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The study of pig dna analysis methods in cosmetics: a review

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ABSTRACT

Collagen is one of the components used for cosmetics. Functionally, collagen is derived from pig fat, which functions as a skin conditioning agent (*Emollient*), emulsion stabilizer (*Emulsifier*), and an ingredient to increase the viscosity of cosmetic preparations. The method that can detect pig content is the PCR method, which can confirm the presence of DNA content in pigs. In this study, an analysis of cosmetic products containing porcine DNA was carried out using the PCR method. This research was divided into two stages: the initial stage was DNA extraction, and the second stage was PCR analysis. This research is a study through literature study using the narrative review technique. This study aimed to collect information and examine the analysis method of identifying pig DNA contained in cosmetics to obtain the right and accurate way through a literature approach. From the literature study results, it can be concluded that the best extraction method for the isolation of porcine DNA in cosmetic preparations is the extraction method with the *Boom* Method, while the DNA analysis used to identify porcine DNA in cosmetics is RT-PCR (Real-time Polymerase Chain Reaction*)*. Conventional PCR is because both methods have their respective advantages. The RT-PCR (Real-time Polymerase Chain Reaction) method can amplify and quantify the number of target DNA molecules. The Conventional PCR Method can form DNA bands based on the amplification value.
Keywords: DNA analysis, DNA extraction, collagen, cosmetics, PCR, journal review.

INTRODUCTION

According to The Pew Forum on Religion Public Life, Indonesia is a country where most of the population is Muslim, with a large population of 209.1 million people, or equivalent to 87.2% of the total population. The high number of Muslim populations in Indonesia affects the halal lifestyle, which is the basis for product selection (Katadata, 2016). Halal includes all stages, from sourcing raw materials, distribution of final products, and delivery to consumers. Not only food, but the term halal can also include all consumables such as medicines, cosmetics, personal care products, toiletries, and others (Che Man & Sazili, 2010).

There are many Indonesian products. Cosmetics is one of the things that need to be understood whether its management is included in halal products or not. The development and change of the times continue, and a beautiful and pleasant physical appearance has become a basic need for humans, especially women. In fulfilling these needs, cosmetics are the main thing to support one's appearance. Cosmetics are materials or preparations used outside the body (epidermis, hair, nails, and external genitalia) or teeth and oral mucosa whose function is to clean and care for the body and change or improve one's appearance.

Some cosmetic products are thought to have been mixed with pig contaminants. The essential ingredients derived from pigs are fatty acids, glycerine, and collagen. In detecting the essential elements derived from pigs, DNA isolation methods are used in cosmetic products. The DNA isolation method has several techniques that are often used to detect pig isolation using the *Double Spin Column* method, CTAB (*Cetyl Trimethyl Ammonium Bromide*) method (Ardi, 2012).

After the isolation, it was continued with DNA-based detection using the *PCR* (*Polymerase Chain Reaction*) method. It is necessary to carry out DNA-based detection with *PCR* (*Polymerase Chain*

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Reaction) to double the number of DNA molecules on specific targets by analyzing new DNA molecules complementary to target DNA molecules through enzymes and oligonucleotides primers in a thermocycler (Widayat et al., 2019). One of the detection methods often used to test pig components and their derivatives in a product is a DNA-based detection method, namely the PCR (*Polymerase Chain Reaction*) method (Adzakiyyi et al., 2020). Detect DNA using PCR (*Polymerase Chain Reaction*) has several types of methods, namely conventional PCR, Multiplex PCR, and RT-PCR (*Polymerase Chain Reaction*) (Rachmawati et al., 2018). The PCR (*Polymerase Chain Reaction*) method is a DNA amplification-based method that has higher specifications and sensitivity than other methods (Fadhlurrahman et al., 2016).

Therefore, this study will examine the method of DNA extraction and accurate DNA analysis to detect the presence of pig DNA in cosmetics through a literature approach to obtain new information about DNA extraction and analysis in cosmetics and become a scientific work in the form of a review. Review articles are divided into two categories: a narrative review and a systematic review. Narrative reviews are written in an easy-to-read format and allow consideration of a broad spectrum of subject matter. However, in systematic reviews, a very detailed and comprehensive literature survey is carried out on the selected topics (Gulpinar & Gucal Guclu, 2014). This research was conducted using the narrative review method, to facilitate the development of new research for the community.

MATERIALS AND METHOD

Data Collection Materials

Data collection is done by searching for published articles on Google Scholar, PubMed, Science Direct, or published by accredited journals using the selected keywords, namely "*extraction*", "*analysis*", "DNA", "*porcine*", "*cosmetic*" and in Indonesian way "ekstraksi", "analisis", "DNA", "babi", "kosmetik".

Based on search results on Google Scholar, PubMed, Science Direct with the keywords "extraction", "analysis", "DNA", "porcine", "cosmetic" and "ekstraksi", "analisis", "DNA", "babi", "kosmetik", researchers found 3,209 journals which divided into 109 Indonesian and 3.109 English journals. According to the inclusion criteria set, journals with different themes were then screened into 35 journals and 7 journals with the same theme and according to the inclusion criteria set.

Methods

The research was conducted by searching for research articles that have been published nationally and internationally from search engines such as Google Scholar, PubMed, and Science Direct.

1. Data Collection Process

   The process of collecting data is done by looking for journal articles related to the journal review that will be carried out, then choosing journals that fit the criteria, then doing an in-depth understanding of the methods and results of several journals studied, the data findings will be analyzed based on the differences generated from the results of the research. several journals, so that it can conclude the results of several journals that are tailored to the research objectives.
2. Inclusion Criteria

   A research journal that focuses on isolation, DNA extraction, DNA analysis in pigs, and cosmetics as samples, from 2011 to 2021, which can be accessed for free, and research journals that have been published in National and International journals in Indonesian and English.

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3. Exclusion Criteria

   Research journals that are not focused on DNA isolation or extraction, DNA analysis in pigs, cosmetics as samples, the period of journals before 2011, journals that are not the result of reviews, and journals that use foreign languages other than Indonesian and English.

Data Analysis

This literature review was analyzed using a literature review study method or systematic literature review by collecting data according to predetermined criteria, then the data was analyzed in a state of art based on the journal findings.

RESULT AND DISCUSSION

Article Information

This study uses 7 journal articles consisting of 3 national or Indonesian journals and 4 international journals. The journal articles collected consisted of 7 extraction methods, 4 methods of porcine DNA analysis using conventional PCR, and 3 methods of porcine DNA analysis using RT-PCR.

Journal Review Results

Based on the results of a review of journals and research journals that have been published nationally and internationally, the results can be seen in Table I. DNA extraction is a process of separating DNA from other cell components such as proteins, carbohydrates, fats, and others. DNA extraction consists of three main stages, namely cell wall destruction (lysis), separation of DNA from other components, and DNA purification (Walker & Rapley, 2008).

Of the 7 journals obtained by the study of journals, various extraction methods were used. Namely, the types of extraction that can be used for cosmetic extraction are the *Double Spin Column* extraction method, the *Phenol Chloroform Isoamyl Alcohol* (PCIA) method, the *Chelex Ion Exchange Resin* method, the *Boom* method, and the CTAB (*Cetyl Trimethyl Ammonium Bromide*) method. The difference between all methods for extraction of cosmetic samples is distinguished from the process of lysis. In the *Double Spin Column* method, various kinds of kits are usually used, such as the Wizard Kit, Progenus Kit, DNA Purification Kit, and Power Prep TM Kit. In the Wizard Kit, *the Nuclei Lysis* reagent was used as the stage of the *lysis* procedure (Munir et al., 2021) and the other Kits did not provide information regarding the reagent used for the lysis. Meanwhile, for other extraction methods such as the *Boom* method using L6 lysis buffer as the lysis material (Aviani et al., 2017), and the CTAB (*Cetyl Trimethyl Ammonium Bromide*) method using MEDTA, M TRIS-HCl as a lysis buffer and the addition of chloroform: isoamyl alcohol (24: 1) (S Abd-Gani et al., 2019).

Various types of extraction methods have their respective advantages, such as the Double Spin Column method which the advantage that it is expected to be able to support the success of the next process (DNA amplification and DNA sequencing), in addition to the use of several special buffer solutions in the Kit that work to bind DNA capable of maintaining the quality of DNA extraction for longer storage (more than 2-3 years). The CTAB (*Cetyl Trimethyl Ammonium Bromide*) method has the advantage that it will produce thick DNA bands and can separate DNA from polysaccharides due to differences in solubility characteristics (*differential of solubility*), and this method does not require more expensive costs than using Kit (Milligan, 1992) The *Boom* method has the advantage that it can isolate DNA in a simple way which can save time and costs (Boom et al., 1990).

Based on the results of the research that has been done, the best extraction method is the *Boom* method. This method has the best results, this is because in this study after extracting DNA, it was followed by purification using a NanoDrop Spectrophotometer, which resulted in good DNA purity values, another thing was because the *Boom* extraction method used non-organic materials, namely *Chaotropic Guanidinium Thiocyanate* which is used as a purification agent and detection of DNA and RNA so that the *Boom* method can isolate properly and simply based on the purity value parameter. In

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addition to being the best extraction method among others, the *Boom* method has a drawback, namely that the procedure requires special skills compared to Kit and other methods (Boom et al., 1990).

The results of DNA extraction isolation with good DNA quality are when it has a purity level in the range of 1.8 – 2.0 from the absorbance ratio at 260 nm and 280 nm wavelength measurements, if the A260/A280 ratio shows results less than 1.8, it indicates the presence of contaminants. in the form of proteins and polysaccharides. Meanwhile, if the A260/A280 ratio shows a result of more than 2.0, the DNA isolate shows the presence of contaminants in the form of RNA (Abdullah et al., 2019). So, it can be concluded that (Aviani et al., 2017) has a purity value that is by the value of DNA quality provisions.

Table I. DNA Extraction Result

Sample	DNA Extraction Method	DNA concentration	DNA Purity	Reference
Lipstick without halal logo	*Promega Genomic Wizard* Kit	0.220 l/ml, 0.224 l/ml, 0.136 l/ml, 0.224 l/ml, 0.092 l/ml	0.479 – 1,000	(Munir et al., 2021)
5 kinds of collagen cream	*Promega Genomic Wizard* Kit	3,662 ng/µl	1,236	(Zabidi et al., 2020)
Capsules, day cream, and beauty soap	*Progenus EasyFast Extraction* Kit *for Pharmaceutical Products 1(Cat ExtrPharma)*	2.9 ng/µL in capsules, 2.4 ng/µL in day cream, 2.2 ng/µL in beauty soap	1.52-4.60 on A260/A280 & on A260/A230 0.38-1.95	(Widayat et al., 2019)
100 mg slimming capsule shell	Modified *boom*	29.8 ng/µL	1.88	(Aviani et al., 2017)
Cosmetic cream contaminated with lard	CTAB modified by Doyle & Doyle	-	-	(S Abd-Gani et al., 2019)
Halal and non-halal cosmetics (cream, liquid mask, powder mask)	*Power Prep TM* Kit	-	-	(Kim et al., 2018)
Lipstick	*Epicenter MasterPure TM* Commercial Complete DNA Purification Kit	-	-	(Ishak & Mutalib, 2018)

Based on the results of the research that has been carried out, after DNA extraction, DNA purity measurements are carried out using or not using the NanoDrop Spectrophotometer, where the tool in principle is to calculate the difference in UV light absorption where double bands of DNA can absorb UV

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light at 260 nm because DNA contains bases. purines and pyrimidines can absorb UV light at a wavelength of 260 nm, while protein or phenol contaminants can absorb light at 280 nm. The data from the DNA analysis method in Pigs using the NanoDrop Spectrophotometer can be seen in Table II.

Measurements with the NanoDrop Spectrophotometer using different extraction methods resulted in different DNA purity and some even did not produce DNA purity. Studies (Aviani et al., 2017; Munir et al., 2021; Widayat et al., 2019; Zabidi et al., 2020), resulted in DNA purity values. While in the study (Ishak & Mutalib, 2018; Kim et al., 2018; S Abd-Gani et al., 2019), there was no DNA purity value. The reason for measuring DNA purity with a NanoDrop Spectrophotometer is because this test is quantitative in nature, it is carried out with a NanoDrop Spectrophotometer with an absorbance ratio of 260/280 to produce an accuracy value and to validate a DNA extraction that has been carried out successfully isolated in addition to seeing the levels of DNA which are then carried out on the *PCR* (*Polymerase Chain Reaction*) stage, and the reason for not measuring DNA purity with the NanoDrop Spectrophotometer because this test is qualitative by looking at the parameters of the presence or absence of DNA.

Data from DNA analysis in pigs using several methods can be seen in Table II. DNA analysis in the journal articles studied, namely Conventional PCR and RT-PCR (*Real-time Polymerase Chain Reaction*). The Data from DNA analysis in pigs using several methods can be seen in Table II. DNA analysis in the journal articles studied, namely Conventional PCR and RT-PCR (*Real-time Polymerase Chain Reaction*). The conventional PCR method is a method that is combined with electrophoresis so that the DNA band can be seen, the RT-PCR (*Real-time Polymerase Chain Reaction*) method is a PCR (*Polymerase Chain Reaction*) technique to amplify and quantify the target number of DNA molecules resulting from the amplification (Yuwono, 2006).

Conventional PCR methods and RT-PCR (Real-time Polymerase Chain Reaction) have the main difference in that Conventional PCR is usually qualitative which only produces positive and negative while Real-time PCR adds potential for quantitative analysis and other differences namely when using the Real-time PCR method, it faster than using the Conventional PCR method. This is because at the time of testing with the *Real-time* PCR method there was no electrophoresis process. *Real-time* PCR detection limits for *Power Prep TM* DNA extraction kits are 100-1000 times higher than other extraction methods (Kim et al., 2018) and for Conventional PCR detection limits with lower presentations resulting in DNA amplification of 0.01% achieved for pig DNA. Each dilution series can provide good amplification up to 0.001 ng/µL but the band intensity decreases at 0.0001ng/µL dilution (Kim et al., 2018).

The working principle of Real-time PCR (qPCR) is the same as conventional PCR. However, to see the real ' real-time ' amplification process, it is necessary to add a fluorescence probe (marker) to the PCR sample target so that the fluorescence light can be caught by the detector contained in the real-time PCR machine. Therefore, if the number of gene copies increases during the reaction, the fluorescence will also increase which indicates the progress of the PCR reaction. The results of the PCR reaction (Polymerase Chain Reaction) using a real-time PCR machine, seen in the form of an exponential curve on computer software where the Y curve shows the amount of fluorescence light captured, while the X curve shows the number of PCR cycles that take place (Handoyo & Rudiretna, 2001).

This journal study on electrophoresis is inseparable from the study of electric fields. The resulting electric field comes from the electrodes which are given electrical energy from the energy sources such as direct current or alternating electric current. *Coulomb's* law *is* the basic principle of the electrophoretic separation method, namely the force at one point of charge is directly proportional to the magnitude of the charge. The electric field is an effect produced by electric charges such as electrons, ions, or protons, in the space around them.

The results of the RT-PCR (*Real-time Polymerase Chain Reaction*) are Cq FAM and Cq VIC where Cq FAM indicates the presence of Pig DNA with a range of approximately 30 while Cq VIC indicates the presence of *Vertebrate* DNA with a range greater than 38 (Widayat et al., 2019). While the result of Conventional PCR was electrophoresis, based on the results of electrophoresis which resulted in pig DNA

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bands being compared with beef DNA bands. The DNA amplified with the reverse bovine primer formed a band measuring 398 bp while in cattle the DNA amplified with the *reverse* bovine primer formed a band measuring 274 bp.

Based on the porcine DNA analysis method in cosmetics, DNA analysis in cosmetic preparations has two methods, RT-PCR (Real-time Polymerase Chain Reaction) and Conventional PCR, which have their respective advantages. The RT-PCR (*Real-time Polymerase Chain Reaction*) method is a method that can amplify and quantify the number of target DNA molecules. While the more accurate and clear stage, because it is combined by electrophoresis so that the DNA bands can be seen, is Conventional PCR. then the sensitive method is the conventional PCR analysis method because it has good amplification results and clear procedures compared to other analyses.

This is because in this study the DNA extraction procedure was followed by a NanoDrop Spectrophotometer and then analyzed by PCR (*Polymerase Chain Reaction*) and the last step was to look at the DNA bands using electrophoresis so that clear and precise DNA bands were obtained (Yuwono, 2006). Research conducted to detect the presence of pig DNA in cosmetic products has not been as much as in food or drug products. From the literature, it was found that most of the detections were carried out on ingredients derived from pork, such as lard and its derivatives. However, few discuss other non-halal ingredients such as placenta, lanolin, albumin, and others. This is due to the lack of awareness and knowledge of the Muslim community regarding the criteria for halal cosmetics.

Table II. Results of DNA Analysis

Sample	DNA Analysis Method	Electrophoresis	CT/CQ	DNA Analysis Results	LOD level	Reference
Lipstick without Halal logo	Conventional PCR	Electrophoresis	-	Negative (-)	-	(Munir et al., 2021)
5 kinds of collagen cream.	Conventional PCR	Electrophoresis	-	Positive (+) 387bp	0.01%	(Zabidi et al., 2020)
Capsules, day cream & beauty soap.	RT-PCR	-	-	Positive (+) on day cream cq FAM 38.23 cq VIC 33.40 & beauty soap cq FAM 37.04 cq VIC 37.36	-	(Widayat et al., 2019)
100mg slimming capsule shell	Conventional PCR uses 4 primers	Electrophoresis	-	Detected Positive in primary: a. PPA 8 in samples A, E, H, I, and N was 126bp	-	(Aviani et al., 2017)

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				b. Primer Pig in all samples positive 130bp		
Cosmetic cream contaminated with lard	RT-PCR	-	-	Cannot be detected.	-	(S Abd-Gani et al., 2019)
Halal & non-halal cosmetics (Cream, Liquid mask, Powder mask)	RT-PCR	-	-	Negative (-)	2.28 x 10^{-} copies /tube	(Kim et al., 2018)
Lipstick	Conventional PCR uses 2 primers	Electrophoresis	-	Positive (+) on the SIMp primer has a 398bp band and on the CYTb primer, there is a 359bp. band	0.01ng	(Ishak & Mutalib, 2018)

CONCLUSION

Based on the results of a review journal can be concluded that after conducting a review of seven journals the best method for the isolation of DNA swine cosmetic preparation is the extraction Boom method, this is because the results of purity in the method produce a good DNA level at around 1.81 -1.94. Not all DNA purity measurements were tested using the NanoDrop Spectrophotometer method. And DNA analysis in cosmetic preparations has two methods, namely RT-PCR (Real-time Polymerase Chain Reaction) and Conventional PCR, which have their respective advantages.

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