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Bioplastic packaging containing *Ceasalpinia sappan* heartwood extract to inhibit *Escherichia coli* causing spoilage of pork jerky

Siridon Rangsihiranrat*, Nantapat Pittayavonganont, Orawan Piyaboon

Department of Biology and Health Science, Mahidol Wittayanusorn School, Nakhon Pathom, Thailand, 73170

*Corresponding author: *siridol.ran_g31@mwit.ac.th*

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Abstract. Pork jerky, a processed pork product, is susceptible to spoilage caused by contamination with *Escherichia coli*. Moreover, the plastic packaging used for these pork sticks contributes to environmental pollution. In response to these concerns, this project aims to develop a bio-based packaging solution by incorporating *Caesalpinia sappan* heartwood extract derived from a medicinal plant known for its antibacterial properties against gram-negative bacteria, specifically *E. coli*, which is responsible for spoilage in pork jerky. The inhibitory activity of the crude extracts from *C. sappan* heartwood against *E. coli* was evaluated using disc diffusion method. The results demonstrated that the crude extracts effectively inhibit the growth of *E. coli*, with a minimum inhibitory concentration of 1,875 ppm and a minimum bactericidal concentration of 7,500 ppm. Accordingly, a bioplastic formulation was developed using 7,500 ppm of *C. sappan* heartwood extract and then tested the physical property of the bioplastics. The tests revealed no significant differences in thickness and water absorption between the bioplastic containing the extract and the bioplastic containing 0.1% dimethyl sulfoxide. The inhibitory activity was further assessed, demonstrating that the bioplastic incorporating *C. sappan* heartwood extract effectively inhibits the growth of *E. coli* on nutrient agar. Based on these promising findings, it can be concluded that the bio-based plastic, mixed with 7,500 ppm of *C. sappan* heartwood extract, has the potential to serve as an effective packaging solution for pork jerky. This innovative approach not only hinders the growth of *E. coli* but also offers the advantage of being environmentally friendly.

Keywords: *Ceasalpinia sappan*, heartwood extract, *Escherichia coli*, pork jerky

1. Introduction

Thailand's pork export in 2022 when some 23,000 tons of pork products worth 3.64 billion baht were shipped out (OIE, 2022). The primary export markets for these products included Japan, Hong Kong, and Singapore. Among the exported items was pork jerky, a delectable delicacy made by shaping and seasoning pork, followed by drying for two days, baking, and frying. Pork jerky boasts a shelf life of approximately one month, after which it becomes susceptible to spoilage caused by bacteria, particularly *Escherichia coli* (*E. coli*), a microorganism commonly associated with food spoilage (Ekici, 2019). To combat this issue, the use of plant-derived crude extracts has emerged as an alternative method for food preservation. *Caesalpinia sappan (C. sappan*) L. (Leguminosae or Fabaceae) is an evergreen shrubby plant. In Thailand, the heartwood has been used as a coloring agent in food, beverages, cosmetics, garments and medicines. Plant heartwood has been used as a traditional medicine with many health benefits (Kimestri et al., 2018). *Caesalpinia sappan* wood is isolated chemical various structural types of phenolic components including xanthone, coumarin, chalcones, flavones, homoisoflavonoids, and brazilin etc. (Nirmal et al., 2015). Brazilin is the major compound in the *C. sappan* heartwood and is reported to possess various biological activities including antioxidant (Saenjum et al., 2010), *anti*-allergic (Yodsaoue et al., 2009), *anti*-inflammatory (Washiyama et al., 2009), and antibacterial (Srinivasan et al., 2012). Brazillins and tannins, exhibits antibacterial properties against negative-gram bacteria (Muangrat et al., 2022). Packaging technology to extend food shelf life can be determined by evaluation of the type and quantity of microorganisms. Bioplastics are importance to the materials on packaging in the future in the world. Because of this, bioplastics are be evaluated for potentially, independence, energy efficiency and Eco-safety (Arikan and Ozsoy, 2015). However, large-scale production of bioplastics is possible problems might come along with the use of bioplastics by high costs, in comparison to synthetic plastics derived from fossil oil, and concerns over functionality (Peelman et al., 2013). Furthermore, the prevalent use of plastic packaging for pork jerky poses challenges due to its slow decomposition and adverse environmental impact. Consequently, the development of bioplastics capable of natural degradation has arisen as a solution to mitigate pollution issues and promote environmental sustainability (Marichelvam et al., 2019).

This study was aims to develop bioplastic packaging containing *C. sappan* heartwood extracts for inhibiting *E. coli* causing spoilage of pork jerky products.

2. Materials and Methods

2.1 Preparation of C. sappan heartwood crude extracts

The extraction method described by Hemthanon and Ungcharoenwiwat (2022) was used with some modifications. The *C. sappan* heartwood powder was dried in a hot air oven at 65 °C for 72 hours to constant weight and dry weight of different components. Subsequently, 1,200 g of dried powder was exhaustively extracted with 6,000 ml of 95% ethanol for 5 days. The resulting 95% ethanol extract was filtered through Whatman paper No.1 and concentrated under reduced pressure at 50 °C using the rotary evaporator. The percent yield, calculated based on the dry weight, was determined to be 5.64%.

2.2 Antibacterial activity

Screening of *C. sappan* heartwood crude extract for antibacterial activity was done by the disc diffusion method (Zaidan et al., 2005), which is normally used as a preliminary check and to select between efficient *C. sappan* heartwood crude extracts. *Escherichia coli* was adjusted to approximately 10^8CFU/ml with sterile saline solution. One hundred microliters of the suspensions were spread over the plates containing nutrient agar (NA) media. The disc diffusion method was utilized to assess the inhibitory activity. The negative control treatment involved the use of 0.1% dimethyl sulfoxide (DMSO), while the positive control treatment consisted of 24% acetic acid. Paper discs were prepared, each containing 30 microliters of *C. sappan* heartwood crude extracts at concentrations of 600, 6,000, and 60,000 ppm. The dried discs were placed on the surface of the medium. Subsequently incubated for about 24 hours at 37°C and the diameter of the circular inhibition zones were measured and the experimentation was done in triplicates. The means were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test comparison test (DMRT). Significance of all the statistical tests was predetermined at $p < .01$.

The antimicrobial efficacy of *C. sappan* heartwood crude extracts were examined using the colorimetric broth microdilution method. The bacterial inoculums were adjusted to the concentration of 1×10^8 CFU/ml. The Minimum inhibitory concentration (MIC) was determined 96-well-microtitre. Two-fold dilutions of plant extracts were prepared in test wells in complete nutrient broth, the final concentrations ranging from 117.19 to 60,000 ppm. The negative control well was prepared 0.1% dimethyl sulfoxide (DMSO) and the positive control well was prepared 24% acetic acid.

Fifty microliters of bacterial suspension were added to 150 µl of plant extracts and control wells containing culture medium. The plate incubated at 37 °C for 24 hours. After each incubation time, 50 µl of resazurin solution was added per well and incubated at 37 °C for

24 hours. A change in color from blue (oxidized state) to pink (reduced state) indicated growth of the bacteria. Minimum bactericidal concentration (MBC) test was performed after MIC assay *via* 10 µl of inoculum subjected to plate method. These plates were then incubated at 37 °C for 24 hours, and the minimum concentration of *C. sappan* heartwood crude extracts that prevented the growth of *E. coli*, indicated by the absence of bacterial colonies, was identified as the MBC.

2.3 Producing of bioplastics

The selected raw materials and method for the production of bioplastics modified by Prasteen et al. (2018) The amount materials of bioplastic production were combined corn starch, glycerol, distilled water, vinegar, and coconut oil in the following proportions: 1.5 g of corn starch, 1 g of glycerin, 1 g of gelatin, 10 ml of distilled water, 1 ml of vinegar, and 1 ml of coconut oil. The mixture was stirred for 8 minutes at 80 °C. Next, 0.84 g of *C. sappan* heartwood crude extracts were added and mixed in bioplastic materials. However, the negative control bioplastic production contained 0.1% dimethyl sulfoxide only and the positive control bioplastic contained 24% acetic acid. Bioplastic production was poured the resulting mixture onto oiled aluminum foil and incubated in a hot air oven at 35 °C for 2 days.

2.4 Bioplastic physical test

Physical property tests on this bioplastic were in the form of thickness, water absorption, tensile strength, elongation analysis.

1) Thickness measurement

The resulting bioplastic was measured in thickness using a micrometer thickness using vernier caliper. The thickness value (mm) is measured from the average of five measurements of the thickness of the bioplastic.

2) Water absorption

Water absorption was investigated by cutting bioplastic with size 2x2 cm and then weighed the mass bioplastic was put into a container filled with distilled water for 10 min. After immersion in water, bioplastic was removed from the water and weighed to measure the wet weight. Water uptake was calculated as follows:

Water uptake = wet weight−dry weight/ wet weight x 100%

3) Tensile strength and elongation analysis (ASTM d412)

Tensile strength was measured using a universal testing machine (UTM, model 10ST, Tinius Olsen). Tensile strength was calculated as follow:

Tensile Strength = Max Load × Gravity

Elongation at break is an indication of bioplastics flexibility, and it’s expressed as a percentage. Elongation at break was calculated as follows:

Percent elongation (%) = (Elongation at rupture)/ (Initial gage length) × 100

The results of our experiments were recorded and conducted statistical analysis by calculating the mean and standard deviation of the diameter measurements. Statistical analysis of the data was conducted using ANOVA one-way test and Duncan values were determined at 0.01 levels.

2.5 The effect of temperature on the physical properties of bioplastic containing C. sappan heartwood crude extracts

Bioplastic mixed with *C. sappan* heartwood crude extracts at a concentration of 7,500 ppm was incubated at four different temperatures: -3 °C (freezer temperature), 4 °C (refrigerator temperature), 33 °C (room temperature), and 50 °C (high temperature) for a duration of 5 days. The physical properties of the bioplastics were tested, including measuring the thickness, water absorption, tensile strength and elongation at break. Data were recorded the results of our experiments and conducted statistical analysis by calculating the mean and standard deviation of the diameter measurements. At three replicates of each measurement were carried out. Statistical analyses were performed using one-way (ANOVA) and Duncan values were determined at 0.01 levels.

2.6 Antibacterial activity of bioplastics containing C. sappan heartwood crude extracts

The antimicrobial tests of the samples were performed disc diffusion test methods was applied to test antimicrobial sensitivity where bacterial cell concentration was 10^8 CFU/ml, bioplastic mixed with *C. sappan* heartwood crude extracts at a concentration of 7,500 ppm and disc size was 6 mm × 6 mm. The negative control treatment involved the use of 0.1% DMSO bioplastic, while the positive control treatment consisted of 24% acetic acid bioplastic. The result was measured the diameter of the inhibitory zone. The experiment was arranged as randomized complete design with 5 replications. Statistical analysis was conducted by calculating the mean and standard deviation of the diameter measurements. The inhibitory zone means were compared using DMRT at $P<0.01$ following One-way ANOVA

3. Results

3.1 Antibacterial activity of Caesalpinia sappan heartwood crude extracts

The extract derived from *C. Sappan* heartwood demonstrated inhibitory effects on the growth of *E. coli* at concentrations of 6,000 ppm and 60,000 ppm. The average diameter of the inhibition zone between the crude extracts at a concentration of 60,000 ppm and 24% acetic acid had no statistically significant difference in Table 1. The 60,000 ppm of *C. Sappan* heartwood extracts showed more activity against *E. coli* compared to other concentration crude extracts.

The antimicrobial activities of plant extracts against *E. coli* examined were assessed by the presence of inhibition zones. The MIC value was active exhibited 1,875 ppm. MBC results for plant extracts were 7,500 ppm

3.2 Bioplastic physical properties

Physical properties of bioplastics containing the *C. sappan* heartwood extract, the bioplastic mixed with 0.1% DMSO, the bioplastic mixed with 24% acetic acid exhibited in Table 2. The average bioplastic thickness for each sample was no significant difference in thickness among these different mixtures. Similarly, when examining the maximum tensile strength and elongation at break, no significant difference was observed between the bioplastic containing the extract and the bioplastic mixed with 24% acetic acid. Furthermore, the water absorption test conducted on the bioplastic containing the *C. sappan* heartwood extract and the bioplastic mixed with 0.1% DMSO also showed no significant difference, as confirmed by statistical analysis.

3.3 The effect of temperature on the physical properties of bioplastic containing C. sappan heartwood crude extracts

Temperature plays observed a significant role in influencing the physical properties of bioplastic containing *C. sappan* heartwood extracts at a concentration of 7,500 ppm. Specifically, when the bioplastic was stored at a temperature of 50°C, the result indicated that the maximum elongation distance and maximum tensile strength differed significantly compared to the samples stored at other temperatures. These differences were statistically significant, indicating the impact of temperature on the physical properties of the biodegradable plastic containing *C. sappan* heartwood extracts at a concentration of 7,500 ppm as illustrated in Table 3. Bioplastics containing *C. sappan* heartwood extracts at concentration of 7,500 ppm in different temperatures were not affect to physical properties

3.4 Antibacterial activity of bioplastics containing C. sappan heartwood crude extracts

The bioplastic containing *C. sappan* heartwood extracts at a concentration of 7,500 ppm possesses antibacterial properties against *E. coli* in Table 4. Furthermore, the result observed

Table 1. The average diameter of the inhibition zone for the inhibition of *E. coli* by various substances

Substances	**The average diameter of inhibition zone** (Mean ± Standard Deviation) (cm)
0.1% DMSO	0.00 ± 0.00[b]
600 ppm *C. Sappan* heartwood extracts	0.00 ± 0.00[b]
6,000 ppm *C. Sappan* heartwood extracts	0.35 ± 0.20[b]
60,000 ppm *C. Sappan* heartwood extracts	1.74 ± 0.24[a]
24% Acetic acid	2.11 ± 0.81[a]

[a-b] Means±SD in the same column followed by a common letter were not significantly different by DMRT (p<0.01).

Table 2. Physical properties of various bioplastics types

Type of plastics	**Thickness (mm)**	**Elongation (%)**	**Tensile strength (MPa)**	**water absorption capacity test (%)**
Bioplastic with 1% DMSO	0.23 ± 0.06[a]	1,486 ± 131[b]	6.03 ± 1.49[a]	60.55 ± 9.39[a]
Bioplastic with 24**%** Acetic acid	0.30 ± 0.00[a]	3,667 ± 402[a]	1.26 ± 2.36[b]	230.00 ± 50.69[b]
Bioplastic with 7,500 ppm *C. sappan* heartwood extracts	0.30 ± 0.00[a]	3,475 ± 412[a]	1.96 ± 0.07[b]	73.07 ± 23.67[a]

[a-b] Means±SD in the same column followed by a common letter were not significantly different by DMRT (p<0.01)

a significantly larger average diameter of the inhibition zone when using the bioplastic with *C. sappan* heartwood extract compared to the bioplastic with acetic acid at a concentration of 24%. The bioplastic containing *C. sappan* heartwood extracts at a concentration of 7,500 ppm indicated more activity against *E. coli* compared to other bioplastics.

4. Discussion

The utilization of 95% ethanol as a solvent for extracting *C. sappan* heartwood has proven to be an effective method. This polar solvent possesses the capability to extract crucial compounds especially brazilin responsible for inhibiting the growth of *E. coli*, a common cause of pork spoilage (Nirmal et al., 2015). Previous research conducted by Muangrat et al. (2022) has further validated the effectiveness of *C. sappan* heartwood extract obtained using 95% ethanol, attributing its antibacterial properties to the presence of brazilin, which disrupts the cell walls of bacteria. Evaluation of the inhibitory potential of *C. sappan* heartwood extract against *E. coli* growth demonstrated its effectiveness in suppressing the bacteria's proliferation.

Comparing the bioplastic containing *C. sappan* heartwood extract and 0.1% dimethyl sulfoxide (DMSO) solution, it was found that the bioplastic containing 0.1% DMSO exhibited significantly lower maximum elongation values. This disparity can be attributed to the presence of water and complex carbon compounds from *C. sappan* heartwood in the bioplastic formulation, resulting in dipole-induced dipole interactions between carbon molecules and water. Conversely, the 0.1% DMSO bioplastic lacks compounds from *C. sappan* heartwood but exhibits stronger hydrogen bonding between water molecules, as hydrogen bonding surpasses dipole-induced dipole interactions in strength (Smith and Ravishankara, 2002). Consequently, the bioplastic

Table 3. Physical properties of bioplastics containing *C. sappan* heartwood extract at concentration of 7,500 ppm in different temperatures

Temperature (°C)	Thickness	Strain (%)	Stress (MPa)	water absorption capacity test (%)
-3	0.4 ± 0.1[a*]	3,256 ± 196[a]	3.59 ± 0.19[b]	69.44 ± 17.35[a]
4	0.4 ± 0.1[a]	3173 ± 180[a]	3.59 ± 0.79[b]	60.00 ± 17.31[a]
33	0.4 ± 0.1[a]	3,253 ± 253[a]	4.04 ± 0.76[b]	79.35 ± 35.37[a]
50	0.4 ± 0.1[a]	2,496 ± 122[b]	7.40 ± 0.47[a]	80.00 ±20.00[a]

[a-b] Means±SD in the same column followed by a common letter were not significantly different by DMRT (p<0.01)

Table 4. The diameter of the inhibition zone of *E. coli* by bioplastic containing *C. Sappan* heartwood extracts

Type of bioplastics	The diameter of the inhibition zone (Mean ± Standard deviation) (cm)
0.1% DMSO	0.00 ± 0.00[b*]
7,500 ppm *C. sappan* heartwood extract	1.86 ± 0.28[a]
24% Acetic acid	0.67 ± 0.21[b]

[a-b] Means±SD in the same column followed by a common letter were not significantly different by DMRT (p<0.01)

containing *C. Sappan* heartwood extract demonstrates a considerably lower maximum elongation value, which is crucial for flexibility.

Subjecting the bioplastic containing with *C. sappan* heartwood extract to a temperature of 50°C resulted in a reduction in the maximum elongation value accompanied by an increase in viscosity. This phenomenon indicates that higher temperatures lead to decreased moisture content in the bioplastic, consequently reducing the maximum elongation while elevating viscosity. This finding aligns with the research conducted by Rachatanpun (2012), which investigated the influence of moisture on chitosan film and demonstrated that water increases the void space between particles.

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