Gemmy Sarina *et al*, Int. Journal of Pharmaceutical Sciences and Medicine (IJPSM),
Vol.8 Issue. 5, May- 2023, pg. 53-57

ISSN: 2519-9889
Impact Factor: 5.9

Analysis of Pork DNA in Non-Halal Certified Jamu Capsule Shells Using Polymerase Chain Reaction (PCR) Method

Gemmy Sarina[1*]; Ridho Asra[1]; Muhammad Haris[1]; Sestry Misfadhila[1]; Ghina Muharrani Yahya[1]

[1]School of Pharmaceutical Sciences (STIFARM) Padang, 25147, West Sumatra, Indonesia
*E-mail address: gemmysarina@gmail.com

DOI: 10.47760/ijpsm.2023.v08i05.008

Abstract

Jamu is an original Indonesian medicine, one of which is formulated in the form of capsules. The rise of products that are not certified halal is a problem that needs to be studied more deeply, especially regarding the halalness of a product. Capsules are produced from animal gelatin, the alleged use of pork gelatin is the core of the problem in this study. The purpose of this study was to determine the presence or absence of pork DNA in the shells of jamu capsules. The sample was taken through one of the e-commerce with certain criteria. From several products, 5 jamu capsules were selected, which were marked with samples A, B, C, D, and E. The five samples were isolated to obtain pure DNA which was then amplified using the right primer to form millions of DNA using the PCR method, and electrophoresis to see the DNA bands produced on agarose media and the number of base pairs (bp) which were then compared with positive controls. From the research conducted, it was found that there were 4 samples B, C, D, and E detected positive for containing pork DNA at 132 bp. Meanwhile, the jamu capsule shell preparation in sample A was negative for pork DNA.

***Keywords**:* Capsule, DNA, PCR, Pork

1. Introduction

Deoxyribo Nucleic Acid (DNA) is the hereditary material present in almost all living things. Most of the DNA is located in the cell nucleus, but small amounts can also be found in the mitochondria. DNA can be replicated, or make copies of itself. DNA contains genetic information related to the appearance of the phenotype. DNA functions as a carrier of hereditary traits from parents to offspring, and also functions in the process of protein biosynthesis (Nurhayati, B., & Darmawati, S., 2017; Helena *et al.*, 2018).

Gelatin is a type of protein with water-soluble properties obtained from natural collagen derived from the skin and bones of cows, pigs and fish. Gelatin can be said to be halal if it is made from materials that come from halal materials and is not contaminated with non-halal materials. Pork gelatin is used more by producers because pork gelatin is cheaper and easier to process (Aviani *et al.*, 2017; Rakhmanova *et al.*, 2018).

Jamu is a traditional medicine that has become part of Indonesia's natural wealth (Andrianti & Wahjudi, 2016). There are many herbal dosage forms in circulation, one of which is the capsule dosage form. Capsules are soluble hard or soft shelled preparations containing one or more medicinal ingredients and are generally made of gelatin (Puteri, 2019; Aliyu *et al.*, 2020).

Indonesia is a predominantly Muslim country. Muslim consumers are obligated to consume halal products so that products such as food, beverages, medicines, cosmetics, chemical products, biological products, genetic engineering products, as well as goods used by people, especially Muslims, must be labeled halal (Soekarba, 2018; Nur, 2021). So that the government has an obligation to provide protection and guarantee the halal products consumed by the public (Hudaefi *et al.*, 2021)

ISSN: 2519-9889
Impact Factor: 5.9

2. Material and Method

2.1. Material

The samples used were herbal capsule shells (hard capsule shells) that were not halal certified. Sample collection was carried out on five herbal capsules marked with samples A, B, C, D, and E with different uses and manufacturers, and one positive control (pork).

2.2. Method

1. Sample Preparation
   The capsule shell was weighed, and then the herbal capsule shell sample were broken into smaller pieces so that the shell dissolved more quickly. The body was freed from any remaining herbs or fish oil by wiping the inside of the cover with a tissue and then placing each sample into a 1.5 mL microcentrifuge tube. A 25-mg weight for pork was used as a positive control. The meat should be cut into smaller pieces using sterile scissors to facilitate the dissolution process during incubation. Then transfer the mixture to a 1.5 mL microcentrifuge tube.
2. DNA Isolation & Purification
   The DNA purification process was carried out using a Thermo Scientific GeneJET Genomic DNA Purification Kit® involving four methods: lysis, binding, washing, and elution. The DNA purity, therefore, was evaluated by using a nanodrop spectrophotometer.
3. DNA Amplification and Visualization
   PCR amplification was performed with the GoTaq® Green PCR Mix procedure, as shown in Table 1

Table 1. PCR reaction composition

No.	PCR Components	Initial Concentration	Final Concentration	Volume (µL)
1.	Go Taq Green Master Mix Promega	2x	1x	12,5
2.	Primer Pork-F	10µM	0,4µM	1
3.	Primer Pork-R	10µM	0,4µM	1
4.	ddH_2O	-	-	6,5
5.	DNA	-	-	4
Final Volume				25

The PCR process in this study was set up for 35 cycles via a multi-step amplification process, including: initial denaturation (95°C 3 minutes); further denaturation (95°C, 30 seconds); annealing (60°C 30 seconds); elongation (72°C 30 minutes); final elongation (72°C 5 minutes). The amplified PCR results were mixed with 5µL GelRed 1x and electrophoresed using 1.5% agarose gel in 0.5x TBE buffer at 110 volts for 50 minutes and eventually visualized via gel documenter to identify the DNA migration.

3. Result and Discussion

3.1. Result

In this study, it was found that some of the samples were capsules derived from porcine gelatin. This can be seen in samples B, C, D, and E showing a band with a value of 132 bp or the equivalent of a positive control, so that it can be stated that samples B, C, D, and E were positive for pork DNA. Whereas for sample A there is no band that is the same as the positive control so that it can be stated that sample A does not contain pork DNA (Figure 1).

ISSN: 2519-9889
Impact Factor: 5.9

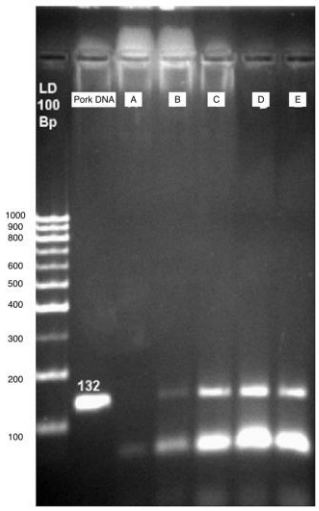

Figure 1. Results of the electrophoresis with 20µL PCR product

3.2. Discussion

DNA isolation from samples and positive controls is needed to obtain DNA from each sample and positive control to be amplified in the PCR. This DNA isolation process begins with lysis of the cell membrane, then the DNA binding stage. The results obtained in the DNA binding process are not completely free of impurities. Therefore, it is necessary to clean up again through the DNA washing stage, and finally, DNA eluting. DNA that is still in the spin column will be moistened with elution buffer which is then incubated and centrifuged so that the clear colored DNA sample can settle down to the bottom of the microcentrifuge tube. The same results were shown by Rizko *et al.*, (2020) on red grapefruit leaves (*Citrus maxima* Merr.) that the DNA sample of red grapefruit leaves that had been isolated was at the bottom of the tube which produced a clear colored sample. The clear color indicates that there is DNA at the bottom of the tube.

The DNA concentration results showed adequate results but not too high, namely in the range of 1.9–31 ng/µL. Estimation of the absorbance ratio at 260 nm to 280 nm can determine the purity of DNA. DNA can be said to be contaminated with protein and RNA if the DNA purity value is lower than 1.8 and greater than 2.0 (Murtiyaningsih, 2017). The high concentration value cannot be a benchmark that the purity is also high. The concentration value will be high if the A_{260} value which is the value for DNA is high and vice versa. The purity value is affected by the contaminant value or the A_{280} value (Iqbal *et al.*, 2016)

Determination of the levels and purity of pork DNA isolates was also carried out by Fathiyah (2015) on vitamin A capsules, the results obtained for the levels of DNA isolates were in the range of 2.435-75.88 ng/µL, and the purity results were obtained in the range of 1.31-2.003. Conducted by Aviani, N (2017) stated that the assay results were in the range of 14.6-69.0 ng/µL, and the purity results were in the range of 1.81-2.00.

Although the DNA results obtained are not so pure. The isolation results will still be continued at the PCR stage, this is because impure DNA will not interfere with the amplification process at PCR (Siswara *et al.*, 2021). This is supported by research conducted by Erwanto *et al.*, (2014) that there are smears on the visualization results of DNA extraction results from meatball samples on the market, but they are still reliable and do not interfere with the PCR process.

To be sure, DNA can be confirmed by observing the amplicon length of the DNA bands which are clearly visible at the electrophoresis stage using 1.5% agarose gel. DNA can be said to be isolated properly if the results of DNA isolation can show a clear and bright band and are above the marker in all wells (Hidayati *et al.*, 2016)
PCR consists of four stages, namely pre-denaturation, denaturation, annealing, elongation and final elongation. Pre-denaturation was carried out at a temperature setting of 95^{o}C for 3 minutes which had been adjusted to the

ISSN: 2519-9889
Impact Factor: 5.9

temperature setting in the kit used. Enzymes contained in the master mix can be affected if the denaturation time is too long and can affect the success of PCR (Kurniawati *et al.*, 2019). Denaturation is carried out at a temperature setting of 95^{o}C for 30 seconds so that the remaining double strands can be converted into single strands so that the results obtained are more even and perfect.

The denaturation temperature is carried out at the optimum temperature, namely in the temperature range of 92^{o}C-95^{o}C, because low temperatures can cause the double-stranded DNA to not open yet which causes the primer to stick to the other side of the DNA strand. Too high a temperature can prevent amplification from occurring because the primer does not stick to the DNA strand (Sinaga *et al.*, 2017). The denaturation process is an important process with a short denaturation time, because it can affect the enzymes in the master mix which can affect the success of PCR (Kurniawati *et al.*, 2019). Then the temperature is lowered to 60^{o}C for 30 seconds where the primer attaches to the DNA that has been specifically cleaved. This decrease in temperature is also known as the annealing step, which is the process of attaching primers to the base (forward) and ends (reverse) of each single-stranded DNA.

Then the temperature was raised to 72^{o}C for 30 minutes. In this process, the primer with the help of enzymes in the master mix will extend the DNA strand according to the split DNA sequence and will form two new single DNA strands. The final process of DNA amplification with PCR is final elongation at 72^{o}C for 5 minutes which is useful to give the unreacted enzymes the opportunity to complete their reaction so that new DNA synthesis can be completed perfectly (Kurniawati *et al.*, 2019). The cycle used in this study was 35 times. After the amplification is complete and the doubled DNA strands are obtained, the sample can be used immediately for electrophoresis or can be stored at -20^{o}C so that the DNA remains fresh and does not experience degradation.

The amplification results were examined using a 1.5% agarose gel. Determination in the use of agarose gel concentration is an important thing. The rate of DNA migration during the electrophoresis process is strongly influenced by the concentration of the agarose gel (Sinaga *et al.*, 2017). The lower the agarose concentration, the further apart the DNA fragments are based on their size and the smaller the gel matrix formed. The use of an agarose gel concentration of 1.5% was also used in the same study by Aviani, N (2017) using beauty supplement capsule preparations as samples.

4. Conclusion

Researchers discovered that some of the capsules used in the study were made with pork gelatin. Bands with values of 132 bp or higher, as seen in models B, C, D, and E, indicate the presence of pork DNA.

References

[1]. Nurhayati, B., & Darmawati, S, (2017), *Bahan Ajar Teknologi Laboratorium Medis (TLM)*. Jakarta: Kementerian Kesehatan Republik Indonesia
[2]. Adrianto, H., (2018), *Biologi Sel dan Molekuler*, Yogyakarta: Deepublish
[3]. Helena, J. M., Joubert, A. M., Grobbelaar, S., Nolte, E. M., Nel, M., Pepper, M. S., Coetzeez, M., & Mercier, A. E, (2018), Deoxyribonucleic acid damage and repair: Capitalizing on our understanding of the mechanisms of maintaining genomic integrity for therapeutic purposes, *International Journal of Molecular Sciences*, 19(4)
[4]. Aviani, N., Mursyanti, E., & Yuda, P., (2017), *Deteksi Cemaran Babi Pada Sediaan Kapsul Suplemen Kecantikan Di Kota Yogyakarta Dengan Metode PCR (Polymerase Chain Reaction).* (Skripsi), Yogyakarta: Fakultas Teknobiologi Universitas Atma Jaya Yogyakarta
[5]. Faridah, H. D., & Susanti, T., (2018), Polysaccharide As Gelatin Substitute Material in Halal Drug Delivery System, *Journal of Halal Product and Research*, 1(2), 15
[6]. Prasetyaningrum. K. D,. Sahubawa. L,. Rahmawati. S. E., (2018), *Pengaruh Konsentrasi Asam Asetat dan Suhu Ekstraksi Terhadap Karakteristik Gelatin Kulit Ikan Mahi-Mahi*, Yogyakarta: Universitas Gadjah Mada
[7]. Rakhmanova, A., Khan, Z. A., Sharif, R., & Lü, X., (2018), Meeting the requirements of halal gelatin: A mini-review, *MOJ Food Processing & Technology*, 6(6), 477–482

ISSN: 2519-9889
Impact Factor: 5.9

[8]. Andrianti., & Wahjudi R. M. T., (2016), *Tingkat penerimaan penggunaan jamu sebagai alternatif penggunaan obat modern pada masyarakat ekonomi rendah-menengah dan atas*, Surabaya: Masyarakat, Kebudayaan dan Politik, 29(3), 133-145
[9]. Puteri, Cut Intan. A. (2019). *Pembuatan dan Evaluasi Cangkang Kapsul Alginat-Kitosan mengandung Teofilin menggunakan Metode Crosslink Trilifosfat*. (Tesis). Medan: Universitas Sumatera Utara.
[10]. Suparman, A. (2019). Karakterisasi dan Formulasi Cangkang Kapsul dari Tepung Pektin Kulit Buah Cokelat (Theobroma cacao L). *Jurnal Ilmiah Farmasi Farmasyifa*, 2(2), 77–83.
[11]. Aliyu R.S., Lawal A.M., Chasta P. & Sharma G.K. (2020). Capsules: Types, Manufacturing, Formulation, Quality Control, Tests and, Packaging and Stotage – A Comprehensive Review. *World Journal of Pharmaceutical and Life Sciences*, 6(8), 93-104.
[12]. Soekarba, S. R. (2018). The Idea of Pluralism in Indonesian Society: A Case Study of Cirebon City as a Cultural Melting Pot. *Journal of Strategic and Global Studies*, 1(1).
[13]. Mohammad, M. F. M. (2021). Pengaturan Sertifikasi Jaminan Produk Halal Di Indonesia. *Kertha Wicaksana: Sarana Komunikasi Dosen dan Mahasiswa*, 15(2), 149–157.
[14]. Nur, F. (2021). Jaminan Produk Halal di Indonesia Terhadap Konsumen Muslim. *Jurnal Likuid*. Bandung: UIN Sunan Gunung Djati Bandung.
[15]. Hudaefi, D., Roestamy, M., Jaka, A., & Adiwijaya, S. (2021). Kepastian Hukum Sertifikasi Halal Pada Obat-Obatan Dikaitkan Dengan Jaminan Produk Halal. *Jurnal Living Law*, 13(2), 122–131.
[16]. Rizko, N., Kusumaningrum, H. P., Siti, R. F., Pujiyanto, S., Erfianti, T., Mawarni, S. N., Rahayu, H. T., & Khairunnisa, D. (2020). Isolasi DNA Daun Jeruk Bali Merah (Citrus maxima Merr.) dengan Modifikasi Metode Doyle and Doyle. *Berkala Bioteknologi*, 3(2), 1–7.
[17]. Murtiyaningsih, H. (2017). Isolasi DNA genom dan identifikasi kekerabatan genetik nanas menggunakan RAPD (Random Amplified Polimorfic DNA). *Agritrop*, 15(1), 84–93.
[18]. Iqbal, M., Buwono, I. D., & Kurniawati, N. (2016). Analisis Perbandingan Metode Isolasi DNA Untuk Deteksi White Spot Syndrome Virus (WSSV) Pada Udang Vaname (Litopenaeus vannamei). *Jurnal Perikanan Kelautan*, 8(1), 54–65.
[19]. Fathiyah. (2015). *Analisis Kandungan Gelatin Babi dan Gelatin Sapi Pada Cangkang Kapsul Keras yang Mengandung Vitamin A Menggunakan RealTime Polymerase Chain Reaction*. (Skripsi). Jakarta: Progam Studi Farmasi Fakultas Kedokteran dan Ilmu Kesehatan Universitas Islam Negeri Syarif Hidayatullah.
[20]. Siswara, H. N., Erwanto, Y., & Suryanto, E. (2021). Kualitas DNA dari Bakso yang Beredar di Pasaran Kabupaten Bojonegoro. *Jurnal Sains dan Teknologi Peternakan*, 3(1).
[21]. Erwanto, Y., Abidin, M. Z., Muslim, E. Y. P., Sugiyono, S., & Rohman, A. (2014). Identification of pork contamination in meatballs of Indonesia local market using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. *Asian-Australasian Journal of Animal Sciences*, 27(10), 1487–1492.
[22]. Hidayati, H., Saleh, E., & Aulawi, T. (2016). Identifikasi Keragaman BMPR-1B (Bone Morphogenetic Protein Receptor IB) pada Ayam Arab, Ayam Kampung, dan Ayam Ras Petelur menggunakan PCR-RFLP. *Jurnal Peternakan*, 13(1), 1.
[23]. Kurniawati, M. D., Sumaryam, S., & Hayati, N. (2019). Aplikasi Polymerase Chain Reaction (PCR) Konvensional dan Real Time-PCR Untuk Deteksi Virus VNN (Viral Nervous Necrosis) Pada Ikan Kerapu Macan (Epinephelus furscoguttatus). *Jurnal Techno-Fish*, 3(1), 19–30.
[24]. Sinaga, A., Agustina, L. P. P., Kata, M. B. (2017). Analisis Pola Pita Andaliman (Zanthoxylum Acanthopodium D.C) Berdasarkan Primer OPD 03, OPD 20, OPC 07, OPM 20, OPN 09. *Jurnal Agoekoteknologi Fakultas Pertanian Universitas Sumatera Utara*. Medan, 5(1): 55-64.

