OPTIMISED EXTRACTION AND FERMENTATION PROCESSES OF CURCUMA CAESIA EXTRACTIVE ON ANTIOXIDANT ACTIVITY FOR POTENTIAL APPLICATION IN COSMECEUTICALS

BY

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INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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ABSTRACT

The global skincare industry offers a wide range of products, however, these products are possible sources of human exposure, to endocrine-disrupting chemicals. Thus, this thesis is a pioneering research on the locally grown *Curcuma caesia* that has been used as a traditional folk medicine but has not been extensively studied, by optimizing its extraction parameters, fermentation parameters and the preparation of anti-acne soap and anti-acne cream from its fermented extract. Deionized water is the solvent of choice based on its *halal*, safe and abundance nature. The 2,2-diphenyl-1-picrylhydrazyl **(**DPPH) scavengers were extracted using the ultrasonic water bath and its suitable extraction frequency was selected based on a one factor at a time (OFAT) study. It can be stated that pH and temperature significantly influenced the extraction yield of phenolics from *C. caesia* and improved the DPPH scavenging activity of the extracted bioactive compounds. The optimization study with varying extraction temperature, pH and time using face centered central composite design (FCCCD) under response surface methodology (RSM) showed that pH 6, a temperature of 60℃ and an extraction time of 30 minutes was the most efficient extraction parameters that exhibited high yield of phenolics and high DPPH scavenging activity. A total number of 20 experimental runs with 6 centre points were carried out. The obtained results were analyzed using design expert and statistical validation indices to check the adequacy of the obtained quadratic models. The analysis of variance (ANOVA) showed that more than 97% of the variation was explained by the models, hence, there was a good agreement between experimental and predicted data at optimum conditions. All the independent variables had a significant effect ($p<0.05$) on all responses which indicated that all extraction parameters employed in this study were important in the optimization process. The R^2 value for DPPH scavenging activity was 0.9782, suggesting that the quadratic polynomial models developed were satisfactorily accurate to be used in analyzing the interactions of the parameters (response and independent variables). The Plackett Burman experimental design was employed in searching for significant media components that influence DPPH scavenging activity amongst the 11 chosen variables and they were optimized using FCCCD by Design Expert software version 6.0.8. Yeast extract, peptone and sucrose were found to be the critical factors influencing DPPH scavenging activity. Optimum DPPH scavenging activity with these factors was deduced using FCCCD. The highest DPPH scavenging activity of 84.25 % was obtained when concentration of yeast extract, peptone and sucrose was at 7, 8 and 10 (g/L), respectively with *C. caesia* concentration of 2 % (v/v) and *L. plantarum* concentration of 2 % (v/v). The supernatant of the optimized media was used to measure the 5–lipoxygenase (5-LOX) inhibition activity as an indicator of anti-inflammatory activity present in the supernatant and the mean activity recorded was 76.84%. Twenty respondents with acne vulgaris were selected to test the efficacy and safety of the anti-acne facial soap and cream formulated from the fermented *C. caesia* supernatant. A split face trial was carried out daily for seven days period. Significant lesion improvements and reduced numbers of acne lesions were observed on the treated side of the face. No erythema, burning, stinging, scaling, drying or edema of the skin or exacerbation of the pre-existing acne were recorded. A student's paired t-test was carried out to test the reliability of the results and the

obtained p value of less than 0.05 indicates that the result is significant and that it can be replicated in a larger population.

خلاصة البحث

عالمياً تم تصنيع مجموعة واسعة من المنتجات للعناية بالبشرة، ومع ذلك، فإن هذه المنتجات هي مصادر محتملة لمواد الكيميائية مسببة لاضطرابات الغدد الصماء عند البشر. وبالتالي، فإن هذه الرسالة هي بحث رائد في الكركم الأسود (*Curcuma caesia*) المزروع محليًا والذي تم استخدامه كدواء شعبي تقليدي، ولكن لم تتم دراسته على نطاق واسع. مثل تحسين معايير الاستخلاص ومعايير التخمير وتحضير صابون أو مرهم مضاد لحب الشباب من مستخلصه المخمر. الماء منزوع الأيونات هو المذيب المفضل بناءً على طبيعته الحلال والآمنة والمتوفر طبيعياً. وتم استخدام مسح (*DPPH*) باستخدام الموجات الصوتية لمستخلص المياه الفاترة وتم استخلاص التردد عن طريق دراسة المتغير الواحد في كل مرة. ويمكن القول أن درجة الحموضة والحرارة تؤثر بشكل كبير على مستخلص الفينولات من نبتة الكركم الأسود وتحسين نشاط مستخرج ال *DPPH* المستخلصة من المركبات البيولوجية النشطة. وأظهرت الدراسة لتحسين النتائج تنوع في الاستخلاص بحسب درجة الحرارة، ودرجة الحموضة والزمن باستخدام واجهة التصميم المركب المركزي وفقًا لمنهجية سطح الاستجابة، فكان الرقم الهيدروجيني 6 ودرجة حرارة 60 درجة مئوية ووقت الاستخراج لمدة 30 دقيقة كانت أكثر معاملات الاستخراج كفاءة وأظهرت إنتاجية عالية من الفينولات ونشاط عالي في مسح (*DPPH*). تم تنفيذ عدد إجمالي 20 تجربة مع 6 نقاط مركزية. تم تحليل النتائج التي تم الحصول عليها باستخدام خبير التصميم ومؤشرات التحقق الإحصائية للتحقق من كفاية النماذج التربيعية التي تم الحصول عليها. وقد أظهر تحليل التباين أن أكثر من 97 ٪ من التنوع تم تحليله بواسطة النماذج، وبالتالي، كان هناك توافق جيد بين البيانات التجريبية والمتوقعة في الظروف المثلى. جميع المتغيرات المستقلة كان لها تأثير معنوي ($p < 0.05$) على جميع النتائج مما يدل على أن جميع معاملات الاستخراج المستخدمة في هذه الدراسة كانت مهمة في عملية التحسين. كانت قيمة (R^2 لنشاط *DPPH* 0.9782) مما يشير إلى أن النماذج التربيعية متعددة الحدود التي تم تطويرها كانت دقيقة بشكل مرضٍ لاستخدامها

في تحليل تفاعلات المعطيات (الاستجابة والمتغيرات المستقلة). تم استخدام تصميم *Plackett Burman* التجريبي في البحث عن مكونات وسائط مهمة تؤثر على نشاط مسح *DPPH* من بين المتغيرات الـ 11 المختارة وتم تحسينها باستخدام *FCCCD* بواسطة برنامج التصميم المختص *Design Expert* نسخه 6.0.8. تم تحقيق أعلى مستخلص للخميرة والببتون والسكروز لتكون من العوامل الحاسمة التي تؤثر على نشاط مسح (*DPPH*) تم استنتاج نشاط المسح الأمثل لـ *DPPH* مع هذه العوامل باستخدام *FCCCD*. تم الحصول على أعلى نشاط لمسح *DPPH* بنسبة 84.25٪ عندما كان تركيز مستخلص الخميرة والببتون والسكروز عند 7 و8 و10 (جم / لتر)، على التوالي، مع تركيز نبتة الكركم الاسود بنسبة 2٪ (تركيز السائل / حجم السائل) ونسبة تركيز (*L. plantarum*) 2٪ (تركيز السائل / حجم السائل). تم استخدام المادة الطافية للوسائط المحسنة لقياس نشاط تثبيط الانزيم ((5-LOX) 5–lipoxygenase) كمؤشر على النشاط المضاد للالتهاب الموجود في المادة الطافية وكان متوسط النشاط المسجل 76.84٪. تم اختيار عشرين مستجيباً مصابين بحب الشباب لاختبار فعالية وسلامة صابون ومرهم الوجه المضاد لحب الشباب المصنوع من السوائل المخمرة المستخلصة من نبته الكركم. تم إجراء تجارب على الوجه يوميًا لمدة سبعة أيام. لوحظ تحسن كبير في الأضرار وانخفاضت أعداد جروح حب الشباب على الجانب المعالج من الوجه. لم يتم تسجيل أي التهاب أو حرق أو حكه أو تقشر أو جفاف أو تقرح في الجلد أو تفاقم حب الشباب الموجود مسبقًا. تم إجراء اختبار (**t**) الزوجي للطالب لاختبار موثوقية النتائج والقيمة الاحتمالية (P value) التي تم الحصول عليها أقل من 0.05 تشير إلى أن النتيجة مهمة وأنه يمكن تكرارها في عدد أكبر من الناس.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

(Siti Fathimah Putery Jemain)

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LIST OF SYMBOLS

µ	Micro
□C	Degree Celsius
%	Percentage
T	Time

LIST OF ABBREVIATIONS

mm	Millimeter
nm	Nanometer
ml	Milligrams
min	Minutes
cm	Centimeter
g	Gram
FCCD	Face Centred Central Composite Design
RSM	Response Surface Methodology
rpm	Revolutions Per Minute
ANOVA	Analysis of Variance
OFAT	One Factor At A Time
ug/L	Micro Gram Per Litre
mg/L	Milligram Per Litre

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The frequency of facial acne vulgaris among teenagers in Malaysia was 67.5% and the condition was more common among males (71.1%) compared to females (64.6%) (Hanisah, Khairani & Shamsul, 2009). Oral antibiotic used in combination with topical application of benzoyl peroxide and retinoid is the usual treatment prescribed (Adawiyah, Priya & Roshidah, 2010).

However, benzoyl peroxide has the ability to stimulate the onset of irritant dermatitis with signs of burning, erythema, peeling, and dryness (Eady et al. 1990) and frequent use of retinoids, a teratogen, could result in liver toxicity and abnormalities in serum lipid profiles (David, Hodak & Lowe, 1988) and the application of topical antibiotics could result in the development of antibiotic resistance (Newman et al. 2011).

Thus, due to the side effects of the conventional prescriptions for acne treatment, safer alternatives derived from plants have been studied which includes *Curcuma longa* (Kapoor and Saraf, 2011), however, *Curcuma longa* would leave a yellow stain on the skin when applied and a time consuming process of producing a colourless derivative of the *Curcuma longa* had to be developed (Majeed,Badmaev & Rajendran. 1999).

According to a survey by the World Health Organisation, 80% of the world's population relies on traditional herbal medicine for their basic health care (Newman, 2012). Malaysia with its treasure of 1300 medicinal plant species in the Peninsular (Burkill, 1966) and 7411 plant species in Sabah (Kulip, et al. 2010) can be a regional

hub offering safe alternatives to the conventional prescription used for skincare regime in general and acne problem in particular.

Traditional prescriptions were mostly not written but were passed orally from generations of traditional Malay medicine men. One such plant that has been used for generations in the Malay culture is the '*kunyit hitam'*. Kunyit hitam has a strong camphoraceous sweet smell and its rhizome has a bluish black colour. Its scientific name is, *Curcuma caesia* and it belongs to the *Zingiberaceae* family. *C. caesia* has been used by the locals as a general energy booster and helps increase blood circulation for both men and women and as a treatment for piles, hernia, severe wound healing and also to treat drug addiction. However, not much scientific study has been conducted on the local *C. caesia*. Its mode of usage among the Malays indicates that it might contain anti-inflammatory substances and phytochemicals that could increase blood circulation.

A research done on *C. caesia* grown in Manipur, India, found that the rhizome contains 30 mg/g weight of flavonoids content, 104.2 mg/g dry weight alkaloid, 47.5 mg/g fresh weight soluble protein and 60 mg/g phenolic compounds (Sarangthem and Mangvung, 2010) and a later research done on the *C. caesia* rhizome grown in Dindhori district of Madhya Pradesh, India, also found that the methanolic extract of *C. caesia*, contains flavonoids, alkaloids, phenolics, tannin and also protein (Paliwal, Pancholi & Patel., 2011). In India, *C. caesia* or '*Kali haldi*' (black turmeric) has been used to cure leprosy, asthma, cancer, epilepsy, wound, menstrual disorder, aphrodisiac, inflammation and gonorrhoeal discharges, smooth muscle relaxant and hemorrhoids (Das, Mondal & Md. Kamaruz Zaman., 2013). Methanolic extract of *C. caesia* was found to show 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (Mangla, et al., 2010; Karmakar, et al., 2011) and the crude extract of *C. caesia*

exhibited stronger DPPH scavenging activity than the enzymatic extract (Yogamaya, Bandita & Sahu, 2012). Methanolic extract of *C. caesia* was also shown to have muscle relaxant effect and antidepressant effect on mice and were also shown to exhibit antitumor and antiulcer activity in mice (Karmakar et al., 2011; Das, et al., 2012; Karmakar et al., 2013).

To the best of our knowledge, as testified by the literature review, no optimization and fermentation studies have been done on the extraction process of bioactive compounds from the Malaysian *C. caesia*. Thus, this research will fill a gap in our knowledge on *C. caesia* by conducting fermentation and process optimization for the extraction of bioactive compounds and for the development of an anti-acne soap and anti-acne cream from fermented *C. caesia* extract.

1.2 PROBLEM STATEMENT

Skincare products includes, cleansers, toners, facial masks, scrubs, serums and whitening creams. According to statistics, the global dermatology industry is forecasted to worth €81.9 billion in 2028, doubling its value from €44.1 billion in 2018. However, according to research done by Meeker et al (2013) and Braun et al (2014), skin care products are a possible source of human exposure, especially adolescent girls, to potentially endocrine-disrupting chemicals, such as phthalates, parabens, and phenols. According to a report by the U.S. Department of Commerce (2016) Malaysia's total trade volume for personal care and cosmetics products was about US$2.24 billion in 2015 and there is an increase demand in organic skincare products amounting to 21% of the market share. Over 50% of this demand was met by US$1.13 billion in imports and that, China, Thailand, France, the EU28, the United States, South Korea and Japan are the main exporters to Malaysia. Thus, it is crucial

that Malaysian government agencies should ensure that products available are safe and also halal in order to meet the growing demand of the Muslim community which made up about 61.3% of the population, as reported by the Malaysian Department of Statistics report in 2015.

Hence, this research aims to provide the solution to the problem that is facing the Malaysian consumer, by utilizing *C. caesia*. The problem is that we do not know the data in the Malaysian context on the production and use of DPPH generated from indigenous *C. caesia.* In view of this, there is an urgent need to conduct research on it locally. Large scale cultivation of *C. caesia* in the country would be able to boost the economy of local farmers in the country due to the competitive price of dry *C. caesia* powder which ranges from USD 68 to USD 73.04 per kilogram, as quoted by trusted suppliers in Indiamart, an online platform.

This research is done in order to study and optimized the process conditions to ensure that the *C. caesia* extract exhibits very high 2,2-diphenyl-1-picrylhydrazyl, DPPH, scavenging activity in a safe and effective way and thus developed it into an anti-acne soap and anti-acne cream.

The fermentation process has been chosen for this study as it has been proven to increase free radical scavenging activity in plant extracts measured by an increase in DPPH scavenging activity in fermented *Echinacea purpurea* (Rizzello, Coda & Marcias., 2013) and fermented mixture of herbal extract consisting of *Puerariae radix, Ephedrae Herba, Zizyphi fructus, Cinnamomi cortex, Paeniae radix, Glycyrrhizae radix* and *Zingiberis rhizoma* (Kim, Um & Ma., 2014), *Chamaecyparis obtusa* (Kwon et al., 2014) and *Myrtus communis* L (Curiel, Pinto & Marzani., 2015). In these research *Lactobacillus plantarum* was used to ferment the extracts.