# EVALUATION OF *Colubrina asiatica* EXTRACT USING MICROWAVE-ASSISTED EXTRACTION AS SAPONIN-BASED FOAMING AGENT

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

School of Chemical and Energy Engineering
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DECEMBER 2019

**ACKNOWLEDGEMENT**

John F. Kennedy had said, "*As we express our gratitude, we must never forget that the highest appreciation is not to utter words, but to live by them*". The voyage towards the completion of this thesis was indeed full of memorable experiences and handful of knowledge attained which indisputably will be beneficial in the future. I would like to extend my warmest appreciation to all souls whom contributed both directly and indirectly in this thesis.

First and foremost, I would like to express my sincerest gratitude to my supervisor, Dr. Eraricar Binti Salleh for their unceasing supervision and guidance in ensuring the success of completion of this thesis. Next, I owe an unfathomable sense of gratitude to laboratory colleague for their enormous support, kindness, and patience in guiding me through the ups and downs in postgraduate life.

I am extremely grateful to my parents, family and fellow friends for their boundless love and encouragement to help me persevere. Truly, it was impossible for me to do this alone without them. Lastly, I thank profusely to all others who are not mentioned but have provided assistance at various occasions in time of production of this thesis.

Last but not least, deepest thankful is expressed to Universiti Teknologi Malaysia for the support of my master project with grant Flagship #14G19\

## ABSTRACT

Since past decades, herbal plants have been used widely by indigenous or even urban people for disease treatment, or taken orally as appetizers. This includes *Colubrina asiatica* (*C. asiatica)* which is also known as Peria Pantai. Previous studies reported that this plant contains high amount of active compounds that are responsible for foaming ability and various bioactivities. However, these active compounds are sensitive towards many factors; temperature, pH, light and other factors in the production line. This study was conducted to optimize the microwave assisted extraction (MAE) process of *C. asiatica* using response surface methodology (RSM) through Box-behnken Design. The foam properties including surface tension, foam ability and stability, film drainage and wetting ability were also evaluated. The parameters used in RSM were irradiation time (3,5 and 7 minutes), solvent to solid ratio (10,20 and 30 mL/g) and power (300,400 and 500 W). The experimental data of MAE from RSM were analysed to identify the factors that affect the extraction yield. The *C. asiatica* extract was characterized by using total saponin content (TSC), foam properties evaluated based on surface tension, foam ability, foam stability, film drainage and wetting ability while the antibacterial activity was assessed by using agar diffusion method. The optimal extraction condition was irradiation time at 6.06 min irradiation time, solvent to solid ratio of 28.31 mL/g and microwave power of 445 W. A non-conventional (UAE) and conventional extraction method (SE) were conducted based on the optimal conditions from previous study. Under the optimal conditions, *C. asiatica* extract yielded 43.1 % of crude extract for MAE, 25.32 % for UAE, and 40.93 % for SE. Total saponin content obtained via MAE, UAE and SE under this condition was 15.36, 13.12 and 10.45 mg ESE/g (milligram Escin/gram) respectively. Evaluation of foam properties showed that surface tension of MAE extract dropped to 44.3 mN/m. Surface tension of MAE dropped nearest to range of good detergent which is 32-37 mN/m. MAE extract showed the highest foam height (10.2 cm) and R5 value of foam height showed the value of 87.3% which indicate that MAE extract possessed good foam ability and stability characteristics. R5 is the ratio of the height of the foam at 5 min to that at 0 min. Rate of film drainage was the slowest for MAE extract (78 %) followed by UAE and SE. No significant difference was observed in rate of film drainage among these three extracts ($P>0.05$). The wetting ability study showed that MAE, UAE and SE extract solution needed 4.2, 7.3, and 16.1 minutes to penetrate the cotton yarn which were too long and this indicated that these extracts had weak wetting ability. These extracts were subjected to antibacterial activity and MAE extract showed excellent inhibition of *E.coli* and *B. subtillis* growth (11 and 13 mm). The quantification of saponin using HPLC analysis also revealed that MAE extract contained saponins from group of oleanolic acid (9.57%), ursolic acid (5.64%) and betulinic acid (3.93%). The extraction of *C. asiatica* leaves by using MAE was proven to provide better quantity and quality of the saponin extract. The *C. asiatica* extracts by using MAE produced the highest amount of extract yield in the shortest time compared to UAE and SE. MAE extract also has good quality in term of foaming properties and antimicrobial activity due to the presence of saponin. The finding of the study revealed that saponin from *C.asiatica* extract has the potential to be a natural foaming agent as an alternative to the conventional chemical-based foaming agent used in personal care, cosmeceutical, pharmaceutical and food industries.

# ABSTRAK

Sejak beberapa dekad yang lalu, tumbuhan herba telah digunakan secara meluas oleh masyarakat orang asli mahupun masyarakat bandar untuk merawat penyakit ataupun dimakan sebagai ulam-ulaman. Ini termasuk *Colubrina asiatica* (*C. asiatica*) ataupun dikenali sebagai Peria Pantai. Kajian sebelum ini melaporkan bahawa tumbuhan ini mengandungi banyak sebatian aktif yang dapat menghasilkan buih dan mempunyai pelbagai bioaktiviti. Walau bagaimanapun, sebatian aktif sensitif terhadap pelbagai faktor; suhu, pH, cahaya dan faktor lain yang terdapat dalam proses pengeluaran. Oleh itu, kajian ini dijalankan untuk mengoptimumkan proses pengekstrakan *C. asiatica* berbantu gelombang mikro (MAE), dengan menggunakan metodologi gerak balas permukaan (RSM) menerusi rekabentuk ujikaji Box-behnken (BBD). Sifat-sifat buih termasuk ketegangan permukaan, kebolehan menjadi buih dan kestabilan buih, saliran filem dan keupayaan pelembapan turut dikaji. Parameter yang digunakan di dalam RSM adalah masa penyinaran (3,5 dan 7 minit), nisbah larutan dan pepejal (10,20 dan 30 mL/g) dan kuasa (300,400 dan 500 W). Data kajian oleh MAE dari RSM dianalisa untuk menentukan faktor yang mempengaruhi jumlah ekstrak. Ekstrak *C. asiatica* dicirikan menerusi jumlah kandungan saponin (TSC), sifat-sifat buih dikaji berdasarkan ketegangan permukaan buih, keupayaan pembuihan dan kestabilan buih, kehilangan saliran filem dan keupayaan pelembapan manakala aktiviti antibakteria diukur menggunakan kaedah penyerapan agar. Keadaan optimal bagi proses pengekstrakan ini adalah pada masa penyinaran 6.06 min, nisbah pelarut kepada pepejal 28.31 mL/g, dan kuasa 445 W. Kaedah bukan konvensional (UAE) dan konvensional (SE) telah dijalankan berdasarkan keadaan optimal daripada kajian yang lepas. Di bawah keadaan ini, ekstrak *C. asiatica* menghasilkan 43.1% peratusan hasil bagi MAE, 25.32% bagi UAE dan 40.93% bagi SE. Jumlah kandungan saponin (TSC) yang didapati bagi MAE, UAE dan SE dibawah keadaan ini adalah 15.36, 13.12 dan 10.45 mg ESE/g (miligram Escin/gram). Kajian tentang sifat-sifat buih menunjukkan ketegangan permukaan bagi ekstrak MAE jatuh kepada 44.3 mN/m. Ketegangan permukaan bagi ekstrak MAE jatuh menghampiri lingkungan kategori bahan pencuci yang baik iaitu 32-37 mN/m. Ekstrak MAE menunjukkan ketinggian buih yang paling tinggi iaitu 10.2 cm, dan nilai R5 untuk ketinggian buih bagi ekstrak MAE adalah sebanyak 87.3 yang menandakan MAE mempunyai ciri-ciri keupayaan dan kestabilan buih yang baik. R5 adalah nisbah ketinggian buih pada 5 minit kepada 0 minit. Kadar kehilangan saliran filem yang paling perlahan adalah dari ekstrak MAE (78%) diikuti oleh ekstrak UAE dan ekstrak SE. Tiada perbezaan yang ketara dilihat pada kadar kehilangan saliran filem bagi ketiga-tiga ekstrak ($P>0.05$). Kajian keupayaan pelembapan bagi ekstrak *C.asiatica* menunjukkan bahawa ekstrak MAE, UAE dan SE memerlukan 4.2, 7.3, dan 16.1 minit untuk menembusi benang kapas yang mana masa yang diambil agak lama dan ini menunjukkan semua ekstrak ini mempunyai keupayaan pelembapan yang lemah. Kesemua ekstrak digunakan untuk penilaian aktiviti antibakteria dan ekstrak MAE menunjukkan perencatan pembiakan yang baik terhadap pertumbuhan *E.coli* dan *B. subtillis* (11 dan 13 mm). Kuantifikasi saponin menggunakan analisis HPLC juga memperlihatkan bahawa sebatian saponin yang terdapat dalam ekstrak *C. asiatica* adalah asid oleanolik (9.57%), asid ursolik (5.64%) dan asid betulinik (3.93%). Pengekstrakan daun *C. asiatica* menggunakan MAE telah terbukti dapat menghasilkan ekstrak saponin dengan kuantiti dan kualiti yang lebih baik. Proses pengekstrakkan *C. asiatica* menggunakan kaedah MAE menghasilkan jumlah ekstrak yang paling banyak dan proses ini memerlukan masa paling singkat berbanding kaedah UAE dan SE. Ekstrak MAE juga mempunyai kualiti yang baik dari segi sifat-sifat buih dan aktiviti antibakteria disebabkan oleh kandungan saponin. Berdasarkan kajian ini, telah terbukti bahawa saponin dari *C. asiatica* berpotensi menjadi agen pembuihan semulajadi sebagai alternatif bagi menggantikan agen pembuihan kimia konvensional yang digunakan dalam produk penjagaan diri, industri kosmetik, farmasi dan makanan.

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| | | |
|---|---|---|
| BBD | - | Box-Behnken Design |
| ESE | - | Escin Equivalent |
| HPLC | - | High Performance Liquid Chromatography |
| MAE | - | Microwave Assisted Extraction |
| SE | - | Soxhlet Extraction |
| SLS | - | Sodium Laureth Sulphate |
| TSC | - | Total saponin content |
| UAE | - | Ultrasound Assisted Extraction |
| V | - | Volume of Solution |
| W | - | Mass of Activated Carbon |
| WHO | - | World Health Organization |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| % | - | Percent |
| $^{o}C$ | - | Degree Celcius |
| CO2 | - | Carbon Dioxide |
| CO | - | Carbon Monoxide |
| g | - | Gram |
| h | - | Hour |
| $H_2O$ | - | Water |
| $H_2SO_4$ | - | Sulphuric Acid |
| kHz | - | Kilohertz |
| L | - | Liter |
| µm | - | Micrometer |
| mg | - | Miligram |
| mg/L | - | Miligram per liter |
| mN/m | - | Millinewton per meter |
| min | - | Minute |
| W | - | Watt |
| $R^2$ | - | Coefficient Values |
| RT | - | Room temperature |
| $T_0$ | - | Time at 0 minute |
| $T_{60}$ | - | Time at 60 minutes |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Nowadays, the majority of world's population use various cleansing agents in their daily lives to maintain proper hygiene and avoid germs or bacterial infections that can cause undesired diseases, especially the millions of people in the wide rural areas of developing countries. Soap might be the oldest cleansing agent that acts as a skin cleanser. Since thousands of years ago, this product has been the result of saponification of oils and fats by alkali. In the beginning of this century, soap was a luxury item that was affordable only to the rich and royalty, but now, it has become the main product for everyday personal hygiene (Friedman & Wolf, 1996).

To fulfil consumers' needs and expectations, a wide range of qualities of cleansing agent are demanded (Friedman & Wolf, 1996). The ability to foam or lather is one of the desired characteristics in some cleansing agents such as cleaning soap, shampoo, detergent, facial cleanser and toothpaste, which are must-have items in each and every house. Thus, the inclusion of foaming agent in the production of cleansing agent is important. Foam is produced due to the reaction between air and liquid where air is trapped inside the liquid.

In the formulation of either cosmetic or pharmaceutical products, surfactant is often present, which is usually used as detergent and emulsifier. The major concern is that most of the ingredients that comprise these cleansing agents are chemical-based, including synthetic surfactant that is harmful to human health. Examples of those chemical-based ingredients are sodium lauryl sulphate (SLS) and ammonium laurel sulphate (ALS). SLS is one of the most famous chemical foaming agents worldwide that is being used in the production of cleansing agent. Even though emulsions are often used to cure inflammatory skin disorders such as atopic dermatitis and eczema,

they might also cause some skin disorders due to the presence of surfactants that act as stabilizers (Barany *et al*., 2000).

There are several products that claimed they contain chemical-free ingredients due to the elimination and alteration of some ingredients. However, the alteration makes the end products become less effective and are unable to satisfy consumers. The increasing demand for cleansing products with natural-based foaming agent especially personal care products such as skin care requires some of those ingredients to be greatly improved in terms of skin tolerance and consumer safety.

The values of medicinal plants have already been introduced by traditional medical practitioners. Some of these medicinal plants are believed to contain antibacterial compounds as well as having the potential of becoming natural foaming agents. The beliefs and practices related to handling diseases that are the products of indigenous cultural development are known as ethnomedicine. Ethnomedicine also refers to the study of traditional medicinal practice. Traditional medicine includes all kinds of folk medicine, unconventional medicine and any kind of therapeutic methods that have been passed down through generations of ethnic groups and communities. A study by Verma *et al.* (2010) revealed that there is a prevalent traditional medicinal system that is practiced by the Nicobarese people in Car Nicobar Island of India.

Aside from the effectiveness of cleansing agent towards dirt and stains, the antimicrobial activity of cleansing agent needs to be considered as well in order to maintain its hygiene. Some of the medicinal plants such as *C. asiatica* have the ability to act as antimicrobial agents (Nivas *et al.*, 2015).

*C.asiatica* was chosen in this study because it believe to contain high saponin content that will posseses foaming properties as reported by Chen *et al*. (2010) that crude saponin content in the seed of *C. oleifera* was 8.34% and its total saponin content in the crude saponin extract was 39.5% (w/w). According to Somdee *et al*. (2016), *C. asiatica* has exceptional advantages in phytomedicine because of the diversity in biological activities including antimicrobial, anti-inflammatory and antioxidant effects. This study aims to optimise the extraction of *C. asiatica* by using microwave-assisted extraction with three main parameters which were time, microwave power and solvent to solid ratio to give the best yield of saponin. Characterisation of optimum extract from different methods including SE, UAE and MAE were performed to compare the properties of the extract in order to evaluate advantages and disadvantages of each method. The saponin extracted at optimum conditions was subjected to determination of saponin and foam properties to prove the ability of the extract to serve as an alternative for a natural foaming agent and antibacterial agent.

Several studies have shown that *Colubrina asiatica* leaves extract possesses antibacterial properties against *E. coli* and *B. subtilis* and has the potential to become a natural foaming agent, which could replace chemical foaming agents. In general, the findings especially on foaming properties of *C.asiatica* that were reported are scarce. Thus, in this study, the characterization of saponin based foaming agent from methanol extracts of *C. asiatica* will be conducted to achieve the objectives in this study.

## 1.2 Problem Statement

Chemical-based foaming agents that are extensively applied worldwide in food, cosmetics and pharmaceutical preparations can be harmful to human health and the environment. It may also lead to skin reactions such as inflammation and can cause irritant contact dermatitis (Lemery *et al*., 2015). Generally, the inclusion of natural foaming agent as a substitute to chemical-based foaming agent will result in a significant improvement to its consumer-friendly and environmentally-friendly qualities. In addition, the presence of natural foaming agent would lead to an increase in ecosystem conservational effort and a decrease in chemical pollution. Thus, the next

logical approach is to replace chemical-based ingredients with natural ones. Besides that, the potential of *Colubrina asiatica* as a natural foaming agent has yet to be widely explored. This could present a new approach in the selection of natural foaming agent and cleansing agent production studies, in a sense that it will produce a product with good and safer properties for human consumption. In addition, some natural foaming agents that have been studied previously have a less stability of foam and possesses weak foam properties. Hence, in this study *C. asiatica* was studied to provide baseline data in producing a new natural foaming agent products in future especially in cosmeceutical and pharmaceutical industries as this plant has the high potential to be a good foaming agents because of its foam stability.

In order for bioactive compounds from plant materials to be obtained for isolation, identification, characterisation and industrial production purposes, standardised and suitable extraction techniques that gives optimum extract yield are required (Reinoso *et al.*, 2017). There exists a need to develop and modify plant extraction protocols resource due to the increasing attention in plant secondary metabolites. Despite that, conventional extraction process such as the Soxhlet method may require long extraction time and high cost, in addition to the method's disadvantages of using huge solvent volume and extended extraction time of compounds producing lower yield. For herbal and nutraceutical medicine-based industries to grow, wider, safer and high quality production with low processing cost and higher yield is called for due to the enlarging demand of herbal products (Belwal *et al.*, 2018). To reach these expectations, an increased market for alternative and modern extraction process have been established such as microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). MAE is capable of extracting multiple samples simultaneously in short time (about 90% time reduction), low cost and minimal usage of solvent volume in an efficient manner (Dean & Tyne, 2012). MAE also has favourable upper hands against UAE which is easier to handle, lower cost equipment and provide higher extraction yield in shorter extraction time. It is also apt for thermolabile constituents and capable of providing agitation during extraction to enhance mass transfer phenomenon (Mandal *et al.*, 2007).

## 1.3 Objectives

(a) To optimize the extraction of *Colubrina asiatica* leaves by using Microwave-Assisted Extraction.

(b) To characterize the saponin-based foam based on foam properties such as surface tension, foam ability and stability, film drainage and wetting ability.

(c) To evaluate the antibacterial activity of *Colubrina asiatica* extracts against *Escherichia coli* and *Bacillus subtilis.*

## 1.4 Scopes of Study

In order to achieve the objectives, the scopes of this research have been identified and divided into a few parts :-

(a) Preliminary study on parameters for microwave-assisted extraction is conducted and methanol is selected as solvent in this research.

(b) The optimization of *C. asiatica* extraction using MAE is performed by using Response Surface Methodology (RSM) with regards to three parameters, which are time (3, 5 and 7 min), solvent to solid ratio (10, 20 and 30 mL/g) and microwave power (300, 400 and 500 W), with extract yield as the response.

(c) The extraction of *C. asiatica* is performed at optimum conditions according to previous research using SE with a solvent to solid ratio of 20:1, methanol as solvent and 6 h extraction time and also using UAE at 54 $^{o}$C temperature, 15 minutes extraction time and a solvent to solid ratio of 50:1 (Wang *et al.*, 2018) and compare with optimized MAE extract.

(d) Characterization of the foams from *C. asiatica* extracts that are extracted using MAE at optimum conditions is in terms of total saponin content (TSC), surface tension, foam ability and stability, film drainage and wetting ability.

(e) Investigation on in vitro study of antimicrobial activity *C. asiatica* is conducted through agar diffusion method by using *Escherichia coli* as gram negative and *Bacillus subtilis* as gram positive.

(f) The saponin contained in the *C. asiatica* extract are evaluated through High Performance Liquid Chromatography (HPLC) analysis.

## 1.5 Significance of Study

This research shall contribute by providing the scientific information required to claim and validate foam and antimicrobial stability of standardized *Colubrina asiatica* extracts. In addition, this research would also provide some functional and phytochemical properties of standardized *Colubrina asiatica* extracts that can be used as guidelines to other researchers in this field and cosmeceutical industries. On the other hand, the gathered and documented information would provide an incentive for commercializing *Colubrina asiatica* plants in Malaysia. The correlation between saponin content in the extracts and antibacterial activities is important to prove that saponin is one of the major bioactive compounds that contributes towards foaming and antibacterial activities.

A natural foaming agent is a better alternative to the chemical-based foaming agent, which is way more harmful to human health and the environment. Therefore, it is intended that this research covers the basic foam and antimicrobial composition of the *Colubrina asiatica* as well as the health benefits of the plant.

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