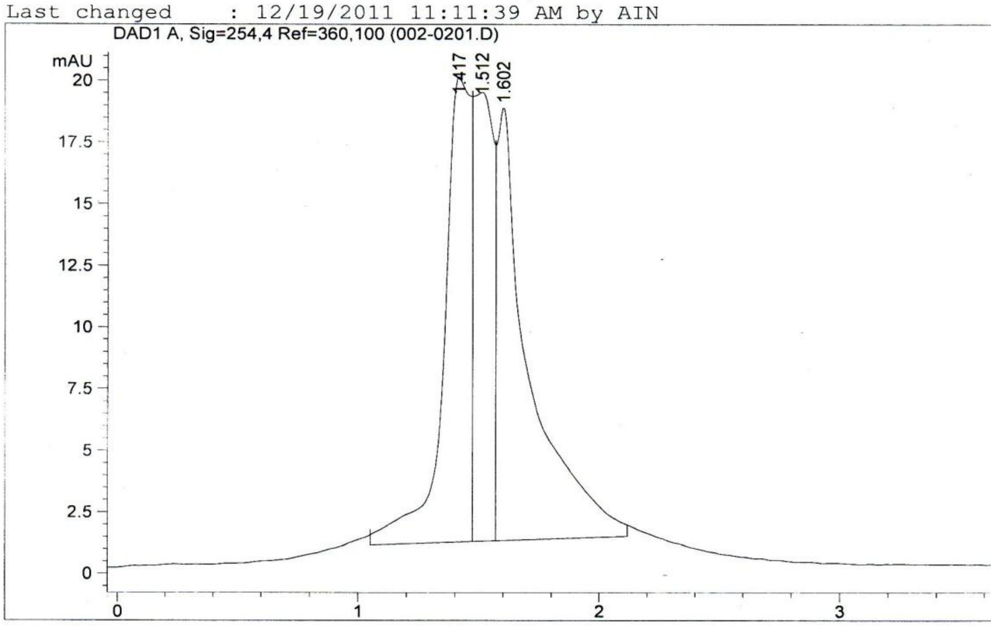

Area Percent Report

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Multiplier           :       1.0000
Dilution             :       1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.417	BV	0.1112	147.54605	18.87509	35.6014
2	1.512	VV	0.0795	102.27316	18.21469	24.6775
3	1.602	VB	0.1213	164.61995	17.56391	39.7211

Totals : 414.43916 54.65370

APPENDIX 4

HPLC result at S=800µm, T=60°C, t=60 min

Injection Date : 12/19/2011 11:26:57 AM Seq. Line : 3
Sample Name : 800micro Location : Vial 3
Acq. Operator : AIN Inj : 1
Acq. Instrument : Instrument 1 Inj Volume : 20 µl
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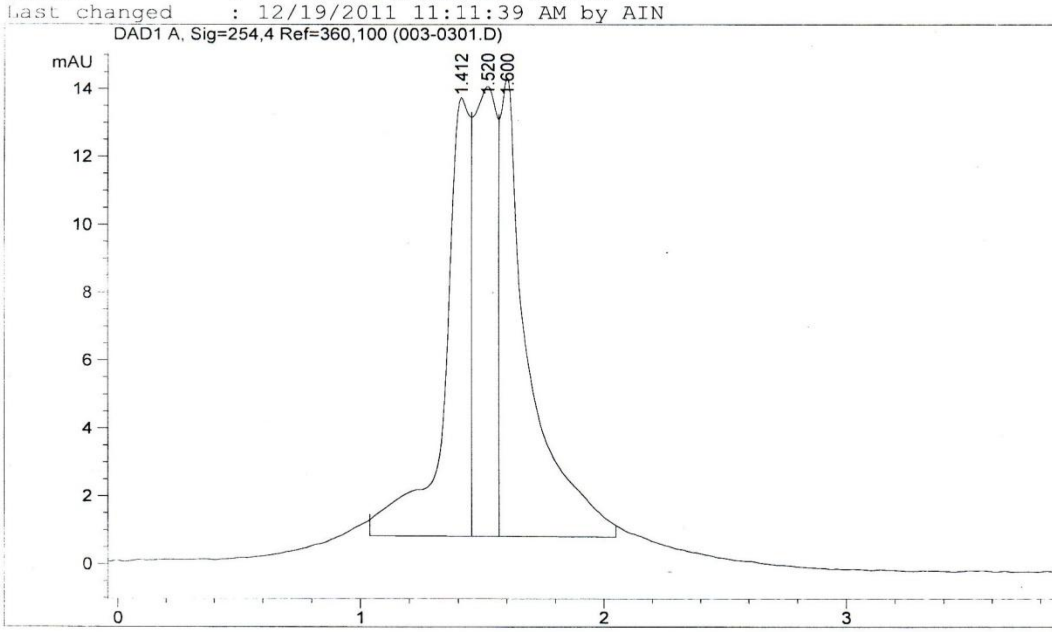
Method : C:\CHEM32\1\METHODS\AIN.M
Last changed : 12/19/2011 11:11:39 AM by AIN



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.412	BV	0.1032	94.42262	12.93688	32.2604
2	1.520	VV	0.0916	85.94224	13.26267	29.3630
3	1.600	VB	0.1087	112.32433	13.55377	38.3767
Totals :				292.68918	39.75332	

APPENDIX 5

HPLC result at S=315µm, T=60°C, t=15 min

Injection Date	: 12/19/2011 12:08:57 PM	Seq. Line :	9
Sample Name	: 15 MIN	Location :	Vial 9
Acq. Operator	: AIN	Inj :	1
Acq. Instrument	: Instrument 1	Inj Volume :	20 µl
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Method	: C:\CHEM32\1\METHODS\AIN.M		
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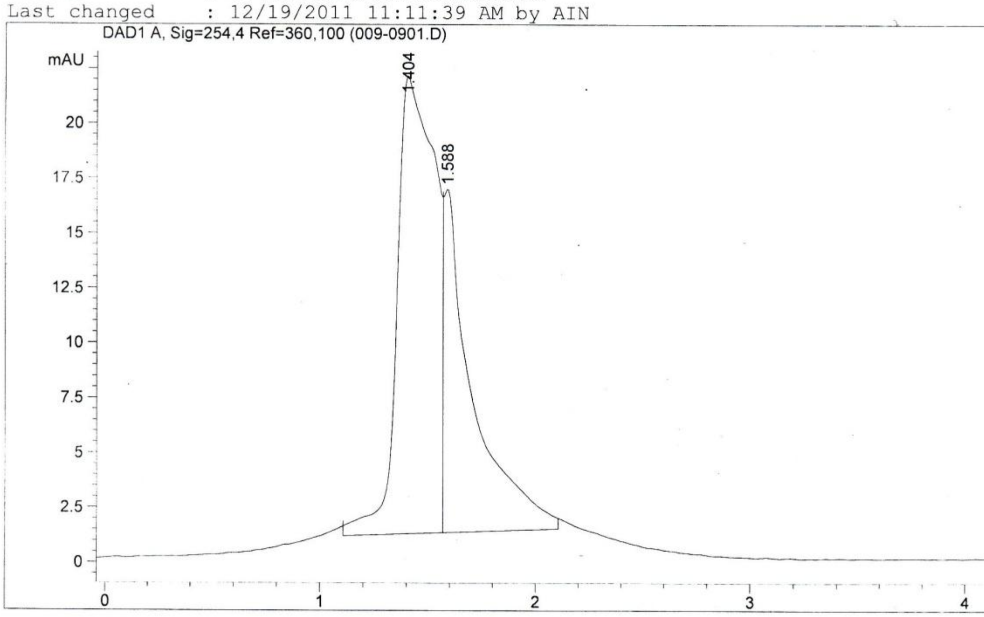

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.404	BV	0.1620	257.72980	20.87508	63.8707
2	1.588	VB	0.1227	145.78842	15.64453	36.1293
Totals :				403.51822	36.51961	

APPENDIX 6

HPLC result at S=315µm, T=60°C, t=30 min

```
Injection Date   : 12/19/2011 12:15:58 PM        Seq. Line :  10
Sample Name      : 30 MIN                         Location : Vial 10
Acq. Operator    : AIN                                 Inj :   1
Acq. Instrument  : Instrument 1                 Inj Volume : 20 µl
Sequence File    : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method           : C:\CHEM32\1\METHODS\AIN.M
Last changed     : 12/19/2011 11:11:39 AM by AIN
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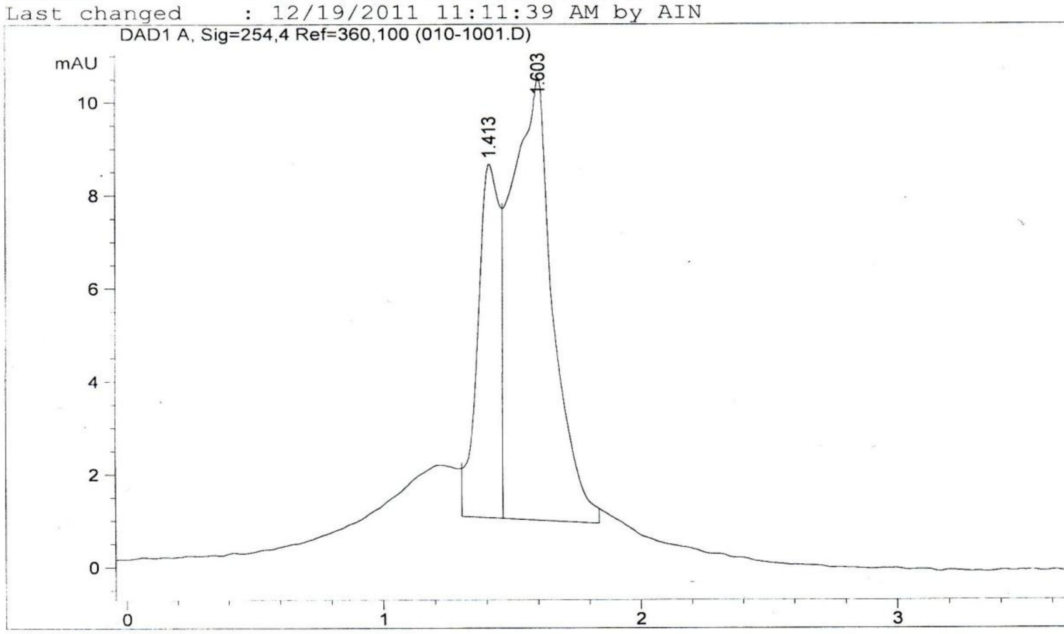

Area Percent Report

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Sorted By             :       Signal
Multiplier            :       1.0000
Dilution              :       1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.413	BV	0.0873	45.29381	7.62042	29.7235
2	1.603	VB	0.1451	107.08978	9.53187	70.2765
Totals :				152.38359	17.15230	

APPENDIX 7

HPLC result at S=315µm, T=60°C, t=45 min

```
Injection Date    : 12/19/2011 12:22:58 PM          Seq. Line :   11
Sample Name       : 45 MIN                           Location : Vial 11
Acq. Operator     : AIN                                   Inj :    1
Acq. Instrument   : Instrument 1                   Inj Volume : 20 µl
Sequence File     : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method            : C:\CHEM32\1\METHODS\AIN.M
Last changed      : 12/19/2011 11:11:39 AM by AIN
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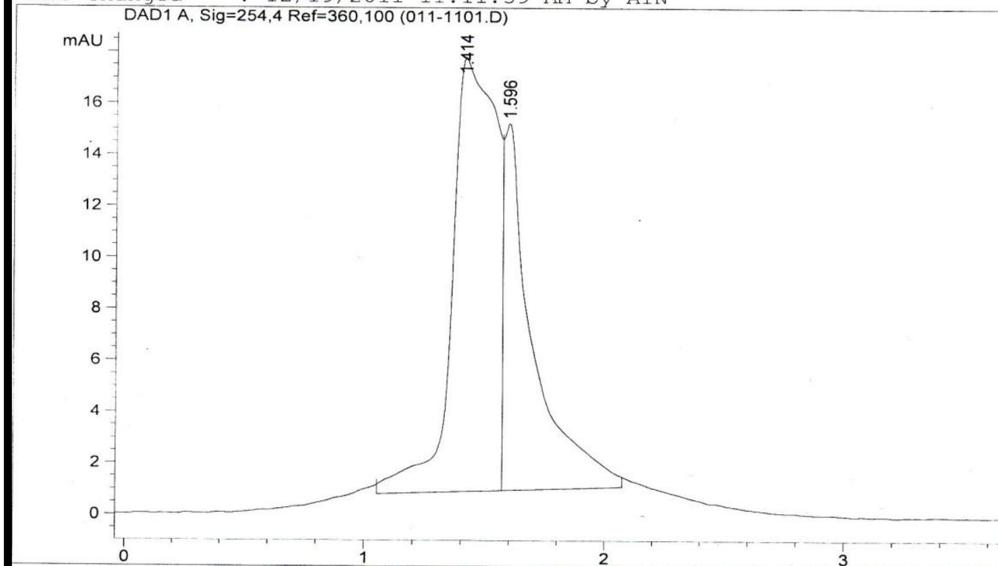


Area Percent Report

```
Sorted By             :        Signal
Multiplier            :        1.0000
Dilution              :        1.0000
Use Multiplier & Dilution Factor with ISTDs

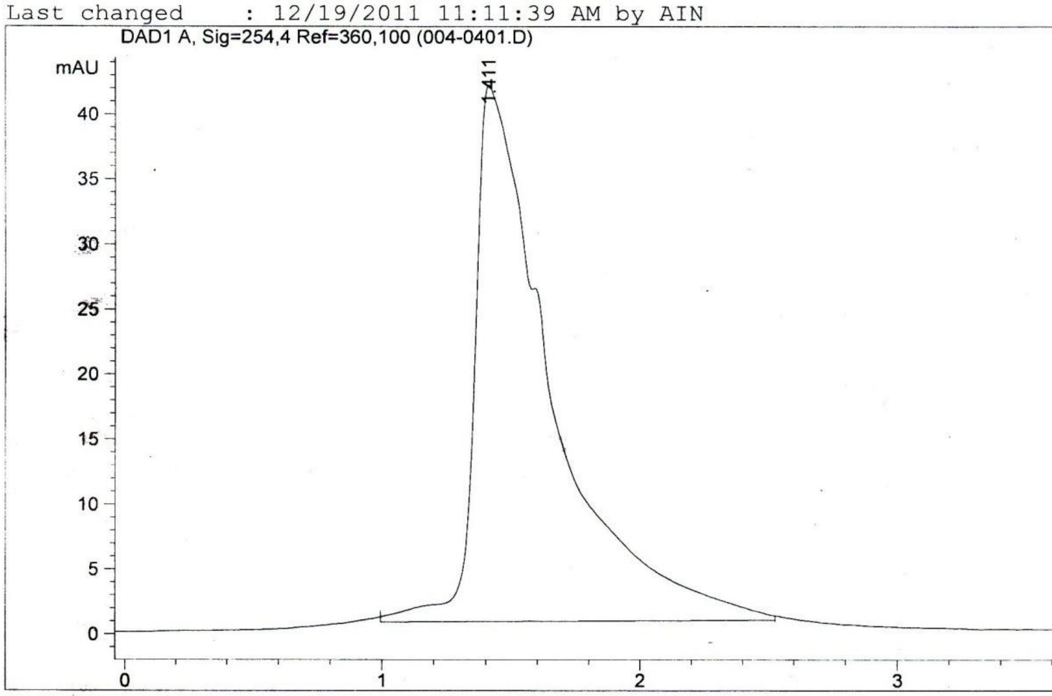
Signal 1: DAD1 A, Sig=254,4 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.414	BV	0.1672	216.13683	16.90461	63.9223
2	1.596	VB	0.1136	121.98740	14.28340	36.0777
Totals :				338.12422	31.18802	

APPENDIX 8

HPLC result at S=315µm, T=30°C, t=60 min

```
Injection Date    : 12/19/2011 11:33:59 AM          Seq. Line :    4
Sample Name       : 30 C                             Location : Vial 4
Acq. Operator     : AIN                                   Inj :    1
Acq. Instrument   : Instrument 1                   Inj Volume : 20 µl
Sequence File     : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method            : C:\CHEM32\1\METHODS\AIN.M
Last changed      : 12/19/2011 11:11:39 AM by AIN
```



Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

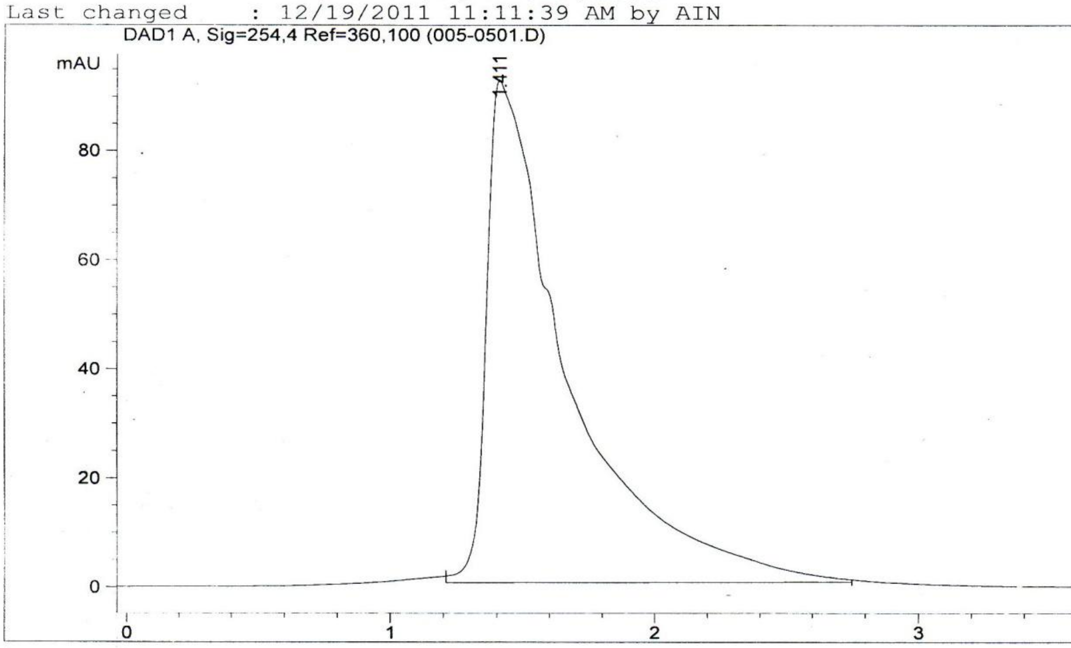
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.411	BB	0.2794	862.60248	41.34071	100.0000

Totals : 862.60248 41.34071

APENDIX 9

HPLC result at S=315µm, T=40°C, t=60 min

```
Injection Date   : 12/19/2011 11:40:58 AM          Seq. Line :    5
Sample Name      : 40 C                             Location : Vial 5
Acq. Operator    : AIN                                   Inj :    1
Acq. Instrument  : Instrument 1                   Inj Volume : 20 µl
Sequence File    : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method           : C:\CHEM32\1\METHODS\AIN.M
Last changed     : 12/19/2011 11:11:39 AM by AIN
```




```
                        Area Percent Report

Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

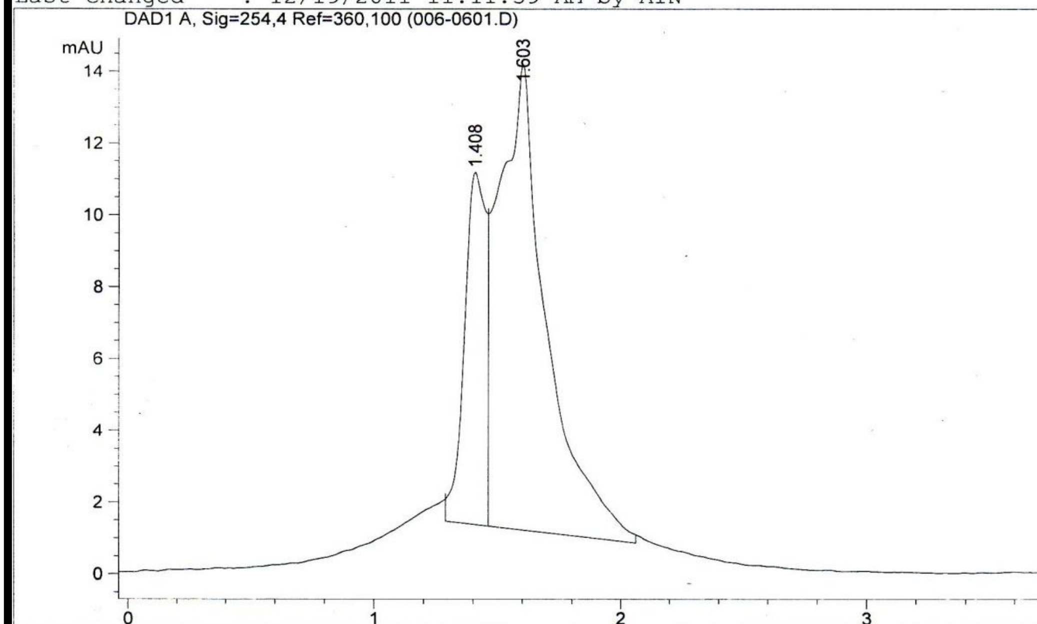
Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.411 BB    0.2698 1991.97668   92.29869 100.0000

Totals :                  1991.97668   92.29869
```

APENDIX 10

HPLC result at S=315µm, T=50°C, t=60 min

```
Injection Date   : 12/19/2011 11:47:56 AM          Seq. Line :    6
Sample Name      : 50 C                             Location : Vial 6
Acq. Operator    : AIN                                   Inj :    1
Acq. Instrument  : Instrument 1                   Inj Volume : 20 µl
Sequence File    : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method           : C:\CHEM32\1\METHODS\AIN.M
Last changed     : 12/19/2011 11:11:39 AM by AIN
```



Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

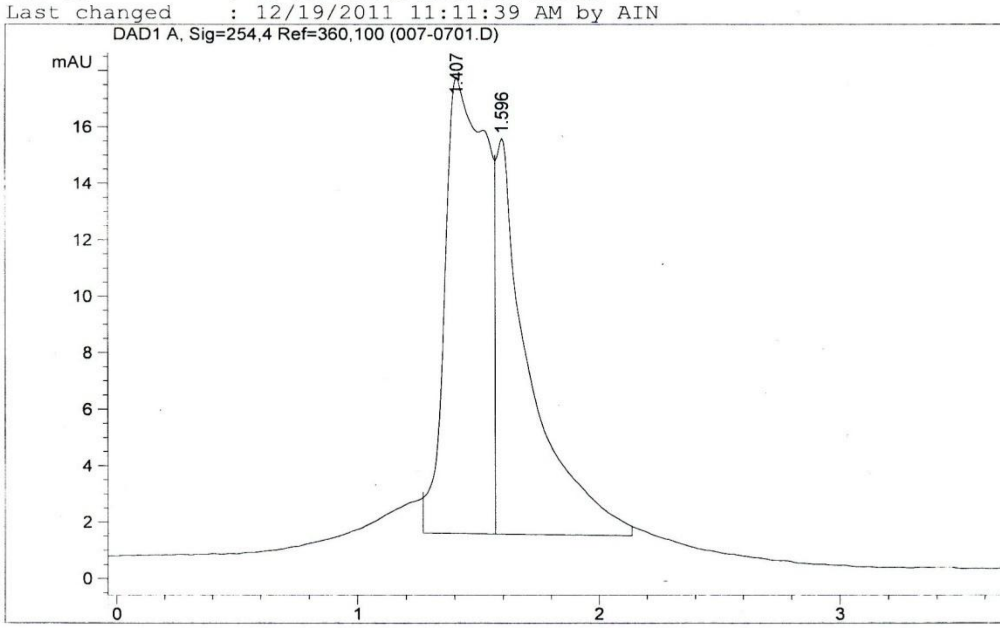
Signal 1: DAD1 A, Sig=254,4 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.408	BV	0.0892	59.89926	9.81693	25.3537
2	1.603	VB	0.1703	176.35518	12.99481	74.6463
Totals :				236.25444	22.81174	

APENDIX 11

HPLC result at S=315µm, T=60°C, t=60 min

```
Injection Date   : 12/19/2011 11:54:57 AM          Seq. Line :    7
Sample Name      : 60 C                             Location : Vial 7
Acq. Operator    : AIN                                   Inj :    1
Acq. Instrument  : Instrument 1                   Inj Volume : 20 µl
Sequence File    : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method           : C:\CHEM32\1\METHODS\AIN.M
Last changed     : 12/19/2011 11:11:39 AM by AIN
```



Area Percent Report

```
Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

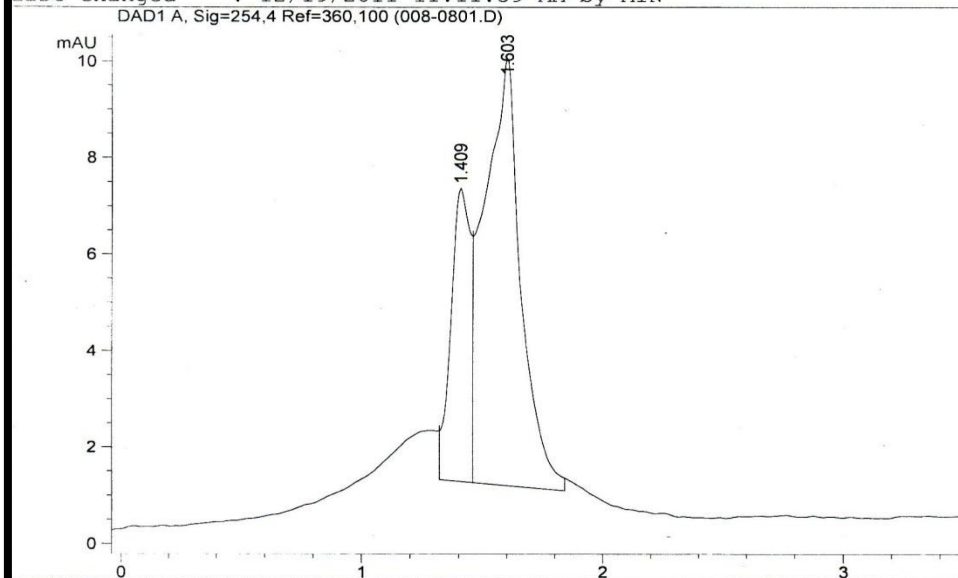
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.407	BV	0.1592	195.88805	16.18547	58.7083
2	1.596	VB	0.1266	137.77499	14.02672	41.2917
Totals :				333.66304	30.21219	

APENDIX 12

HPLC result at S=315µm, T=70°C, t=60 min

```
Injection Date    : 12/19/2011 12:01:56 PM          Seq. Line :    8
Sample Name       : 70 C                             Location : Vial 8
Acq. Operator     : AIN                                   Inj :    1
Acq. Instrument   : Instrument 1                   Inj Volume : 20 µl
Sequence File     : C:\CHEM32\1\SEQUENCE\CLASS\AIN.S
Method            : C:\CHEM32\1\METHODS\AIN.M
Last changed      : 12/19/2011 11:11:39 AM by AIN
```



Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.409	BV	0.0816	34.18647	6.08094	26.7900
2	1.603	VB	0.1368	93.42276	8.87391	73.2100

Totals : 127.60923 14.95485

APPENDIX 13

Result for different size of particle on extraction yield

Size of particle (µm)	Final Volume	After rotary	Yield (%)
315	97ml	0.330	6.6
630	96ml	0.315	6.3
800	95ml	0.30	6.0

APPENDIX 14

Result for effect of different sonication time on extraction yield

Time,t	Final volume	After rotary (g)	Yield(%)
15	99ml	0. 320	6.4
30	97ml	0. 335	6.7
45	96ml	0.345	6.9
60	94ml	0.347	6.94

APPENDIX 15

Result for effect of different temperature on extraction yield

Temperature,T	Final Volume	After rotary(g)	Yield (%)
30	100ml	0.315	6.3
40	100ml	0.317	6.4
50	98ml	0.325	6.5
60	97ml	0.330	6.6
70	85ml	0.330	6.6