INVESTIGATING THE RELEASE OF ETHANOL EXTRACT OF *JENGKOL* (*ARCHIDENDRON PAUCIFLORUM*) FRUIT PEEL ENCAPSULATED IN PLGA NANOPARTICLES IN SIMULATED INTESTINAL FLUID

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ABSTRACT

The oral administration of herbal medicine is easier and yields excellent results; however, the release of the medicine can be hindered due to degradation processes in the digestive tract. To maintain the stability of the medicine, PLGA nanoparticle encapsulation is employed. Ethanol extract of Jengkol fruit peel (EEOJFP) is known to have potential for development as an antidiabetic agent. This study aims to determine the influence of PLGA nanoparticle encapsulation on the release concentration of EEOJFP in the digestive tract. Release testing of EEJFP from PLGA nanoparticle encapsulation was conducted in vitro in simulated intestinal fluid (Simulated Intestinal Fluid pH 6.8) over 72 hours. The EEOJFP concentration was measured using HPLC with a mobile phase of methanol and water (8:2) and a C18 column (250 mm x 4.6 mm, 5 µm pore size). The research results indicate that the concentrations of EEOJFP released were 0.03 ppm at 1 hour, 0.128 ppm at 2 hours, 0.201 ppm at 4 hours, 0.343 ppm at 8 hours, 0.374 ppm at 16 hours, 0.486 ppm at 24 hours, 0.528 ppm at 48 hours, and 1.118 ppm at 72 hours. The concentration of EEOJFP released from PLGA nanoparticle encapsulation in simulated intestinal fluid was notably low. In conclusion, PLGA nanoparticles can protect EEOJFP from degradation processes in the digestive tract.

Keywords*: Encapsulation, Nanoparticles, PLGA, Simulated Intestinal Fluid.*

1. INTRODUCTION

Traditional herbal medicines are increasingly utilized in treating various ailments due to their lack of side effects and cost-effectiveness compared to synthetic drugs. The World Health Organization (WHO) reports that 80% of the global population relies on medicinal plants for health [1]. One such plant used as a herbal remedy is the *Jengkol* plant (*Archidendron pauciflorum* (Benth.) I.C. Nielsen). Traditionally, the *Jengkol* plant (*A. pauciflorum*) is used to treat Diabetes Mellitus [2]. The use of herbal medicines for treating Diabetes Mellitus is increasing due to their affordability and low side effects [3].

Researchers have focused on developing oral administration of antidiabetic medications due to its ability to deliver effective treatment results [4]. Oral administration is widely used as it is easy to administer, cost-effective, and preferred by patients compared to other drug delivery systems [5]. Oral drug delivery offers several advantages, but achieving controlled drug release orally is challenging due to external barriers in the digestive tract designed to break down substances entering the body. Barriers to oral drug delivery include the acidic environment of the digestive tract, digestive enzymes, mucous layers lining most of the digestive tract, and tight junctions of the epithelium. One major barrier is the varied and harsh acidic environment of the digestive tract, including the

stomach and intestines. Gastric acidity ranges from pH 1.0 to 2.5, which increases to pH 6.6 to 7.5 in the proximal small intestine and decreases to pH 6.4 to 7.0 in the large intestine. These variations in acidity pose challenges in maintaining drug integrity along the digestive tract [6].

The challenges of oral drug delivery can be addressed using nanoparticles. Nanoparticles are particles ranging in size from 10 to 1000 nm. Their small size allows for a higher surface area-to-volume ratio, providing higher adsorption capacity. The advantages of using nanoparticles include protecting drugs, peptides, or nucleic acids from degradative enzymes, enhancing mucosal adhesion, and improving retention in the digestive tract [6]. Encapsulating these molecules in nanocarrier systems can improve drug solubility and stability [7]. Biodegradable polymer macromolecules are used in nanotechnology systems (polymer-based nanoparticles) and have been extensively developed for drug delivery. One commonly used polymer is Poly (lactic-co-glycolic acid) (PLGA) [8].

Research on the stability of PLGA nanoparticles and drug release from PLGA nanoparticle encapsulation in vitro in simulated digestive fluids has been widely conducted. The levels of estradiol hormone released from PLGA nanoparticle encapsulation with polyvinyl alcohol (PVA) stabilizer formulation in pH 7.4 simulated intestinal fluid, with a released hormone concentration of 38.94 ng/ml from a total encapsulated estradiol concentration of 1 mg/ml after 12 hours of experimentation, indicating the potential of PLGA nanoparticles as effective oral drug carriers [9].

This study will conduct release testing of ethanol extract of *Jengkol (Archidendron pauciflorum)* fruit peel (EEOJFP) encapsulated in PLGA nanoparticles in vitro in intestinal fluid (Simulated Intestinal Fluid) to evaluate its effectiveness as an oral antidiabetic drug delivery system.

2. Materials and Methods

2.1 Preparation of Ethanol Extract of *Jengkol* Fruit Peel

The extraction process is carried out by maceration using 70% ethanol solvent. The steps for preparing the extract include washing and drying the *Jengkol* fruit peel, followed by grinding it into a crude powder using a blender. The obtained crude powder is placed in a container and mixed with 70% ethanol in a ratio of 1:10 (w/v). Subsequently, it is left to stand for 3 cycles of 24 hours each, and then the macerate is collected. The obtained macerate is concentrated using a rotary evaporator at a temperature of 40°C [10].

2.2 Preparation of PLGA Nanoparticles-Ethanol Extract of *Jengkol* Fruit Peel

The fabrication of PLGA nanoparticles-EEOJFP was conducted using the nanoprecipitation technique as described by Fessi et al. [11] under optimal conditions. The procedure for producing PLGA nanoparticles-EEOJFP began by weighing 50 mg of PLGA and 10 mg of EEOJFP, which were then dissolved in 3 ml of acetone. The mixture was slowly added dropwise (0.5 ml/minute) into a solution containing 20 ml of 1% stabilizer (polyethylene-polypropylene glycol) (F68; w/v). Subsequently, the mixture was stirred at 400 rpm using a stirrer at room temperature until complete evaporation of the organic solvent occurred. The stirred mixture was then centrifuged at 25,000 rpm at 4°C for 30 minutes. The resulting pellets were resuspended in Milli-Q water and washed three times. The solid particle suspension was dried using an oven at 30°C for 24 hours.

2.3 Preparation of Simulated Intestinal Fluid (SIF)

The medium used in the in vitro testing of PLGA nanoparticle-EEOJFP release is Simulated Intestinal Fluid (SIF) with a pH of 6.8, following the United States Pharmacopeia 26 (USP 26) standards [12]. The composition of the medium is presented in Table 3.1. The SIF medium is prepared by mixing reagents into deionized water with constant stirring until a clear solution is obtained. The pH of the SIF medium solution is measured using a pH meter and adjusted to pH 6.8 ± 0.05 by adding 0.1 M NaOH solution or 0.1 M phosphoric acid solution as needed.

Table 1. Composition of melting medium for in vitro testing of polymer nanoparticle formulations.

Medium	Composition	
Simulated Intestinal Fluid pH 6.8 (SIF sp) USP 26	KH_2PO_4	6,805 g
	NaOH	0,896 g
	Deionized water	1 L

2.4. Preparation of Standard Solution of Ethanol Extract of *Jengkol* Fruit Peel

The standard solution of EEOJFP is prepared by dissolving 0.5 grams of EEOJFP, which has been formed into a paste, in 500 ml of methanol to obtain a stock solution with a concentration of 1000 ppm. Subsequently, from the stock solution, five different concentrations are prepared. Specifically, 0.02 ml, 0.04 ml, 0.06 ml, 0.08 ml, and 0.10 ml of the stock solution are transferred into separate 10 ml volumetric flasks, and methanol is added to the mark to obtain solutions with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

2.5. In Vitro Testing of PLGA Nanoparticles-Ethanol Extract *Jengkol* Fruit Peel in Intestinal Fluid

The in vitro testing of PLGA nanoparticles-EEOJFP in the intestine is conducted using High Performance Liquid Chromatography (HPLC) method. Methanol and water are used as the mobile phase in a C18 column (250 mm x 4.6 mm, pore size 5 µm) [14]. A total of 37.5 mg of PLGA nanoparticles-EEOJFP are dissolved in 50 ml of simulated intestinal fluid (Simulated Intestinal Fluid (SIF) pH 6.8) without enzymes to achieve a concentration of encapsulated EEOJFP of 750 ppm. This solution is then divided into 8 Falcon tubes and continuously shaken using a shaker at 120 rpm at 37°C. A 100 µL aliquot is taken and centrifuged at 10,000 rpm for 10 minutes at specified time intervals (1, 2, 4, 8, 12, 24, 48, and 72 hours). After discarding the supernatant, the resulting pellet is resuspended in 1 ml of methanol for HPLC analysis [5].

3. Results and Discussion

Release testing of EEOJFP from PLGA nanoparticles is essential to determine whether PLGA nanoparticles can protect the EEOJFP from intestinal fluid pH. This test is conducted in vitro using a medium that mimics the digestive system conditions in intestinal fluid over 72 hours. The concentration of EEOJFP released from PLGA nanoparticles is expressed in ppm and obtained through linear equation of the standard curve of the tested ethanol extract solution using High Performance Liquid Chromatography (HPLC). The linear curve of EEOJFP is determined by conducting linearity tests on the stock solution of EEOJFP with 5 different concentrations measured using HPLC. Linearity testing is performed to assess the method's ability to provide a good response at various analyte concentrations on a calibration curve to generate a straight line [13]. The stock solution with a concentration of 1000 ppm is diluted with methanol solvent to obtain solutions with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The results of the linearity test of the stock solution of EEOJFP are presented in Table 2.

Table 2. Linear Test Results of Stock Solution of EEOJFP

No	Concentration (ppm)	Area Size
1	2	56001
2	4	76740
3	6	95015
4	8	111692
5	10	131180
Linear Regression Equation		Y = 9265.5x + 38533
Coefficient of Correlation		0,9988

Based on the data in Table 2, the linear curve of the stock solution of EEOJFP can be determined (Figure 1).

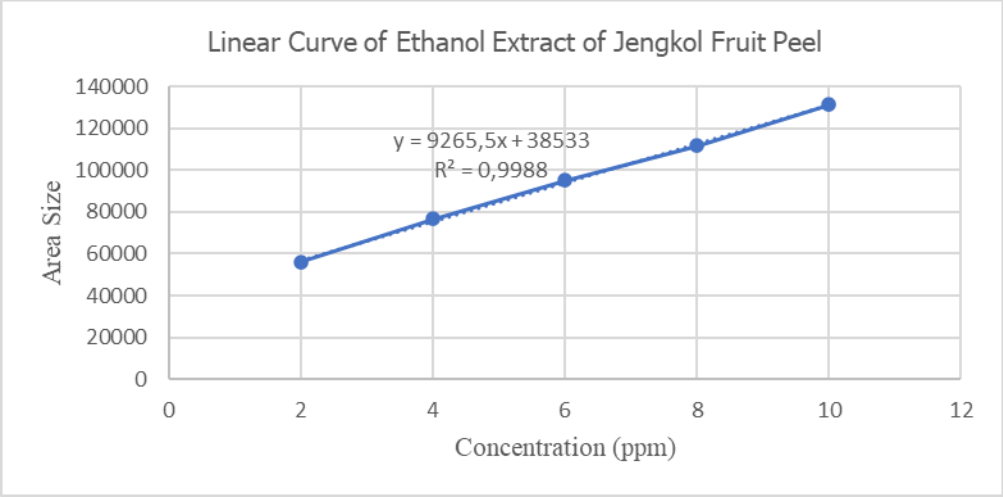

Fig 1. Linear Curve of EEOJFP

The equation of the straight line (linear regression) is depicted as the relationship between the concentration of the ratio of chromatogram area to analyte, then the correlation coefficient (R) is calculated. Based on the linearity test,

an R value of 0.9988 was obtained, indicating that with a confidence level of 99.8%, there is a linear relationship between extract concentration and the formed area, where higher extract concentrations lead to larger areas. The linearity test results of an analytical method are considered valid if the correlation coefficient (R) obtained is greater than 0.98 [15]. The equation of the standard linear curve used is y = ax + b where y represents the area and x represents the concentration of EEOJFP released. The results of the release test of EEOJFP nanoparticles in simulated intestinal media (Simulated Intestinal Fluid USP 26 pH 6.8) at 1, 2, 4, 8, 16, 24, 48, and 72 hours using High Performance Liquid Chromatography are shown in Table 3.

Table 3. Results of Release Test of EEOJFP from PLGA Nanoparticles in Simulated Intestinal Fluid

Hour	Retention Time (minutes)	Area Size	Released Extract Concentration (ppm)
1	1.156	38561	0.003
2	1.153	39721	0.128
4	1.150	40398	0.201
8	1.151	41710	0.343
16	1.138	42002	0.374
24	1.157	43032	0.486
48	1.140	43428	0.528
72	1.148	48908	1.118

Based on the test results, it can be observed that the concentration of EEOJFP released from PLGA nanoparticles in simulated intestinal medium pH 6.8 is very low compared to the total PLGA nanoparticles-EEOJFP in simulated intestinal medium, which is 750 ppm. The released extract concentrations after 1 hour were 0.003 ppm, at 2 hours it increased to 0.128 ppm, at 4 hours it was 0.201 ppm, at 8 hours it was 0.343 ppm, at 16 hours it was 0.374 ppm, at 24 hours it was 0.486 ppm, at 48 hours it was 0.528 ppm, and at 72 hours it was 1.118 ppm. These results indicate an increase in the concentration of EEOJFP released as the testing time progresses in simulated intestinal medium pH 6.8 (Figure 2).

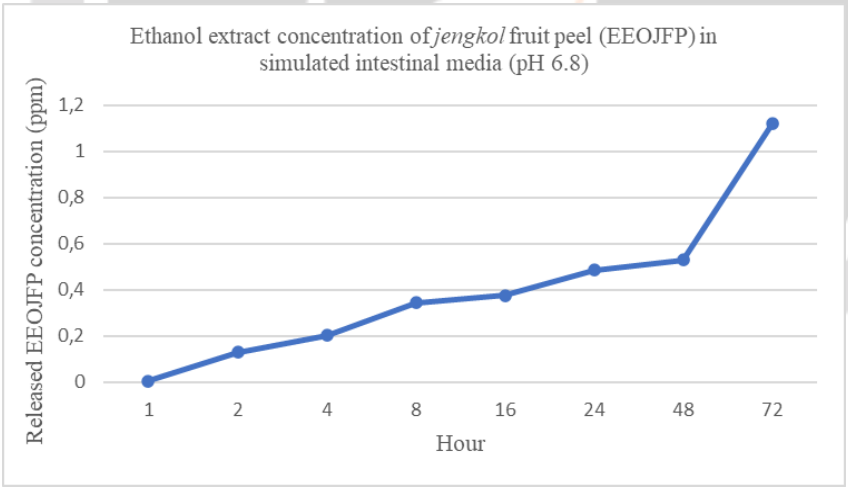

Fig 2. EEOJFP Released from Encapsulated PLGA Nanoparticles in Simulated Intestinal Fluid over 72 hours.

The release of EEOJFP encapsulated in PLGA nanoparticles is caused by the destabilization of the PLGA nanoparticle structure due to environmental conditions in simulated intestinal medium. The acidity level affects the stability of the nanoparticle structure. This finding is supported by Liu et al. [16] who stated that the acidity level of artificial intestinal fluid stimulates the release of nuciferin extract from PLGA-Nuciferin nanoparticles due to increased water absorption by the GA groups, which induces water penetration towards the core of the nanocomposite.

Acidic environmental conditions (such as gastric acidity with a pH range of 1.2) cause protonation of the carboxyl groups of PLGA nanoparticles and nanoparticle aggregation, forming a stable structure that limits the release of extract from PLGA nanoparticles. Increased acidity in intestinal media triggers extract release due to

increased water absorption by the GA groups, enabling water penetration into the nanoparticle core, resulting in a small amount of extract being released from nanoparticles in the digestive tract. Most of the encapsulated extract can maintain its therapeutic effectiveness [16].

PLGA polymer has ester (=O) groups in its backbone structure that allow hydrolysis in aqueous environments or environments containing water. PLGA can degrade through ester group hydrolysis in water-containing environments, with the process initiated by hydration or water entering the nanoparticle structure [17]. The degradation rate is influenced by several parameters, including environmental pH. At neutral pH (in vitro aqueous environment without enzymes), Poly(lactic-acid) nanoparticles can remain relatively stable for up to 10 days. In vitro and in vivo environments such as gastric and intestinal fluids can cause rapid degradation of PLGA nanoparticles if there are enzymes catalyzing hydrolysis. Additionally, the nature of the encapsulated drug also affects nanoparticle degradation. Drugs that are acidic or basic can influence the hydrolysis process [18].

EEOJFP contains several active compounds, including alkaloids that are basic in nature. Alkaloids are nitrogen-containing compounds that are basic and have pharmacological activities [19]. The basic nature of the active compounds in EEOJFP is believed to slow down the hydrolysis process, which leads to degradation of the PLGA nanoparticle structure, thereby minimizing the release of EEOJFP into the medium. According to Li et al. [20]. basic drugs can catalyze ester group hydrolysis but can also neutralize carboxyl acid chains at the end of PLGA polymers, which can slow down the hydrolysis process.

PLGA nanoparticles are internalized into cells partly through liquid phase pinocytosis and clathrin-mediated endocytosis. PLGA nanoparticles in the body can quickly escape from endolysosomes and enter the cytoplasm after 10 minutes of incubation, facilitating nanoparticle interactions with vesicular membranes that allow nanoparticle release into the cytosol [21]. Based on the release test results showing low concentrations of released extract in intestinal media from PLGA nanoparticles and the short incubation time within the body for cell entry, controlled drug release efficiency can be achieved with PLGA nanoparticle encapsulation. Based on the release test results of from PLGA nanoparticle encapsulation, it can be concluded that the concentration of released EEOJFP encapsulated in PLGA nanoparticles into intestinal fluid is very low. Therefore, most of the extract encapsulated by PLGA nanoparticles can maintain its effectiveness in reaching the target organ.

4. CONCLUSIONS

Based on the release test results of EEOJFP from PLGA nanoparticle encapsulation, it can be concluded that the concentration of released EEOJFP encapsulated in PLGA nanoparticles in simulated intestinal fluid (Simulated Intestinal Fluid pH 6.8) is very low, ranging from 0.003 ppm at 1 hour to 1.118 ppm at 72 hours of experimentation. Thus, PLGA nanoparticles can protect EEOJFP from digestive environmental conditions in the intestines.

5. REFERENCES

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