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MOLECULAR ANALYSIS FOR HALAL VERIFICATION: SCREENING PORCINE DNA IN CHARMS COSMETIC SKINCARE PRODUCTS

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Nursyuhada Othman[1], Hidayah Haris[1], Nur Hartini Sariyati[1], Farah Farhana Ramli[1], Norhayati Muhammad[2], Mohd Zul Hilmi Mayzan[3], Muhammad Suhaimi Sulong[4], Muhammad Abu Bakar Abdul-Latiff[1]*

[1] Environmental Management and Conservation Research Unit (eNCORe), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Muar 84600, Johor, Malaysia;
[2] Advanced Herbal and Ethnomedical Research (AdHerb), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Muar 84600, Johor, Malaysia;
[3] Ceramic and Amorphous Group (CerAm), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Muar 84600, Johor, Malaysia; [4]Faculty of Technical and Vocational Education (FPTV), Universiti Tun Hussein Onn Malaysia, Batu Pahat 86400, Johor, Malaysia

*Corresponding author: latiff@uthm.edu.my

ABSTRACT

The growing Muslim population around the world has led to an increase in demand for Halal products such as cosmetic. Previously, the cosmetics business was dominated by non-halal cosmetic enterprises, as many products contained pig-derived substances, which are forbidden in Islam. In 2012, Charms Skincare Cosmetics, a Malaysian cosmetic brand, was established with a focus on producing Halal-certified products. Thus, the aim of the study is to demonstrate that the seven products from the brand are free from porcine DNA, following halal verification. All the cosmetic products were isolated using the Agilent Porcine Detection Kit, and the amplification was done by the real-time polymerase chain reaction (RT-PCR) method targeting porcine-specific DNA sequences from the Cytochrome b region. The result showed no amplification of the Cytb gene in gel visualization and was supported by the absence of porcine DNA using RT-PCR from all seven Charms cosmetic products, representing the absence of any pig derivative substances. These findings indicate that the products comply with Islamic requirements, and the PCR method proves to be a sensitive and reliable approach for the detection of porcine DNA in halal authentication processes.

Keywords: Animal-derived; Cosmetic manufacture; Cytb; Malaysia; Shariah compliance

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INTRODUCTION

The rapid growth of the halal industry worldwide is evident due to the increasing awareness among the Muslim population. This industry encompasses a wide range of products, including not only food and beverages but also healthcare, travel and tourism, financial services, cosmetics, and pharmaceuticals. According to Zabidi et al. (2020), halal food and cosmetics play a significant role in the global halal market, catering to the demands of consumers. Halal products must be produced in accordance with Islamic law, which forbids the use of specific meat cuts, particular animal species, and the Islamic method of animal slaughter (Abdallah et al., 2021; Sugibayashi et al., 2019). With the advancement of technology, the halal cosmetic market is also increasing as demand is expected to rise due to the ever-growing Muslim population, which is predicted to account for 26.4% of the world's population by 2030. This surge is attributed to the strong awareness and knowledge among the Muslim community (Hashim et al., 2020; Nasab & Banejad, 2016).

In recognition of the fact that many cosmetic formulas contain ingredients derived from animals, producing halal cosmetics must adhere to Shariah regulations. At the moment, non-halal cosmetics companies control most of the manufacturing process (Anuar and Tukiran, 2022). To improve skin health and attractiveness, however, pig-derived components such collagen, fatty acids, and glycerin are frequently used in the creation of cosmetics worldwide (Kim et al., 2018; Zabidi et al., 2020). According to Sionkowska et al. (2020), collagen's inherent humectant and moisturizing qualities make it a popular ingredient in cosmetics. Pork (pig flesh), lard made from adipose tissue, and porcine gelatin are common pig-derived materials used in cosmetic manufacture (Erwanto et al. 2018). These materials are frequently less expensive than halal-certified alternatives like bovine (Rohman and Che Man, 2012). As of raw materials and the manufacturing process of cosmetic ingredients is crucial for Muslim consumers.

In recent years, non-halal ingredient detection technologies have seen significant advancements to assist religious authorities in certifying halal compliance and identifying the presence of non-halal components. Extensive research has been conducted on detecting non-halal components, especially porcine-based products, in the food sector (Lubis et al. 2016; Sajali et al., 2022). Various detection techniques have been developed, including Fourier transform infrared (FTIR) spectroscopy, comprehensive two-dimensional gas chromatography hyphenated with time-of-flight mass spectrometry (GCxGC-TOF-MS), and gas chromatography-mass spectrometry (GCMS) for identifying gelatin, alcohol, fats, and oils in cosmetic products (Hashim et al., 2010). In terms of species detection specificity and sensitivity, PCR-based techniques have proven to be the most successful, offering cost and time effectiveness. This technique has been widely used for detecting porcine DNA in meat and meat products (Erwanto et al. 2018), bakery products (Norrakiah et al., 2015), capsule production in the pharmaceutical industry (Nikzad et al., 2017) and cosmetic ingredients (Zabidi et al., 2020). Additionally, PCR-based methods can overcome potential challenges arising from extensive heat processing and chemical treatment when detecting porcine DNA (Muflihah et al., 2023).

The lack of previous studies on porcine detection in cosmetics served as the foundation for this investigation. Then there is the expanding demand for halal cosmetics around the world, which has raised awareness of the issue of porcine ingredients in cosmetics. Thus, this study intends to focus on assessing the accuracy and effectiveness of PCR in detecting in detecting pig ingredients in cosmetics. Therefore, this study aims to prove that the makeup made by the Malaysian company Charms Beauty is free of porcine DNA and appropriate for use by Muslims who follow halal certification.

METHODOLOGY

Sample preparation and DNA extraction

The seven Charms cosmetics consist of a cleanser, toner, serum, essence, night cream, moisturizer, and facial scrub that were extracted using Agilent Porcine Detection Kit (Agilent Technologies, USA). The extraction of DNA was performed according to the manufacturer's protocol. Before the extraction of DNA, the Proteinase K Digestion Buffer was pre-warmed to 65℃ for 5 minutes in the incubator and was inverted to mix. A 220 µL of the Proteinase K working solution was added to release the nucleic acids into the solution and incubated at 65℃ for 1 hour. Then, Nucleic Acid Binding

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Buffer was added to each pipette before transferring to a separate DNA Binding Spin Cup. The spin cups were retained, the filtrates were discarded, and for each sample, the spin cup was replaced in the receptacle tube, then 500 µL of 1X High Salt Wash Buffer was added, followed by centrifugation at approximately 75% of the maximum speed for one minute. The samples were washed three times using 80% ethanol to remove the contaminations. Finally, the 100 µL Elution buffer was added to recover DNA by incubating in one minute. The extracted DNA was stored at 4℃ and ready to use for quantification process. The test for authenticating of halal cosmetic were conducted with the collaboration with ALS Technichem (M) Sdn Bhd in Selangor, Malaysia.

DNA quantification and qualitative analysis

The quality and quantity of the extracted DNA were determined by using Thermo Multiskan GO Microplate Spectrophotometer (ThermoFisher, USA). The concentration of DNA was determined by UV absorbance at 260 nm. The purity of the extracted DNA was determined by the absorbance ratio at 260/280. Besides, DNA was also analyzed by gel electrophoresis to determine the presence of DNA and the size of the PCR Product. Agarose gel 2.0% was used by loading a 100 bp ladder as the size marker and was run at 80V for 1 hour. The process was continued with gel visualization using the built-in software of Syngene NuGenius gel imager, and the digital image was obtained.

Real-time PCR assay

The extracted DNA was amplified and quantified using real-time PCR assay where the detection is based on a porcine-specific DNA sequence from the Cytb gene. The assay with aliquots of 25 µL reaction mixture per reaction consisting of 12.5 µL of 2 x QPCR Master Mix, 2.5 µL of Porcine Detection Assay Mix, and 10 µL of DNA template. The real-time PCR was carried out in CFX96 Touch Real-Time PCR Detection System (Bio-Rad Technologies, USA). A two-step amplification program consisted of pre-denaturation at 95℃ for 10 minutes, followed by 40 cycles of denaturation at 95℃ for 30 seconds, and annealing and extension at 60℃ for one minute was used in this study. All the samples were run in two replicates for each cosmetic product.

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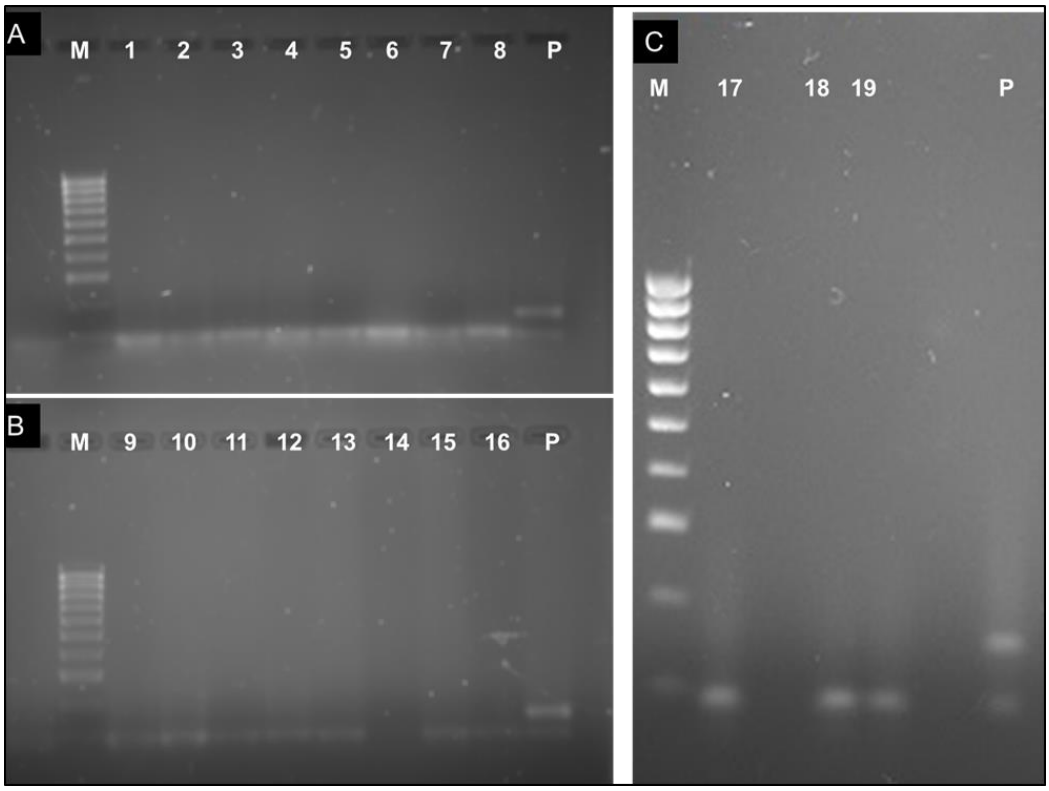
RESULTS

Optimization of DNA extraction

All seven samples were successfully extracted using Agilent Porcine Detection Kit. The extracted DNA was analyzed using gel electrophoresis, as stated in Figure 1(A) to Figure 1(B). We also confirmed the extracted DNA concentration ranging from 0.1 ng/µL to 51.7 ng/µL as two replicates from each sample were performed (Table 1). The highest concentration of DNA was found in serum (37.75 ng/µL), followed by night cream (35.55 ng/µL), moisturizer (14.3 ng/µL), toner (4.1 ng/µL), essence (2.35 ng/µL), face scrub (1.85 ng/µL) and cleanser (1.15 ng/µL). Based on the gel in Figure 1, showed the DNA band for the positive control sample in well P that was successfully amplified at ~200 bp while for the skincare products, no amplification is shown in gel image including the negative control.



*M=100bp DNA ladder, P= positive control (porcine DNA), 1&17= negative control (PCR water), 2,9= extraction blank control, 3,4&10=cleanser, 5&6=toner, 7&8=essence, 11&12=serum, 13&14=night cream, 15&16=moisturizer, 18&19=face scrub

Figure 1. The gel electrophoresis image of Charms Cosmetic products where 100bp marker was used using 2% agarose with porcine DNA as positive control (P). (A) the gel image consists of cleanser (3 &4), toner (5&6) and essence (7&8). (B) the gel image consists of serum (11&12), night cream (13&14) and moisturizer (15&16). (C) the gel image of face scrub (18&19).

From Table 1, DNA concentration from the seven samples is considered high compared to the previous study conducted by Zabidi et al. (2020) that only focused on collagen cream cosmetic products that range around 3.252-4.222 ng/µL. Besides, the presence of other contaminants of proteins can be seen due to the absorbance reading at A260/A280, where it is primarily below than 1.8 because the ratio of 'pure' DNA is between 1.8-2.2 (ThermoFisher). The purity of DNA might be affected because cosmetic products are classified as highly processed products that are treated by various components which can modify or degrade by thermal, physical, or chemical treatment during the manufacture (Kim et al. 2018).

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Table 1. The reading of DNA concentration based on samples

Samples	Replicate	DNA concentration (ng/µL)	A260/A280	A260/A230
Cleanser	A	1.6	1.2	0.3
	B	0.7	0.7	0.0
	Average	1.15	0.95	0.15
Toner	A	3.7	1.4	0.5
	B	4.5	1.5	0.1
	Average	4.1	1.45	0.3
Essence	A	0.1	0.2	0.0
	B	4.6	1.4	0.1
	Average	2.35	0.8	0.05
Serum	A	26.1	1.3	0.6
	B	49.4	5.6	0.6
	Average	37.75	3.45	0.6
Night cream	A	19.4	1.2	0.6
	B	51.7	2.5	0.4
	Average	35.55	1.85	0.5
Moisturiser	A	22.2	1.2	0.6
	B	6.4	1.0	0.1
	Average	14.3	1.1	0.35
Face scrub	A	1.6	1.3	0.2
	B	2.1	1.2	0.3
	Average	1.85	1.25	0.25

Table 2. Cq values of the real-time PCR assay

Samples	Cq value
Positive control (Porcine DNA)	20.23
Cleanser	Absent
Toner	Absent
Essence	Absent
Serum	Absent
Night cream	Absent
Moisturiser	Absent
Face scrub	Absent

Detection using real-time PCR

The real-time PCR (rt-PCR) or qualitative PCR (qPCR) assay was used to determine the presence of pig-derived ingredients in Charms cosmetics from the extracted DNA. All seven products were tested and showed the negative result, indicating the absence of porcine DNA and contradicting with positive control containing porcine DNA (Table 2). Cyt*b* gene was used to detect the trace amounts of porcine in cosmetics products as Agilent Porcine Detection Kit can perceive as low as 300fg of porcine DNA.

DISCUSSION

In order to detect the presence of porcine DNA, especially in cosmetic products, the DNA extraction kit's choice will affect the result's efficiency. In this study, the efficiency of Agilent Porcine Detection Kit can be proved as previously the study used Wizard Genomic DNA Purification System kit from Promega (Zabidi et al. 2020) and Power Prep™ DNA extraction kit to detect the porcine DNA (Kim et al. 2018). In 2018, Kim et al. compared five commercial DNA extraction kits (CTAB method, Power Prep™ DNA extraction kit, QIAamp DNA stool mini kit, Wizard Genomic DNA Purification System kit, TIANamp genomic DNA kit, Nucleo spin food kit) as they concluded Power Prep™ DNA extraction kit is the most suitable to use for cosmetics. Thus, the suitability of the DNA extraction method depends

on the types of products; which most of the studies were focused on food detection, while for the method used for cosmetic products is still not well known (Kim et al. 2018).

Mitochondrial DNA based method is usually used in detecting and identifying the animals (Abdul-Latiff et al. 2017; Othman et al. 2021). This is because DNA is stable and resistant even in high temperatures where loci, 12S rRNA, and NADH dehydrogenase subunit 5 (ndh5) genes were mostly used for porcine detection in cosmetic products as successfully proved by Zabidi et al. (2020) and Kim et al. (2018). For the Cyt*b* gene, it is mostly was used to detect porcine in the food industry especially on meat products (Hamzah et al. 2013). Previously, even though the assay is sensitive and capable of producing an accurate result, the choice of a specific primer and probe is also essential to detect the presence of pig-derived materials, especially in highly processed products due to various ingredients such as alcohols, pectin, and detergents that can interfere while conducting the PCR (Laila-Liyana et al. 2018; Kim et al. 2018). Thus, the finding from this study able to show the effectiveness of Cyt*b* gene is applicable in cosmetic products to detect the porcine in order to develop the halal cosmetics.

Furthermore, PCR-based technique used for Charms beauty products also able to prove help the absence of porcine-derived ingredients which is crucial in the label of commercial halal products as mentioned by Kang et al. (2018). The evaluation of halal authenticity needs the accurate analytical method especially when it is involving the processed production from food, cosmetic and pharmaceutical to ensure the safety of product and product efficacy. In addition, the fraud and mislabeling of products can be avoided in cosmetic products as previously most of cases were reported from food products (Panprommiin and Manosri, 2022). The usage of gelatin from animal origin can be applied widely in all the sectors in parallel to the advancement technologies in molecular as it is one of the most controversial ingredients in modern Halal and Kosher industry (Uddin et al. 2021).

Besides, the certified Halal logo need to be implemented in all cosmetic products similar to halal food industry. In Malaysia, cosmetic products are permitted to use halal logo by Islamic Development Department of Malaysia (JAKIM) that is a certified authority safeguarding the Halal status for products in Malaysia as indicated by Sajali et al. (2022). Based on Isa et al. (2023), the global halal logo can build consumer's trust and confidence as they believed halal cosmetics offered quality and safety. Indirectly, the resonance can contribute significantly to robust marketing strategies, providing a competitive edge over non-certified counterparts, as mentioned Mohezar et al. (2016). Hence, the outcomes of this study offer valuable insights, guiding the establishment of halal authentication protocols through the detection of porcine DNA in unidentified cosmetic products, aligned with Islamic perspectives in Malaysia.

CONCLUSION

In conclusion, from the PCR performed to amplify the fragments of Cyt*b*, there is no detection of porcine in all seven Charms cosmetic products as no amplification happened. Overall, the mitochondrial gene Cyt*b* is proven applicable to facilitate the detection of any pig derivative substances in cosmetic products. Consequently, the Charms products have been substantiated to be devoid of porcine DNA presence, thereby affirming their safety for use, particularly among Muslims in Malaysia.

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